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Understanding the physiology of folliculogenesis serves as the foundation for perfecting diagnosis and treatment of ovulatory defects

J.H. Check - Camden, NJ (USA)

Understanding the physiology of folliculogenesis can help to develop new techniques to improve pregnancy rates in infertile women.

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Transient ligation of umbilical vessels elevates placental tissue oxygenation by near-infrared spectroscopy (NIRS) in clawn miniature pig animal model.

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Acute generalized exanthematous pustulosis (AGEP) during the puerperal period is reported.
Understanding the physiology of folliculogenesis serves as the foundation for perfecting diagnosis and treatment of ovulatory defects

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Summary
Purpose: To discuss updated physiologic information concerning the mechanism of folliculogenesis. Methods: Physiology studies involving the growth of primordial follicle growth and pre-antral growth to the development of the corpus luteum are discussed. Results: Benefits in aiding fertility potential and pitfalls of these drugs in preventing embryo implantation are discussed with reference to the physiologic processes required for folliculogenesis. Conclusions: Knowledge of the physiology of folliculogenesis can provide further understanding of luteal function when taking follicle maturing drugs and complications, as premature luteinization and the luteinized unruptured follicle syndrome. Also, this knowledge helps to create novel therapies to prevent ovarian hyperstimulation syndrome, endometrial receptivity defects, and treating women with diminished oocyte reserve.

Key words: Antral follicle; Pre-antral follicles; Luteinizing hormone; Follicle stimulating hormone; Ovarian oocyte reserve.

Introduction
Ovulation defects have been conveniently divided into anovulation with estrogen deficiency [1], anovulation with normal estrogen [2], and ovulation defects despite regular menses [3]. Some of the subtle ovulation disorders that can be found in women who have regular menses and an adequate serum progesterone (P) one week before menses include: a short follicular phase [4, 5], premature luteinization [6], luteinized unruptured follicle syndrome [7-10], and luteal phase defects [11].

Diagnosing these subtle defects in ovulation can be challenging, and one has must to wonder in women who show a subtle ovulatory problem, and when there seems to be an adequate number of corrected cycles, why do some women still fail to conceive? Is it bad luck? Another subtle uncorrected cryptic problem outside of ovulation, such as sperm factor, or tubal factor that is not being detected. Or could there still be a subtle ovulatory defect that is not being detected?

What is frightening is that a subtle defect in the formation of the dominant follicle and a metaphase II oocyte can even lead to a normal-appearing embryo that will not successfully implant following embryo transfer (ET). A vivid example of this is the failure of many top in vitro fertilization (IVF) centers to achieve pregnancies despite the ET that appear morphologically normal, following controlled ovarian hyperstimulation (COH) in women with diminished oocyte reserve, and yet high pregnancy rates are achieved in these same women when milder drug stimulation is used [12-14]. The evidence suggests that the defect is at the oocyte level leading to an embryo that will not implant [15]. Since frozen embryos similarly fail to implant (in contrast to women with normal oocyte reserve where the adverse effect of the follicle-maturing drugs in a much lower percentage of patients seems to be at the endometrial level), the data suggest a defective implantation factor that is directly associated with the embryo [16-18]. Alternatively, high dosage follicle stimulating hormone (FSH) drugs or raising further already-elevated endogenous serum FSH levels may lead to an increased risk of meiosis errors leading to aneuploidy [16-18].

The physiology of follicular maturation and the selection of one dominant follicle out of a cohort of antral follicles is a very complex process involving a complex interaction between hormones secreted by the ovary, pituitary, hypothalamus, and other areas of the brain, hormone receptors, growth factors and cytokines. It is with the understanding of the normal physiology that new methods of diagnosing and treating infertility-related to ovulatory factors will be discovered. Furthermore by studying these factors one may learn how to mitigate some of the adverse aspects of follicle-maturing drugs or lead to the discovery of other novel methods to develop mature follicles. Thus this editorial will attempt to summarize folliculogenesis and discuss some of the newest concepts in the physiology of folliculogenesis.

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Folliculogenesis

Germ cell migration to the genital ridge begins at six to eight weeks. There are about \(6 - 7 \times 10^6\) oocytes (maximum number) at 16 - 20 weeks. They exist as primordial follicles which are oocytes arrested in diplotene stage of meiotic prophase surrounded by single layer of spindle shaped granulosa cells. The number of primordial follicles is fixed shortly after birth.

Fate of primordial follicles

The primordial follicles either grow or undergo atresia. There are no interruptions in this process from birth to menopause. The most rapid decline by atresia of oocytes is from 20 weeks to birth (only 10% have survived, i.e., about \(1 - 2 \times 10^6\)). From birth to puberty is the next most rapid decline – only about 300,000 oocytes are left at puberty. Only 400 - 500 follicles will ovulate out of this pool during a lifetime.

Apoptosis and early pre-antral growth

Most primordial follicles are destined for atresia (or apoptosis). Some follicles over an 85-day period develop into follicles that have the potential to become dominant follicles.

It is unknown what process inhibits the apoptosis factor of these pre-ovulation follicles, but paracrine and autocrine factors in the microenvironment seem responsible. This developmental process over these 85 days is independent of FSH. Most of these preantral follicles of two to five mm in size can develop to dominant follicles if they can be rescued from apoptosis by FSH.

The cohort of antral follicles

Those pre-antral follicles now becoming antral (five to ten mm) follicles first show an increase in oocyte size and a change in shape of the granulosa cells from squamous to cuboidal shape. Gap junctions open between the granulosa cells allowing exchange of nutrients and regulatory molecules. The granulosa cells produce factors that inhibit the final maturation of the oocyte until the luteinizing hormone (LH) surge. Follicular growth is regulated by regulatory molecules from the oocyte itself.

Functions of the gap junction

The channels that compose the gap junction are composed of proteins known as connexins. Connexin expression in ovarian follicles is up-regulated by FSH and down-regulated by LH. FSH keeps the channels of gap junctions open and LH closes them.

Development of primary follicles from primordial follicles

When mitosis of the cuboidal layer of granulosa cells in the primordial follicle reaches about 15 granulosa cells (or may begin with as few as three cells), the follicle is now considered a primary follicle. The granulosa cells are separated from the stromal cells by a basement membrane known as the basal lamina. The surrounding stromal cells differentiate into concentric layers referred to as the theca. This occurs at the time when continued mitosis produces three to six layers of granulosa cells. The stroma cells closest to the basal lamina is called the theca interna and the theca externa is the outer portion.

Role of FSH in early folliculogenesis

Although the majority of the primary follicles begin to grow, they quickly undergo atresia. By the beginning of the menstrual cycle, after about 70 days of development, there is a cohort of follicles that if exposed to sufficient FSH, have the capacity to develop into a dominant follicle.

The decline from mid-luteal phase of corpus luteum steroidogenesis (when there is maximal suppression of gonadotropins and inhibin A), allows a rise in FSH beginning a few days before menses. Bioactivity of FSH also increases.

Development of the preantral follicle

Oocyte enlargement (to 0.8 mm) and continued granulosa cell proliferation produces a multilayer effect. The oocyte and granulosa cells are now surrounded by another membrane called the zona pellucida. The theca layer continues to organize by the surrounding stroma and the follicle attains a size of two mm (about four times larger than the primordial follicle).

Steroidogenesis by the preantral follicle

FSH receptors on granulosa cells develop. Granulosa cells can produce estrogen, progesterone, or testosterone. The production of estrogen is FSH-dependent since the aromatase enzyme is FSH-dependent. Estrogen production is limited by FSH receptor content. The predominant steroid produced by the preantral follicle is estrogen.
FSH and the preantral follicle

FSH combines synergistically with estrogen to exert a mitogenic action on granulosa cells to stimulate their proliferation and also increase the number of FSH receptors. FSH receptors quickly reach a concentration of about 1,500 receptors per granulosa cell. The early appearance of estrogen within the follicle allows the follicle to respond to relatively low concentrations of FSH. This is considered an autocrine function of estrogen within the follicle. Not all granulosa cells have FSH receptors: cells with receptors can transfer a signal by gap junction causing protein kinase activation in cells lacking FSH receptors.

Androgen and the preantral follicle

Specific androgen receptors are present in the granulosa cells. They serve as precursors for estrogen through the FSH dependent aromatase enzyme. In low concentration, androgens can further enhance aromatase activity.

Androgens and the preantral follicle

With higher concentration of androgens the preantral follicle favors the conversion to five alpha reduced androgens, for example, dihydrotestosterone (DHT), which cannot be converted to estrogens. 5α reduced androgens inhibit the aromatase enzyme and inhibit FSH induction of LH receptors and the follicles will eventually undergo atresia. The success of a follicle depends on its ability to convert an androgen-dominated microenvironment to an estrogen-dominated microenvironment.

Development of the antral follicle – importance of follicular fluid

Estrogen and FSH cause an increase in follicular fluid which accumulates in the intercellular spaces of the granulosa cell layer and eventually becomes a cavity. Follicular fluid provides a mechanism for nurturing the oocyte and its surrounding granulosa cells (known as the cumulus oophorus). Estrogen becomes the dominant substance in the follicular fluid in the presence of FSH vs androgens in the absence of FSH-FSH receptor interaction. Thus follicles with less FSH receptors become androgen dominant and undergo atresia. If premature luteinization occurs and LH appears in follicular fluid before mid-cycle, mitotoxic activity is activated in the granulosa layer and degeneration occurs, associated with a rise in intrafollicular androgen levels.

LH and FSH receptors – preantral follicle

LH receptors are present on theca cells only. FSH receptors are on granulosa cells only. LH stimulates theca cells to make androgen. FSH induced aromatase enzymes convert thecal androgens to estrogen in the granulosa cells. FSH and estrogen help to induce more FSH receptors in the granulosa cells.

The dominant follicle and FSH

The follicle that has the most estrogen is the dominant follicle and is destined to ovulate. Estrogen increases the sensitivity of the follicle to FSH. The negative feedback effect of estrogen on hypothalamic pituitary FSH secretion causes the other follicles with less FSH receptors to undergo atresia as serum FSH decreases. The dominant follicle is usually established between days five to seven and serum estradiol levels begin to rise by cycle day seven.

The gonadotropins and estrogen are not the only hormones involved in the creation of the dominant follicle. The following hormones also play a role: inhibin A, inhibin B, activin, follistatin, insulin-like growth (IGF) factor I, IGF II. Inhibin B is secreted by granulosa cells in response to FSH. It directly suppresses pituitary FSH secretion. Activin originates in both pituitary and granulosa cells and augments FSH secretion and action in the early follicular phase especially aromatase activity. IGF I enhances all actions of FSH and LH.

Development of LH receptors on granulosa cells

FSH with the help of estrogen induces LH receptors on the granulosa cells of the large antral follicles. LH provides support for the final maturation and function of the dominant follicle. Not only do granulosa cells increase by day nine, the thecal vascularity is also twice as high in the dominant follicle than any other follicles.

Transition from suppression to stimulation of LH release from pituitary

The estrogen initially causes a suppression of LH release from the pituitary, but the transition from suppressor to stimulator of LH occurs as estradiol (E2) rises during mid-follicular phase. The final LH surge requires both a critical E2 level (~200 pg / ml) and certain length of exposure to this higher concentration of E2 (~50 hours).

A less sustained elevation of E2 leads to diminished or no LH surge. Estrogen increases sialic acid content of FSH and LH, thus increasing their bioactivity at mid-cycle.
Role of LH in late stages of follicular development

The increased concentration of estrogen in the dominant follicle enables FSH to shift its focus from making more FSH receptors in granulosa cells, but now increases induction of LH receptors which are critical for eventual corpus luteum function, especially progesterone secretion. Thus LH may have an important role in the final maturation and function of the dominant follicle, thus leading to a “healthier” oocyte. With the development of recombinant FSH products, some initial studies suggested that COH regimens using all recombinant FSH, produced higher pregnancy rates (this concept was partially commercially generated). However, most IVF centers have returned to adding some LH, especially in the later stages of follicular development.

Preovulatory follicle

E2 rises rapidly and usually peaks at 36 hours before ovulation, sometimes even 24 hours before ovulation. This peak in E2 induces the initiation of the LH surge.

LH binds to its receptor in the granulosa cells and initiates P secretion from the granulosa cells. LH also stimulates P receptors in granulosa cells and interaction of P with its receptor inhibits granulosa cell mitosis.

Role of progesterone in the preovulatory follicle

After the pituitary gonadotropin cells have had adequate exposure to estrogen, the small rise in P facilitates the positive feedback effect on LH rise, helping to reach a peak LH surge. If the P level exceeds 2 mg/ml, it may have a negative effect and thwart a proper LH surge. The low levels of P secreted by the dominant follicle helps facilitate the mid-cycle FSH surge. The mid-cycle FSH surge in turn helps to facilitate adequate development of LH receptors on granulosa cells.

Role of androgens on the preovulatory follicle

Some of the follicles that undergo atresia become part of the stroma again and make androgens. The rise in androgens are needed to be made into estrogens by the corpus luteum. The increase in thecal androgens is mostly related to the rise in LH, but with the drop in E2 following the LH surge inhibin increases, which enhances LH stimulation of androgens.

LH surge (important in timing intrauterine insemination (IUI)

The onset of the LH surge is usually 34 - 36 hours before follicle rupture. The LH surge lasts 48 - 50 hours. A certain level of LH concentration must be maintained for at least 14 - 27 hours for full maturation of the oocyte to occur. The LH surge tends to occur at 3:00 a.m. and also between midnight and 8:00 a.m. in 67% of women.

Importance of the LH surge

The LH surge initiates the continuation of meiosis (though not completed until after the sperm has entered the oocyte and the second polar body released). It also helps to expand the cumulus cells. Furthermore, it aids in luteinization of the granulosa cells. Finally, it aids in the synthesis of prostaglandins which are needed to allow the oocyte to release from the follicle.

Preovulatory follicle – factors inhibiting premature oocyte maturation and premature luteinization

LH induced cyclic adenosine monophosphate (AMP) activity overcomes the local inhibitory action of oocyte maturation inhibitor (OMI) which comes from granulosa cells. LH-induced cyclic AMP overcomes local luteinization inhibitor (possibly endothelin a product of vascular endothelial cells). Premature luteinization is also inhibited by activin which suppresses production of P by luteal cells.

The oocyte controls functions of the granulosa cells

The cumulus oophorus differs from other granulosa cells: first there are no LH receptors, and also the cumulus oophorus does not make P. The cumulus oophorus acts as a suppressor of FSH-induced LH receptors in the contiguous granulosa by the oocyte itself.

LH and progesterone interaction during the LH surge

The LH surge initiates a rise of P from granulosa cells. P may enable degeneration of collagen in the follicular wall, enabling the follicular wall to become thin and stretch, especially related to the action of plasminogen which induces collagenase.

Plasmin production

There are two plasminogen activators secreted by granulosa and theca cells in response to LH. The plasminogen activity in granulosa cells activate plasminogen in follicular fluid to make plasmin. The plasminogen activator is mainly
under LH influence, but also by the FSH surge and growth factors. The LH surge leads to suppression of plasminogen inhibitor.

Prostaglandin in the pre-ovulatory follicle

Prostaglandin E and F series and other eicosanoids increase tremendously in the preovulatory follicular fluid. Peak prostaglandin concentration is reached at the time of ovulation. A luteinized unruptured follicle may occur from suppression of prostaglandin production. The role of prostaglandin is to free proteolytic enzymes in the follicular wall. They may also aid in the extrusion of the oocyte-cumulus cell mass by causing smooth muscle contraction.

Steroids and the LH surge

Estradiol levels drop with LH surge possibly by LH down-regulation of its own receptors in the follicle. A decrease in LH may be related to negative feedback of rising serum P level. The decrease in LH may also be secondary to decreased pituitary LH and FSH synthesis secondary to change in gonadotropin releasing hormone (GnRH) pulsatility secondary to E2 and P feedback.

Another mechanism to prevent premature luteinization

FSH stimulates granulosa cells to produce a gonadotropin surge inhibiting factor (GnSIF). Its highest concentration is at mid-follicular stage. Its main function is to prevent premature luteinization.

The luteinized unruptured follicle syndrome

A follicle must be at the proper stage of maturity to enable oocyte release to a given LH surge. The rise in E2 allows for the positive effect on LH release from the pituitary. Follicle maturing drugs causing a higher serum E2 level from multiple follicles can cause a release of LH before any follicles are fully mature.

A brief summary of the corpus luteum and luteal phase

Appropriate luteal steroid secretion requires optimal preovulatory follicular development and continued tonic LH support. The corpus luteum in the early luteal phase requires vascular endothelial growth factor (VEGF) to cause increased angiogenesis. Corpus luteum regression is associated with a decrease in VEGF. P, E2, and inhibin A suppress gonadotropins and new follicular growth. Early pregnancy produces human chorionic gonadotropin (hCG) which rescues the corpus luteum.

Future research potential gained from knowledge of physiological mechanism involved with the process of ovulation

The knowledge of growth factors required for normal ovulation and comparison of changes that occurs with these factors with follicle-stimulating drugs could lead to novel treatment regimens and a better understanding why in some instances apparent multiple ovulation does not lead to perfect embryos, or does not lead to a perfect endometrium, so that proper embryo implantation and pregnancy does not ensue.

IGF II

IGF-II is more abundant in follicles than IGF-I. IGF II is produced in thecal cells, granulosa cells, and luteinized granulosa cells. IGF II enhances FSH and LH. Also IGF II stimulates granulosa cell proliferation. Furthermore IGF II stimulates aromatase activity.

IGF I

The gonadotropins stimulate IGF I and IGF II. IGF I receptors are present in thecal and granulosa cells. IGF I is not present in luteinized granulosa cells. IGF II activates both IGF I and IGF II receptors. FSH inhibits IGF I and IGF II binding protein synthesis and thus maximizes the presence of IGF.

Epidermal Growth Factor (EGF)

EGF in conjunction with gonadotropins causes proliferation of granulosa cells. Et suppresses the up-regulation of FSH on its own receptor.

Transforming Growth Factor (TGF)

TGF-beta is secreted by theca cells. TGF-B enhances FSH induction of LH receptors on granulosa cells. TGF-B in the theca inhibits androgen production.

Fibroblast Growth Factor (FGF)

FGF has the opposite effect of TGF-B. It stimulates mitosis in granulosa cells, angiogenesis, and plasminogen activator. However FGF inhibits FSH up-regulation of its own receptor. It also inhibits FSH-induced LH receptor expression and it inhibits E2 production.
Vascular endothelial growth factor (VEGF)

VEGF is a cytokine produced by granulosa cells. It is important for vascularization of the follicle. If one blocks VEGF, this will suppress angiogenesis of the theca cells and inhibit follicular growth and development.

Luteinized granulosa cells respond to hCG with increased VEGF output, probably contributing to the increased vascular permeability associated with ovarian hyperstimulation that can occur with controlled ovarian hyperstimulation.

Other important cytokines growth factors and immunosuppressant

Angiopoietin 1 and 2 inhibit angiogenesis. Platelet-derived growth factor may modify prostaglandin production within the follicle. Tumor necrosis factor alpha is produced by leukocytes and is important in apoptosis, thus aiding in follicular atresia and disintegration of the corpus luteum.

Effect of follicle stimulating drugs

The use of anti-estrogen drugs in the early follicular phase and the use of FSH or FSH/LH drugs from early follicular phase disrupts the precision and interrelationship of all these complex interactions that have been described. What is amazing is that despite the alterations of these biochemical events, the oocytes produced are reasonably normal. This is evidenced by normal or supranormal pregnancy rates per cycle in women who are anovulatory using follicle maturing drugs. Furthermore, we are all aware of the very high pregnancy rates that have been achieved by recipients using donor oocytes that have been achieved by COH.

It would appear that the main effect of the precise feedback effect, both positive and negative, and the interaction of all of these factors on each other, and on the developing dominant follicle, and the pituitary, is to select one and maybe the best follicle each month to provide the single best oocyte in the cohort.

Some problems that are increased in frequency with follicle stimulating drugs are: 1) greater need for luteal phase P supplementation, 2) a greater risk of premature luteinization, 3) a greater risk of the luteinized unruptured follicle, 4) a greater risk of ovarian hyperstimulation syndrome (OHSS) and 5) a greater risk of creating a hostile uterine environment leading to poor implantation [11-13].

Diminished oocyte reserve

There is one exception to the statement that in general COH does not adversely effect the quality of the oocyte and the ability of the resulting embryo to implant in a normal endometrial environment. This exception may be the woman with increased day three FSH and diminished inhibin B and anti-Mullerian hormone where raising the serum FSH too high leads to oocytes that fertilize normally but produce embryos that do not implant even in a good endometrial environment [14-18].

Discussion

It is hoped that the knowledge of the complex interactions of steroids, gonadotropin receptors, growth factors, and cytokines made by both the ovary and the pituitary and suprasellar structures, that we may improve the ability to identify a subtle ovulatory defect more precisely that we can do today. Similarly, we can try to determine what factors differ in stimulated vs natural cycles to gain some insight as to whether follicle maturing drugs fail to perfectly correct the ovulatory defect, and to thus fail to establish a pregnancy. Thus it is important to establish a good fund of knowledge of the hypothalamus and biogenic amines from higher brain centers, the pituitary, the ovaries, and the endometrium.

As mentioned, it seems that the main adverse effect of follicle-maturing drugs on subsequent conception is on the endometrium and not on the oocyte itself [19, 20]. However, this adverse effect is probably from the secretion of hormones, cytokines, growth factors and receptor stimulation, and suppression that leads to this adverse effect on the endometrium. It is already known that adding extra P in the luteal phase can mitigate some of the adverse effects on the endometrium [21].

The pulsatility of the gonadotropins in the precise amplitude and frequency patterns is mostly for the purpose of down-regulating and restoring FSH receptors to allow the “best” follicle in the cohort to develop. In women with gonadotropin deficiency, the use of LH/FSH gonadotropins can restore ovulation without the need for providing these drugs in a pulsatile manner. However, it is difficult to mature only one follicle. Nonetheless, the knowledge of the susceptibility of FSH receptors to down-regulation led to the idea of trying to restore ovulation by suppressing serum FSH, even in women in apparent menopause with successful pregnancies achieved [14].

There is no question that oocyte quality is more related to age than oocyte reserve [22]. Of course part of this is related to a greater tendency for oocytes from women of advanced reproductive age to be more prone to meiosis I and II errors. Comparative studies of various substances in the serum or follicular fluid of women of more advanced reproductive age versus younger women may perhaps show differences. Finding some key differences in certain cytokines, growth factors, and others in the serum follicular fluid or serum between these two groups, could lead to novel therapies that could improve success with other oocytes from women of advanced reproductive age.
References


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ICSI cycle outcomes in oligozoospermia

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Summary

Aim: The aim of this study was to evaluate the sole effect of sperm concentration on fertilization, embryo quality and pregnancy rates in patients undergoing ICSI cycles. Materials and Methods: 560 ICSI cycles performed for male factor infertility were divided into four groups according to sperm concentration retrospectively. Group 1 consisted of 86 couples whose sperm concentration was less than $1 \times 10^6$, group 2 consisted of 169 couples whose sperm concentration ranged between $1 \times 10^6$ and $5 \times 10^6$, group 3 consisted of 95 couples whose sperm concentration ranged between $5 \times 10^6$ and $10 \times 10^6$, and group 4 consisted of 210 couples whose sperm concentration ranged between $10 \times 10^6$ and $20 \times 10^6$. Results: Fertilization rate was significantly lower in the first three groups compared to the last group ($p < 0.05$). The first three groups were comparable with each other. There were no differences according to ovarian response to stimulation, embryo quality and clinical pregnancy rates between the four groups. Conclusion: Lower sperm concentration has detrimental effects on the outcomes of ICSI cycles. This situation is more evident in men with severe and extremely severe oligozoospermia.

Key words: ICSI; Oligozoospermia; Fertilization rate.

Introduction

Great progress has been made in male subfertility by the introduction of ICSI by Palermo et al. in 1992 [1]. From then on, only sperm has been thought to be enough for a successful pregnancy independent of the sperm defect severity. In the following years investigations of the effect of sperm defects on the outcomes of ICSI were published. Morphology is the most evaluated parameter of semen analysis with conflicting results; some report adverse effects [2-6] and others comparable results [7-10]. However there is a paucity of data regarding the effect of sperm concentration on the outcomes of ICSI cycles [11, 12].

The aim of this study was to evaluate the sole effect of sperm concentration on fertilization, embryo quality and pregnancy rates in patients undergoing ICSI cycles.

Materials and Methods

We retrospectively reviewed the records of patients who underwent ICSI for male factor infertility with oligozoospermia as the sole cause at Hacettepe University, Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Fertility and Reproductive Endocrinology between July 2001 and January 2010. Before the beginning of data collection, institutional review board approval was obtained.

The patients were divided into four groups according to sperm concentration which was analyzed according to Kruger’s strict criteria [13]. Group 1 consisted of 86 couples whose sperm concentration was less than $1 \times 10^6$, group 2 consisted of 169 couples whose sperm concentration ranged between $1 \times 10^6$ and $5 \times 10^6$, group 3 consisted of 95 couples whose sperm concentration ranged between $5 \times 10^6$ and $10 \times 10^6$, and group 4 consisted of 210 couples whose sperm concentration ranged between $10 \times 10^6$ and $20 \times 10^6$. All patients underwent controlled ovarian hyper-stimulation using luteal-long leuprolide acetate (LA; Lucrin; Abbott, Cedex, Istanbul, Turkey) and recombinant FSH (Gonal-F; Serono, Istanbul, Turkey) using the step-down protocol. The starting dose of gonadotropin was determined based on the woman’s age, body mass index (BMI), antral follicle count at baseline transvaginal sonography (TVS), and day 3 FSH and E2 levels. Ovarian response was monitored with frequent serum E2 measurements and TVS. The criterion for hCG (Profasi; Serono, Istanbul, Turkey) administration was the presence of three or more follicles exceeding 17 mm in diameter. Oocyte retrieval was carried out under local anesthesia using vaginal ultrasound-guided puncture of follicles 36 hours after hCG administration.

Semen samples of the male patients were collected by masturbation after two to seven days of sexual abstinence on the day of egg retrieval. ICSI was performed for all metaphase II oocytes, as described by Van Steirteghem et al. [14]. Spermatozoa were selected for injection based on motility. Nonmotile sperm were used when motile sperm were not collected. Fertilization was controlled 16 to 18 hours after ICSI for the presence of distinct pronuclei and two polar bodies [15].

Embryos were graded on day 3 according to a 1-4 scoring system (with 1 being the best), which was based on fragmentation, cell symmetry and blastomere number. The embryos with even blastomeres and no fragmentation were graded as grade 1, embryos with even blastomeres and < 20% fragmentation as grade 2a, embryos with uneven blastomeres and no fragmentation as grade 2b.
as grade 2b, and embryos with uneven blastomeres and < 20% fragmentation as grade 2ab. Embryos with 20-50% fragmentation and > 50% fragmentation were graded as grade 3 and 4 embryos, respectively [16]. Grades 1-3 were considered as transferable embryos. All the procedures of embryo transfer were performed with a soft catheter under TVS. The luteal phase was supported by daily vaginal progesterone suppositories (Crinone, Serono, Istanbul, Turkey) starting one day after oocyte pick-up.

Clinical pregnancy was determined by ultrasound demonstration of a gestational sac at TVS.

Statistical analyses were performed using Statistics Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL). The normal distribution of variables was tested with Kolmogorov-Smirnov. Parametric and numeric variables were compared with the Independent samples t-test. The χ² test was used to analyze nominal variables in the form of frequency tables; p values of 0.05 or less were considered statistically significant. Values are expressed as mean ± SD, unless stated otherwise.

Results

A total of 560 ICSI cycles were included in the analysis. The baseline characteristics of patients and contents of the groups are given in Table 1. The demographic characteristics and responses of the females to controlled ovarian hyperstimulation were comparable between groups (Tables 1 and 2).

Fertilization rate was significantly lower in the first three groups compared to the last group (p < 0.05, Table 2). The first three groups were comparable with each other.

There was no difference according to ovarian response to stimulation, embryo quality and clinical pregnancy rates between the four groups (Tables 1 and 2).

Discussion

There is not enough accurate data about the effects of sperm defects on ICSI cycle outcomes. Morphology, motility and sperm concentrations are the basic characteristics of a routine semen analysis. Many researchers are trying to find out the effect of male factor on treatment outcome by using these parameters.

According to our study embryo quality and clinical pregnancy rates are comparable in oligozoospermic couples. Only fertilization rate was significantly decreased in couples who had sperm concentration less than 10 M/ml (Table 2).

Hashimoto et al. evaluated the effect of severity of oligozoospermia on ICSI cycle outcome in the way we did and they also observed a decreased fertilization rate similar to our study, but the difference was significant in couples who had sperm concentration less than 1 M/ml [11]. Fertilization rates were similar in the other three groups. In our study the fertilization rate was decreased in the first three groups who had sperm concentration less than 10 M/ml compared to the last group (Table 2).

Strassburger et al. investigated the effect of extremely low sperm counts on outcome of ICSI cycles in which sperm concentration ranged between cryptozoospermia and 1 M/ml [12]. The couples with cryptozoospermia had the worst outcomes. Fertilization and clinical pregnancy rates decreased and abortion rate increased in the couples in whom no visible spermatozoa were found before or after centrifugation which was called cryptozoospermia. Spermatozoa could only be discovered after extended sperm preparation in these patients.

Hashimoto et al. and Strassburger et al. evaluated the effects of oligozoospermia on ICSI cycle outcome by first investigating severe oligozoospermia and second extreme oligozoospermia; neither study had a control group with normal sperm analysis [11, 12]. They both demonstrated adverse outcomes with decreasing sperm counts similar to our study, especially for fertilization rates.

Several authors have reported increased sperm chromosome abnormalities with decreasing sperm concentration [17-20]. Nagvenkar et al. reported that men with severe oligozoospermia (sperm concentration < 5 M/ml) have

<table>
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<th>Table 1. — Baseline characteristics.</th>
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<tr>
<td>Group 1</td>
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<tr>
<td>(SC 0 &lt; Ml/ml)</td>
</tr>
<tr>
<td>n = 86</td>
</tr>
<tr>
<td>Female age (years)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>Duration of infertility (mo)</td>
</tr>
<tr>
<td>Antral follicle count</td>
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SC: Sperm concentration

<table>
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<th>Table 2. — Ovarian response, embryo quality and treatment outcomes.</th>
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<tr>
<td>Group 1</td>
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<tr>
<td>(SC 0 &lt; Ml/ml)</td>
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<tr>
<td>n = 86</td>
</tr>
<tr>
<td>E2 level on hCG day (pg/ml)</td>
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<tr>
<td>No. of metaphase II oocytes</td>
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<td>No. of 2-pronucleated oocytes</td>
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<td>Fertilization rate (%)</td>
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<tr>
<td>No. of day 3 grade 1 embryos</td>
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<tr>
<td>No. of transferred grade 1 embryos</td>
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<tr>
<td>No. of transferred grade 2 embryos</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
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<tr>
<td>Clinical pregnancy/embryos</td>
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</table>

SC: Sperm concentration
higher frequencies of sperm aneuploidy compared to oligozoospermic (sperm concentration < 5 M/ml and 20 M/ml) and normozoospermic men [17]. They demonstrated that pregnancy and ongoing pregnancy rates were significantly lower in couples with severe oligozoospermia, however the fertilization rate was similar to our study and the above studies. These results were related to increased implantation failure and miscarriage of chromosomally abnormally developed embryos.

In a study reported by Won Bak et al. 24.6% of men with severe oligozoospermia (sperm concentration < 5 M/ml) developed azospermia in a time period greater than six months and they advised considering sperm freezing in such patients [21].

It is clear from the literature that lower sperm concentration has detrimental effects on the outcomes of ICSI cycles. This situation is more evident in the men with severe and extremely severe oligozoospermia. Clinicians should consider these effects when performing ICSI cycles on couples with oligozoospermia.

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General Section

The role of sperm banking in fertility preservation

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Summary

Objective: To investigate factors that influence sperm banking before cancer therapy and assess the use and disposal of banked sperm after cancer treatment. Study Design: Database exploratory study combined with questionnaire survey of a cohort of 55 men who cryopreserved their sperm at an Andrology Clinic. Main Outcome Measure(s): Rate of use, disposal and abandonment of banked sperm, current fertility, and patient satisfaction with sperm banking. Results: Using logistic regression, we analyzed the factors associated with use and disposal of banked sperm, current fertility status, reproductive outcomes and quality of life in 55 survivors of cancer therapy who cryopreserved sperm at our facility. Most (93%) of the patients undergoing sperm banking before cancer treatment did not use their samples and 33% requested sperm disposal following completion of cancer therapy. Married status and fatherhood before cancer therapy were associated with higher rates of sperm disposal. Sperm disposal was requested because the subjects remained fertile, spontaneously fathered a child, or completed their family. The families of four patients (7%) who died from their cancer also requested disposal of the stored sperm. Six (11%) patients could not be located or failed to contact the clinic and were considered to have abandoned their banked sperm. Only 7% of the patients used their cryopreserved sperm for assisted reproduction. Most of the patients that banked sperm achieved pregnancy with their partners through spontaneous conception compared to through the use of cryopreserved sperm. Conclusions: The rates of disposal and abandonment of banked sperm were high following cancer therapy. Retention of fertility appears to contribute to the low utilization of banked sperm, which emphasizes the need for appropriate consent and directives regarding disposal of unused cryopreserved sperm.

Key words: Fertility preservation; Sperm banking; Sperm use and disposal.

Introduction

Therapeutic advances during the past decades have improved the long-term survival of patients with malignant diseases. The overall 5-year survival rate for cancer in young individuals has risen to 60%; the rates being much higher for Hodgkin’s disease (82%) and testicular cancer (95%), two of the most common tumors in men in the reproductive age [1-7]. With increasing numbers of cancer survivors, quality of life issues are receiving more attention. Fertility is one of the major concerns of men and women surviving cancer. Cancer treatment, whether by surgery, radiotherapy or chemotherapy, can have severe and adverse long-term iatrogenic effects on male and female fertility. Chemotherapy and radiation therapy compromises fertility through their cytotoxic effects on gametogenesis. The degree of gonadotoxic effect is governed by the regimen used (i.e., type, dose, regimen) and duration of the treatment [8]. Further, men with newly diagnosed cancer often have poor semen quality that is associated with limited success in achieving pregnancy after semen banking and subsequent intrauterine insemination. In addition, the process of cryopreservation results in further reduction in semen quality. Fertility may return after cancer treatment in some but not in all individuals, and who will be affected cannot be predicted. For some patients, a method to preserve fertility potential is feasible. Therefore, patients need to be counseled on fertility-sparing opportunities before commencing potentially sterilizing cancer regimens.

The present clinical means for preserving the potential reproductive capacity of men at risk is cryopreservation of sperm before the treatment begins, followed by artificial insemination or assisted reproductive techniques (ART) when pregnancy is desired. Because most reports that have been published focus on fertility outcomes with assisted reproduction using cancer patients’ cryopreserved sperm [8-11], there is inadequate information to guide patient counseling to encourage sperm banking particularly for those with advanced cancer and poor semen quality that may not be suitable for cryopreservation. Preserving the fertility of younger, prepubertal patients raises special concerns about the welfare of offspring resulting from an expected reduced life span of the parent [12]. Further, ethical issues regarding the disposal of the frozen semen and the use of sperm posthumously raise ethical and emotional issues that have not been sufficiently addressed [13, 14]. The aim of this study was to investigate factors that influenced sperm banking at our center. We also sought to elucidate factors associated with disposal of banked sperm by young individuals diagnosed with cancer. Our study sought to provide insights into the rationale for sperm storage from the perspective of patients that may guide the reproductive care of young men with cancer.

Methods

Participants in this study were 70 males diagnosed with malignant diseases and referred for sperm banking at the Andrology Clinic of the Department of Obstetrics, Gynecology and Reproductive Sciences at the Royal University Hospital, Saskatoon, during a 13-year period (1991 to 2004), before...
undergoing surgery, chemotherapy or radiotherapy for their cancer. A detailed database of clinical and sperm variables has been maintained in our unit since 1990. At referral, semen analysis was done to assess pretreatment alterations in sperm numbers, motility and post-thaw motility recovery that influenced the likelihood of successful cryopreservation. The patients signed written consent for sperm banking including indicating their preferences for sperm disposal in the event of death. Patients were followed by the cancer centers to monitor their cancer status, were advised to be in contact with the Andrology Clinic and were sent annual notification concerning continuation or disposition of their cryopreserved sperm.

Following approval from the University of Saskatchewan Human Research Ethics Committee, a consent form explaining the purpose of the study and a questionnaire were mailed to the participants. This was followed by attempts at contacting the patients by telephone. The medical records of all the patients who banked sperm were reviewed. Demographic and clinical data reviewed included age, marital status, cancer diagnosis, modality of cancer treatment, reasons for sperm banking, semen quality, number of banked sperm samples, date of sperm banking, reasons for and date of sperm disposal, and use of frozen sperm. In addition, the outcomes of cancer therapy, current fertility and fatherhood status, and overall satisfaction with sperm banking were evaluated.

**Quantitative indices**

A University of Saskatchewan Fertility after Cancer (USASK-FAC) questionnaire was designed by the authors to evaluate the role of sperm banking in fertility preservation for patients diagnosed with cancer. There were 12 questions in the questionnaire, categorized into three groups: pre-cancer, post-cancer and future, as illustrated in Table 1. The first four questions related to the time periods before sperm banking and cancer treatment, while questions 5 through 9 were designed for the post-treatment follow-up period. The last three questions (10 through 12) were used to identify patient intentions regarding use or disposal of their banked sperm samples. Each question was evaluated by one or two numerical numbers that represented different choices associated with that question.

**Statistical analysis**

All data were stored in an information database and were analyzed using software (SPSS, Chicago, IL). Descriptive statistics were used to characterize the baseline information and to elucidate the information described in the introduction section. Histograms or pie charts were used to show the utilization frequency or the outcomes of sperm banking in clinical practice. The correlation between important factors in sperm banking was assessed using cross-table analysis and chi-square measures. Discriminate analysis was used to investigate the effects of multiple factors, four in this study, on conception or birth after cancer treatment.

**Results**

Fifty-five of the 70 (78.5%) patients who were referred by their cancer specialists to our Andrology Clinic completed sperm banking. The patients varied in age from 15 to 46 years, with a mean age of 34 years and standard deviation of 4.7 years. Fifteen patients were excluded from the survey. These included five patients who had already had surgery and chemotherapy and were excluded because their semen samples were inadequate for cryopreservation. Four patients who were too ill to produce samples for initial semen analysis were excluded. Another four patients who were offered the service declined sperm banking for personal reasons. Two pre-pubertal boys were excluded for lack of sperm samples. The clinical records of the 55 patients were reviewed and the USASK-FAC questionnaire was sent to each of the patients by registered mail with stamped return envelopes. A response rate of 54.5% (30 of 55 participants) was achieved through this postal survey. An additional 15 responses were obtained through follow-up telephone inquiry, for an overall response rate of 81.8% (45 of 55 participants). Ten patients could not be directly reached and four of the patients (7.3%) were known to be deceased. Six patients (11%) that could not be reached were deemed to have been lost to follow-up and were considered to have abandoned their cryopreserved sperm.

Considering the time span that was reviewed in this study, it is possible that some of the patients that could not be reached were also no longer alive.

On the basis of the patients’ information in the questionnaire and their clinical records, we organized our findings into the following two groups, which represent different time lines in the process of cancer treatments.
The role of sperm banking in fertility preservation

Rationale for sperm banking before cancer treatment

In the USASK-FAC questionnaire, there were two questions (3 and 4 in Table 1) related to patients’ reasons for seeking sperm banking. Figure 1 demonstrates that more than 80% of patients intended to have children in future after cancer treatment, and only 2.6% of patients did not plan to. The remaining 18% of patients were unsure about future parenthood. One possible reason is that some of these patients were still very young and uncertain of future desires regarding parenthood. The pie chart in Figure 2 reveals different reasons that patients had for choosing sperm banking. A large proportion of patients (82%) sought sperm banking because of their interest in future parenthood after cancer treatment. Of these, 64% have not fathered a child at the time of cancer diagnosis and another 18% of patients who had children wanted more after cancer treatment.

Use and disposal of banked semen following cancer treatment

The utilization of banked sperms presents an interesting finding. Of the 55 patients who banked their sperm, only four (7.3%) who were not parents before cancer treatment used their cryopreserved sperm for assisted reproduction (3 for artificial insemination and 1 for in vitro fertilization). Two of these were successful. Approximately 93% of patients did not make use of their cryopreserved sperm. Fourteen patients (25.5%) achieved spontaneous conception after cancer therapy without using their banked sperm samples. The time interval from treatment to conception varied from one to eight years. Only ten patients (18.2%) used follow-up semen analysis to ascertain their fertility potential following cancer therapy. Of these patients, three had azoospermia, two had oligozoospermia, and five had normal semen parameters. Eighteen (32.7%) of the patients requested disposal.
of their sperm samples because they remained fertile, spontaneously fathered a child or completed their family. We attempted to elucidate the factors that influenced the possibility of pregnancy or birth after cancer treatment. Figure 3 shows that having “children before cancer” treatment was a strong indicator for achieving pregnancy after cancer treatment. It could be inferred that if a patient had children before cancer treatment, he would likely be more successful in achieving a pregnancy with his partner after cancer treatment. On the contrary, only one in four patients achieved a pregnancy if they had no children before cancer treatment. A crosstabs analysis in Table 2 shows that $p$ value of the Pearson chi-square (asymptotic significance) is just around the threshold of statistical significance (i.e., $p = 0.05$). This suggests that having “children before cancer” treatment has a strong correlation with achieving pregnancy after cancer treatment.

Figure 4 presents the overall satisfaction with the sperm banking service, in which we use a ten-point grading system to assess the level of satisfaction. Grade 1 refers to very low level of satisfaction while grade 10 means a high level of satisfaction. Average satisfaction of patients who chose the banked sperm service was 7.5 with a standard deviation of 2.2. Overall, 65% of patients were satisfied with current sperm banking services.

**Discussion**

We found a high rate of request for disposal of cryopreserved sperm following cancer therapy because most patients remained fertile. Rates of sperm disposal were highest among patients who were either married or were parents at the time of sperm banking. The rate of abandonment of banked sperm was highest among those who were single.

Long-term sperm banking began in our Andrology Clinic in 1990. This study reported our experience with 55 patients who banked their sperm samples up to 2004. The results of this study demonstrate that the majority of patients accepted sperm banking as important to preserving their fertility before cancer therapy. The key motive that seems to guide the decision for semen cryopreservation is ensuring the opportunity for biological parenthood and not risking the prospect of sterility. One important finding in this study is the contradiction between high motivation for sperm banking, the low rate of utilization for subsequent reproduction and high rate of request for disposal of the cryopreserved semen. While on the one hand a majority of patients had a strong interest in sperm banking, on the other hand, the same group of patients rarely utilized their banked sperm for subsequent reproduction. This fact raises a question about the practical utility of sperm banking in the overall management of patients with malignant diseases.

Considerable difficulty was encountered in the follow-up of patients banking semen before cancer therapy. Consistent with earlier studies, a substantial number (11%) of our patients who consented to semen cryopreservation and indicated interest in future reproduction failed to maintain contact with the Andrology Clinic and could not be contacted to make their wishes known regarding continued storage or disposition. This raised the question of whether to consider that the banked semen has been abandoned and poses an ethical dilemma regarding their disposition or continued storage. Because of legal uncertainty and a lack of clear guidance on this issue, our practice is to continue indefinite storage. The Ethics Committee of the American Society for Reproductive Medicine is of the opinion that gametes or embryos deemed abandoned should not be donated to other couples or used for research without prior consent [15].

Our results suggest that several factors may have influenced the utilization of cryopreserved sperm after the cancer treatment. These include marital status prior cancer therapy, paternity before cancer diagnosis and treatment, fertility status following cancer therapy and general health and cancer survivor status.

More patients achieved pregnancy or birth though spontaneous conception as compared to the use of banked semen. The ratio between these two groups is about one in ten, that is, for every patient who achieved the pregnancy or birth by utilizing the banked sperms, there are ten patients who succeeded in pregnancy or birth through the spontaneous conception after the cancer treatments.

Our findings would suggest that patients who are married or have fathered a child prior to cancer therapy have a higher likelihood of achieving fatherhood after cancer treatment, regardless of whether or not sperm banking service is used. On the contrary, patients who have not fathered a child prior to cancer therapy had a reduced likelihood of achieving a conception with their partner after cancer treatment. Therefore, it is important to consider sperm banking for this group of patients prior to cancer treatment.

Our study has certain limitations including the short follow-up period. Further, the views and reproductive outcomes of the substantial numbers of patients that we were unable to reach might differ from those of the respondents and influence the overall findings. In addition, the questionnaires addressed reproductive issues that some might consider too personal to elicit elaborate responses. These limitations notwithstanding, the results from this study are striking, particularly in their demonstration of the substantial proportion of patients who requested disposal of their banked sperm following cancer treatment, the substantial number of patients who remained fertile and achieved spontaneous conception, and the high frequency of abandonment of banked sperm. These results emphasize the need for adequate counseling before sperm banking and provision of clear directives regarding sperm disposition after cancer treatment.

**Acknowledgements**

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The role of sperm banking in fertility preservation

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Cesarean section with relative indications versus spontaneous vaginal delivery: short-term outcomes of maternofetal health

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title

Summary

Aim: The aim of the study was to compare maternal and perinatal mortality and short-term outcomes of maternal and perinatal health between a cesarean group with relative indications and a vaginal delivery group. Methods: A total of 1,119 patients were included; 582 were delivered by spontaneous vaginal birth and 537 delivered by cesarean section without labor. The indication for cesarean section was tocophobia and fear of childbirth for all patients. Maternal and perinatal morbidity and mortality were compared between the groups. Results: No maternal mortality was recorded. Maternal morbidity was significantly lower in the vaginal birth group than the cesarean group (7 vs 30, p < 0.05). Perinatal mortality (2 vs 0) and perinatal morbidity were not significantly different between the two groups (33 vs 17). The vaginally delivered group had significantly higher newborn hospitalization rates than the cesarean group (p < 0.05), but hospitalization time did not differ. Newborns with the first minute Apgar score below 7 were higher in the cesarean group (p < 0.05). Fifth minute Apgar scores and umbilical cord pH values were similar. Cesarean neonates weighed more than vaginally delivered ones (p < 0.05). Conclusion: Short-term maternal complications were more frequently seen in cesarean deliveries with relative indications than spontaneous vaginal deliveries but no difference was found in perinatal mortality and morbidity. There is a clear need for research on health outcomes for mothers and infants associated with cesarean delivery without any medical indication.

Key words: Planned cesarean delivery; Mortality; Morbidity; Short-term outcomes.

Introduction

Cesarean delivery rates continue to rise and rates vary from country to country [1]. It is estimated that 4% of primary cesarean deliveries are elective [2]. The terms “patient choice cesarean” or “maternal request cesarean” are defined as primary cesarean delivery without any obstetric or medical indications. After the first report on prophylactic cesarean at term by Feldman et al., it has become the most controversial topic in obstetric medicine [3]. In reported series cesarean delivery on request ranged from 4% to 18% of all cesareans and 14% to 22% of elective cesareans [4-8]. At the National Institutes of Health State-of-the-Science Conference on cesarean delivery on maternal request in 2006, a panel concluded that there is insufficient evidence to fully evaluate the benefits and risks of cesarean delivery on maternal request and that more research is needed [9, 10]. Risks of cesarean delivery for healthy women are considered very low by many reports, thus making elective cesarean an alternative birth method for women who have tocophobia or fear of childbirth [11-19].

In this study we aimed to compare maternal, perinatal mortality and short-term outcomes of maternal and perinatal health between a cesarean group for which the indication was tocophobia or fear of childbirth and a spontaneous vaginal delivery group.

Methods

This study included a total of 1,119 patients who delivered at the Zonguldak Karaelmas University Faculty of Medicine, Department of Obstetrics and Gynecology, between 2005 and 2010. Data collection was performed retrospectively from the hospital records. The study was approved by the ethical committee of the university. Of the patients 582 delivered by spontaneous vaginal birth and 537 patients delivered by cesarean section without labor; all patients were nulliparas. The indication for cesarean section was tocophobia or fear of childbirth for all the patients. Patients with systemic illnesses as hypertension, diabetes mellitus, renal or cardiovascular disorders were excluded from the study. High-risk pregnancies as intrauterine growth restriction (IUGR), congenital anomaly of the fetus, multiple gestations, preterm labor and cases of oligo- and polyhydramnios were excluded from the study. Maternal and perinatal morbidity and mortality were compared between the groups. Maternal morbidity was accepted as one of the following: postpartum hemorrhage, blood transfusion, fever, wound infection, genitourinary infection, thromboembolic event and operative complications. Perinatal morbidity was accepted as one of the following: birth, trauma, neurologic injury, respiratory distress syndrome (RDS), sepsis, intracranial hemorrhage, necrotizing enterocolite and jaundice. Also infant weight, Apgar scores, umbilical cord pH values and neonatal intensive care unit admission were compared between the groups.

Results

Totally 1,119 patients were included in this study; 582 delivered vaginally and 537 women had cesarean section without labor. The selected patients had no systemic illness or high-risk pregnancies or fetal abnormalities.
Mean gestational age of the women was 37.4 ± 2.9 weeks and 37.6 ± 2.5 weeks for the vaginal delivery group and cesarean group, respectively. All pregnancies were singleton.

There was no maternal death recorded in the 1,119 patients between 2005 and 2010. Maternal morbidity was significantly lower in the vaginal birth group than the cesarean group (7 vs 30, \( p < 0.05 \)) (Table 1).

There were two perinatal deaths in the vaginal delivery group but they were not significant (Table 1). Perinatal morbidity also had no significant difference between the two groups (33 in the vaginal and 17 in the cesarean group) (Table 1). Newborn hospitalization rates were significantly different between the groups (4.7 in the vaginal and 2.9 in the cesarean group, \( p < 0.05 \)) but no significant values were seen in hospitalization days (6.2 for vaginal and 7.8 for cesarean groups) (Table 1).

First minute Apgar scores below 7 were compared between the groups and they were significantly at a lower percentage in the vaginal group than the cesarean group (9.5% vs 14.6%, \( p < 0.05 \)) but no similar difference was noted between the 5th minute Apgar scores (2.6% vs 1.2%) and umbilical cord pH values below 7.10 (14 vs 10) (Table 1). Interestingly, newborn weights were lower in the vaginal group than the cesarean group (3064 ± 580 g in the vaginally delivered babies and 3268 ± 513 g in cesarean delivered babies \( p < 0.05 \)) (Table 1).

Discussion

Rates of elective cesarean deliveries without obstetrical indications are rising worldwide. Unquestionably there is need to assess the risks of maternal and perinatal complications associated with elective cesarean delivery. As a result of a small sample size there were no maternal deaths in our study. In earlier studies maternal mortality had a marked increase with cesarean delivery but these studies were performed in the 1960s to 1970s and do not only reflect the actual risk of cesarean itself but also reflect preexisting disease [20-22]. Maternal mortality or morbidity was not evaluated in patient subgroups, like elective cesarean, cesarean after labor or emergency cesarean. Llford et al. compared elective versus non elective cesareans with respect to vaginal deliveries and showed that maternal mortality rate from elective cesarean was 23 per 100,000 procedures in contrast with 6 per 100,000 vaginal deliveries [23]. The relative risk (RR) of death from elective cesarean was 3.8. In a recently published review of the literature Vadnais et al. found overall maternal mortality rate to be 6 to 54 deaths per 100,000 live births from analysis of nine publications [24]. The RR of direct obstetrical death with cesarean delivery for any reason compared with vaginal delivery ranged from 3 to 13. Also RR of death with elective cesarean delivery as compared with vaginal delivery was reported as 0.77. A meta-analysis of eight studies which compared elective repeat cesarean versus trial of labor with a prior cesarean delivery reported three maternal deaths out of 27,504 women but could not find a significant difference in maternal mortality based on method of delivery [25]. The Report on Confidential Enquiries into Maternal Deaths 1997 to 1999 reported 8.23 direct maternal deaths per 100,000 total cesarean deliveries and 1.69 maternal deaths per 100,000 vaginal deliveries. Cesarean deliveries were classified as emergent, urgent, scheduled, elective, perimortem, and postmortem. The RR of mortality with scheduled cesarean delivery compared with vaginal delivery was 0.8 and this was statistically insignificant [26]. The greatest risk was for emergency cesarean delivery (12.0). The authors reported increased RR with scheduled and elective cesarean (RR 2.8), and also with emergency and urgent cesarean (RR 4.3) when compared with vaginal delivery between 2000-2002 [27]. Liu et al. compared 46,766 planned cesareans with 2,292,420 planned vaginal deliveries. No women died in-hospital in the planned cesarean group but 41 women died in the planned vaginal delivery group (1.8 per 100,000 deliveries, \( p = 0.87 \)); the highest in-hospital maternal mortality rate was recorded in the emergency cesarean delivery group [28]. An ideal study to assess maternal mortality rate would require a large number of women. Moreover it would require many years of follow-up to evaluate long-term complications and effects of primary elective cesarean delivery.

In our study maternal morbidity was accepted as one of the following: postpartum hemorrhage, blood transfusion, fever, wound infection, genitourinary infection, thromboembolic event and operative complications. Postpartum complications were significantly higher in the cesarean group than the vaginally delivered group (30 vs 7, \( p < 0.05 \)). In the literature some studies were against and some were for cesarean section. Two studies that evaluated rehospitalization within 60 days of delivery found women delivered by cesarean or operative vaginal delivery were more likely than those delivering spontaneously to be readmitted for uterine infection, surgical wound complications, genitourinary conditions, cardiopulmonary disorders, thromboembolic phenomena or appendicitis. However these studies had limitations in

### Table 1. — Maternal and fetal outcomes.

<table>
<thead>
<tr>
<th>Description</th>
<th>Vaginally delivered Cesarean section</th>
<th>Cesarean section</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SD)</td>
<td>26.9 ± 5.2</td>
<td>27.2 ± 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age (weeks, mean ± SD)</td>
<td>37.4 ± 2.9</td>
<td>37.6 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Maternal mortality (n)</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Maternal morbidity (n)</td>
<td>7</td>
<td>30</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Perinatal mortality (n)</td>
<td>2</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Perinatal morbidity (n)</td>
<td>33</td>
<td>17</td>
<td>NS</td>
</tr>
<tr>
<td>Newborn hospitalization (%)</td>
<td>4.7</td>
<td>2.9</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Newborn hospitalization time (days ± standard deviation)</td>
<td>6.2 ± 3.4</td>
<td>7.8 ± 6.4</td>
<td>NS</td>
</tr>
<tr>
<td>First minute Apgar &lt; 7 (%)</td>
<td>9.5</td>
<td>14.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fifth minute Apgar &lt; 7 (%)</td>
<td>2.6</td>
<td>1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Umbilical cord Ph &lt; 7.10 (n)</td>
<td>14</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Newborn weight (g ± standard deviation)</td>
<td>3064 ± 580</td>
<td>3268 ± 513</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
identifying cesareans as elective or in labor [29, 30]. Allen et al. studied maternal morbidity between 1998 and 2001 in Nova Scotia Atlee Perinatal Database [17]. Overall complication rates were similar in elective cesarean (7%) and spontaneous vaginally delivered women (6.2%). Cesarean in labor and assisted vaginal delivery had a higher rates of morbidity (16.3% RR 0.4, 95% CI 0.3, 0.6 and 12.9% RR 0.6, 95% CI 0.4, 0.7). The term breech trial, a randomized multicenter study comparing planned cesarean versus planned vaginal delivery of breech presentation at term, found no significant differences in specific complications including hemorrhage, genital tract injury, wound breakdown, infections or depression [12]. Mozurkewich and Hutton reported increased risks of febrile morbidity, transfusion and hysterectomy at elective repeat cesarean versus trial of labor in the metaanalysis of controlled trials [25]. Liu et al. compared low-risk planned cesarean versus planned vaginal delivery and reported an increased risk of most of the complications in the cesarean except for hemorrhage requiring transfusion (odds ratio 0.4, p = 0.005) and uterine rupture (odds ratio 0.4, p = 0.048) [28]. The planned cesarean group had a significantly longer duration of hospital stay. Wax examined five retrospective cohort studies [31-35] of planned cesarean versus planned vaginal delivery of breech presentation fetuses in a metaanalysis [36] and found that including cystitis as a morbidity indicator, adverse maternal outcomes ranged from 12%-28% in planned cesareans and 8%-23% in planned vaginal deliveries. Excluding cystitis and performing a fixed effect metaanalysis there was no difference seen in morbidity by planned delivery route. Compared with planned cesarean, planned vaginal delivery also incurs a somewhat increased risk of hemorrhage, attributable to operative vaginal delivery and unplanned cesarean in labor. In contrast, planned cesarean is consistently associated with more frequent infectious morbidity, cystitis, and endometritis than planned vaginal delivery. Despite these differences in morbidity-specific risks, composite morbidity is similar in women undergoing planned vaginal and planned cesarean delivery. A Cochrane Database Systematic Review of three randomized trials [12, 37, 38] comparing planned cesarean with planned vaginal delivery noted somewhat increased overall maternal morbidity in the planned cesarean group (9.1% vs 8.6%, RR 1.29; 95% CI 1.03, 1.61) [39]. A recently published indication matched cohort study found the incidence of total complications 2.2 times higher in the cesarean group [40]. The cesarean section group had a RR of 5.6 for postpartum hemorrhage. Rates of puerperal infection or postpartum fever did not show significant differences. Most common problems after one-year discharge such as anemia, reproductive tract infection, wound complications and waist/back pain did not find differences between the two groups.

The effects of elective cesarean delivery on the fetus are less clear than for the mother. Performing elective cesarean will result in iatrogenically premature infants but on the other hand continuing pregnancy may end with stillbirth. Nielsen et al. observed that the Swedish cesarean rate rose from 5.5% to 12.4% between 1973 and 1981, while at the same time a decline in perinatal mortality of 12 per 1,000 to 7.1 per 1,000 [41]. However the authors concluded that decrease in perinatal mortality could not only be explained by increasing cesarean rate; neonatal practice changes, antenatal steroids, tocolytics and antepartum fetal surveillance are the other influencing factors. Signore et al. calculated that approximately 1,440 elective cesareans would have to be performed to prevent one perinatal death by a decision analysis method [42].

In our study we could not find significant differences between the two groups in perinatal mortality and morbidity. The vaginally delivered group had significantly higher newborn hospitalization rates than the cesarean group, but hospitalization time did not differ. Newborns who had a first minute Apgar score below 7 were higher in the cesarean group (p < 0.05). Fifth minute Apgar scores and umbilical cord pH values were similar. Cesarean babies were heavier than vaginally delivered ones (p < 0.05). The first suggestion of elective cesarean was if it could avoid neurologic injury or not. Cerebral palsy effects 2-3 per 1,000 births and 10% could be attributable to intrapartum events [43]. Despite the marked increase in cesarean rate, cerebral palsy rates remained stable. Therefore cesarean is not neuroprotective for the fetus [44, 45]. Towner et al. reported that infants delivered by prelabor cesarean showed no differences in frequencies of subdural, intraventricular, subarachnoid hemorrhage, facial nerve, brachial plexus injury or seizures compared with spontaneous vaginally delivered infants [46]. Also prelabor cesarean was associated more with common occurrences of CNS depression, feeding difficulty and mechanical ventilation. Puza et al. found that in contrast to rising cesarean rate during several years nerve palsies and fractures did not decrease [47]. Labor is responsible for clearing greater than 75% of liquid filling the fetal lungs during vaginal delivery in sheep [48]. Vaginally delivered infants establish final lung volumes more rapidly than those delivered by cesarean [49, 50]. After 37 weeks of gestation respiratory distress syndrome and transient tachypnea of the newborn are seen in order of decreasing frequency after prelabor cesarean, cesarean in labor, and labor with vaginal delivery [51]. Hook et al. reported an incidence rate of 7% for respiratory problems in a cesarean delivery group compared with 4% in a vaginal delivery group (p < 0.03) [52]. Vaginal delivery remains the mode of delivery associated with the lowest risk of neonatal respiratory distress [53].

This study has some limitations such as small sample size, not evaluating long-term morbidity, capacity of future fertility, risk of ectopic pregnancy, stillbirth and spontaneous abortion, risk of abnormal placentaion and future hysterectomy. Moreover pelvic floor, anal and urinary incontinence were outside of the interest of this study. Short-time maternal complications were seen more frequently in cesarean delivery with relative indications
than in spontaneous vaginal delivery but no difference was found in perinatal mortality and morbidity. There is clear need for research on health outcomes for mothers and infants associated with cesarean delivery without any medical indications.

References


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Transient ligation of umbilical vessels elevates placental tissue oxygen index (TOI) values measured by near-infrared spectroscopy (NIRS) in clown miniature pig animal model

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Summary
We recently found a significant elevation in placental tissue oxygen index (TOI) values in cases of fetal growth restriction using near-infrared spectroscopy (NIRS), indicating high oxygenation in the placental tissue. We hypothesized that insufficient fetoumbilical blood flow is causatively associated with high oxygenation levels in placental tissue. We transiently (for 15 sec) ligated the whole umbilicus, umbilical arteries, or veins of pregnant Clawn miniature pigs (102-113 days of gestation) and assessed the changes in TOI values of the placenta and fetus. The ligation significantly increased placental TOI values (p < 0.01, respectively), but concomitantly decreased fetal TOI values (p < 0.01, respectively), suggesting a decline in oxygen inflow from the maternal to fetal circulation in the placental tissue to be causative of the elevated placental TOI values. These observations suggest the promising clinical use of placental TOI values measured noninvasively by the transabdominal application of NIRS to assess the fetoplacental circulation.

Key words: Placenta; Pregnancy; Near-infrared spectroscopy (NIRS); Umbilical.

Introduction
The placenta is a critical organ for fetal development as it transports oxygen and nutrients from maternal to fetal blood. Therefore, it is plausible that a chronic and/or acute malfunction of fetomaternal transportation in the placenta leads to a deterioration in fetal well-being, such as fetal growth restriction and/or hypoxia. Indeed, both fetal growth restriction [1] and hypoxic-ischemic encephalopathy [2] are closely associated with the mortality as well as morbidity of newborns and important clinical issues even in modern medicine. However, no gold standard for the intrauterine diagnosis of fetal hypoxia has been established, despite recent improvements in neonatal medical care, partly because of the methodological complexity in clarifying the physiology and pathophysiology of changes in placental tissue oxygenation. Maternal and fetal blood circulate separately throughout the structure of the placenta, which makes it difficult to assess overall placental tissue oxygenation by direct measurement with a pulse oximeter, an electrode [3], tissue or blood sampled by needle biopsy etc.

Jobsis first reported a technique for the noninvasive monitoring of tissue oxygenation in intact organs, near-infrared spectroscopy (NIRS) [4]. NIRS has since been utilized in various clinical fields all over the world [5, 6]. Recently, we transabdominally applied NIRS to the human placenta, successfully evaluated concentrations of oxyhemoglobin (HbO2) and hemoglobin (Hb) [7], and proposed a placental tissue oxygen index (TOI), calculated according to the formula [HbO2/HbO2 + Hb] x 100 (expressed as a percentage) [8], as an index for the assessment of placental oxygenation [8, 9]. Subsequently, we observed a significant elevation of placental TOI values in cases of fetal intrauterine growth restriction (IUGR) [8, 9], especially those complicated by chorangiogenesis, a distinct pathological change of the placenta [10]. The TOI values positively correlated with tissue oxygenation in a wide range of organs, such as the brain [11, 12], muscle [13], and liver [14, 15] in both humans and animal models. Therefore, our recent observations indicate the co-existence of paradoxically high levels of oxygenation in the placental tissues, because some cases of IUGR [16], and especially those with placental chorangiogenesis [17, 18] are potentially associated with long-standing, rather low-grade, fetal hypoxia.

In the placenta, maternal blood, mainly from uterine arteries, supplies oxygen to and receives carbon dioxide from fetal blood. Studies with both human subjects [19] [20] and animal models [21, 22] have suggested that insufficient maternal blood flow in the uterine arteries was causatively associated with fetal hypoxia. However, as not only maternal, but also fetal blood circulates in the placental tissues, it is possible that fetoplacental circulation affects placental tissue oxygenation, contributing to an exquisite regulatory system to maintain appropriate oxygenation in the fetoplacental compartment.

In the present study, we hypothesized that insufficient fetoumbilical blood flow reduces the amount of oxygen transported from the maternal to fetal circulation in the placenta, thereby contributing to a high level of oxygenation-
tion in placental tissues, as indicated by the elevated TOI values in our recent studies. To test the hypothesis, we temporarily obstructed the umbilical vessels of pregnant Clown miniature pigs and assessed the changes in placental tissue oxygenation by measuring TOI values using NIRS. We found that acute obstruction significantly increased placental tissue oxygenation, which is to our knowledge the first evidence supporting the concept that the feto-umbilical circulation is causatively linked with the regulation of placental tissue oxygenation, in addition to the changes in maternal blood supply.

Materials and Methods

**Pregnant miniature pigs as an animal model**

All animal experiments were carried out at Kobe Medical Device Development Center (Kobe, Japan) with the permission of the institutional animal experiment committee. A total of six pregnant Clown miniature pigs at 109, 112, 113, 102, 108, and 105 days of gestation (39.6±48.4 kg; term: 114 ± 3 days of gestation) were anesthetized with an intramuscular injection of 15 mg/kg of ketamine (Daiichi Sankyo Co., Ltd., Tokyo, Japan), 10 mg/kg of xylazine (Bayer Medical Ltd., Tokyo, Japan), and 0.1 mg/kg of atropine (Tanabe Co., Ltd., Osaka, Japan) followed by ventilation with 100% oxygen gas mixed with 2-3 % isoflurane (Merck Japan, Tokyo, Japan). In each animal, a longitudinal incision was made in the abdomen, and six to eight fetuses (155 to 676 g) were used for the experiments. Maternal blood oxygen saturation was continually monitored transcutaneously using a DS-7141 Patient Monitor (Fukuda Denshi, Co., Ltd., Tokyo, Japan).

**Assessment of oxygenation by NIRS**

Assessment of tissue oxygenation was carried out as reported for human placenta [8]. In brief, spectral changes in near-infrared rays of four different wavelengths (775, 825, 850, and 905 nm), reflecting changes in oxyhemoglobin (HbO2) and deoxyhemoglobin (Hb) concentrations in placental tissue, were
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 monitored using an NIRO200 spectrophotometer (Hamamatsu Photonics, Hamamatsu Japan). TOI values were calculated according to the formula \(\[\text{HbO}_2/\text{HbO}_2 + \text{Hb}\] \times 100 (expressed as percentages) \[8\].

Measurement of placental and fetal TOI values

A small longitudinal incision was made in the lateral side of the uterus with an electric scalpel at the fetal compartment. Placental TOI values were measured by attaching the NIRS probe to the surface of the uterine wall just outside the placental attachment (Figure 1A), while a thin layer of aluminum was inserted between the placenta and fetus to prevent near-infrared rays from reaching the fetal circulation. Fetal TOI values were measured by placing the NIRS probe directly on the fetus (Figure 1B).

Table 1. — Representative patterns of changes in oxyhemoglobin, deoxyhemoglobin, and total hemoglobin underlie the increased placental and decreased fetal TOI values. Horizontal arrows indicate unchanged values. Up and down arrows indicate increased and decreased values, respectively. Two and only one arrows indicate large and small changes.

<table>
<thead>
<tr>
<th></th>
<th>Ligation of whole umbilical cord</th>
<th>Ligation of umbilical artery</th>
<th>Ligation of umbilical vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta</td>
<td>Oxyhemoglobin</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td></td>
<td>Deoxyhemoglobin</td>
<td>↓↓</td>
<td>→</td>
</tr>
<tr>
<td></td>
<td>Total hemoglobin</td>
<td>→</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>TOI values</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Fetus</td>
<td>Oxyhemoglobin</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td></td>
<td>Deoxyhemoglobin</td>
<td>↑↑</td>
<td>↑↑</td>
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<tr>
<td></td>
<td>Total hemoglobin</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>TOI values</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

The whole umbilical cord, umbilical arteries or veins were ligated for 15 sec using silk string, and the changes in placental and fetal TOI values were continuously recorded (Figure 1C). TOI values of the placenta and fetus at the end of the ligation were compared to those just before the ligation.

Statistical analysis

Values are expressed as the means ± SD. Significant differences were assessed using the Wilcoxon signed rank test for comparison of paired TOI values just before the ligation with those at the end of the ligation (15 sec); \(p\) values less than 0.05 were regarded as significant.

Results

Changes in TOI values with the ligation of the whole umbilicus

Acute ligation of the whole umbilicus significantly increased the placental TOI values (59.0 ± 7.5 [%] vs 64.0 ± 8.5, \(n = 23\) ligations/12 fetuses, \(p < 0.01\)), but simultaneously decreased fetal TOI values (45.6 ± 3.7% vs 39.2 ± 4.6%, \(n = 12\) fetuses, \(p < 0.01\)) (Figure 2A).

A small increase in oxyhemoglobin, decrease in deoxyhemoglobin, and unchanged total hemoglobin resulted in a significant increase in placental TOI values (Figure 2B; Table 1). A decrease in oxyhemoglobin, increase in deoxyhemoglobin, and unchanged hemoglobin resulted in a significant decrease in fetal TOI values (Figure 2C; Table 1). There were no significant changes in oxygen saturation in the dams (data not shown).

Changes in TOI values with the ligation of umbilical arteries

Acute ligation of umbilical arteries significantly increased the placental TOI values (62.0 ± 3.4% vs 64.9 ± 4.9%, \(n = 14\) fetuses, \(p < 0.01\)), but simultaneously decreased fetal TOI values (47.7 ± 3.9% vs 45.1 ± 2.9%, \(n = 20\) ligations/14 fetuses, \(p < 0.05\)) (Figure 3A).

A small increase in oxyhemoglobin, decrease in deoxyhemoglobin, and decrease in total hemoglobin resulted in
a significant increase in placental TOI values (Figure 3B; Table 1). A small increase in oxyhemoglobin, large increase in deoxyhemoglobin, and large increase in total hemoglobin resulted in a small but significant decrease in fetal TOI values (Figure 3C; Table 1). There were no significant changes in the oxygen saturation in the dams (data not shown).

Changes in TOI values by transient ligation of umbilical veins

Acute ligation of umbilical veins significantly increased the placental TOI values (60.2 ± 4.8% vs 63.8 ± 6.9%, n = 10 fetuses, p < 0.01), but simultaneously decreased fetal TOI values (43.0 ± 4.1% vs 39.1 ± 5.1%, n = 10 fetuses, p < 0.05) (Figure 4A).

An increase in oxyhemoglobin, stable deoxyhemoglobin, and increase in total hemoglobin level resulted in a small but significant increase in placental TOI values (Figure 4B; Table 1). A decrease in oxyhemoglobin, increase in deoxyhemoglobin, decrease in total hemoglobin resulted in a significant decrease in fetal TOI values (Figure 4C; Table 1). There were no significant changes in oxygen saturation in the dams (data not shown).
Discussion

In the present study, we showed that acute ligation of the umbilicus significantly decreased fetal TOI values and concomitantly increased placental TOI values (Figure 2; Table 1). Since the TOI values positively correlated with tissue oxygenation in various kinds of research models [11-15], these data indicate the co-existence of low and high levels of oxygenation in the fetus and placenta, respectively. It is reasonable that complete blockage of both the inflow and outflow of fetal blood decreased fetal oxygenation, as shown by the reduction in fetal TOI values, which will decrease the oxygen inflow from maternal blood in the placental tissues. Therefore, the synchronized elevation in placental TOI values strongly supports our hypothesis that a decline of oxygen inflow from the maternal to fetal circulation in the placental tissue is one of the factors responsible for the elevated placental TOI values. Indeed, the local coordinated changes to oxyhemoglobin and deoxyhemoglobin levels underlie the increase in placental TOI values (Table 1). The stable maternal oxygen saturation (data not shown) suggested the high placental oxygenation to be caused independently of the general maternal status of oxygenation. Acute ligation of uterine arteries significantly decreased both placental and fetal TOI values (data not shown), supporting the hypothesis that placental TOI values properly represent placental oxygenation in this animal model.

Acute ligation of the umbilical arteries (Figure 3; Table 1) or veins (Figure 4; Table 1) caused a similar increase and decrease in placental and fetal TOI values, suggesting that a decrease in the inflow or outflow of fetal blood to the placenta reduces oxygen absorption from the maternal blood, contributing to the elevation of placental TOI values. Therefore, disturbances of fetal circulation, the flow either to or from the placenta, immediately raised the placental TOI values, which also supports our hypothesis that a deterioration of fetal circulation is causatively associated with the augmentation of placental TOI values.

Irradiating near-infrared rays pass through the entire placenta; therefore, maternal as well as fetal blood could affect placental TOI values. In the human placenta, we recently demonstrated that placental TOI values mainly represent the oxygenation of maternal blood [10]. Although porcine studies have provided excellent information concerning the physiology and pathophysiology of the placenta [23-26], the porcine placenta is “epitheliochorial” and not exactly the same as the “hemochorial” human placenta [27]. Therefore, the contribution of fetal blood to placental TOI values in this animal model is unclear. However, the paradoxical increase in TOI values with the obstruction of the fetoumbilical circulation itself suggests that they too mainly represent the oxygenation of maternal blood in the placental tissues, although no information on the real oxygen saturation of maternal blood in the porcine placenta has been published to our knowledge.

Nylund et al. injected a radio-isotope in pregnant women before delivery and demonstrated a 50% or more reduction in uteroplacental blood flow in cases of fetal growth restriction [28]. Macara et al. reported that structural analysis of placental terminal villi of growth-restricted fetuses suggested a decreased oxygen transfer from the intervillosus space to the fetal circulation [29]. Paradi et al. reported that uterine venous oxygen saturation of growth-restricted fetuses was significantly higher than that of appropriate growth for gestational age [30]. Kingdom and Kaufmann proposed a hypothetical concept of “postplacental hypoxia” as one of origins of fetal hypoxia, when the placental villi is exposed to a higher oxygen tension than under normal circumstances [31]. The findings of the present animal study supports the concept despite the morphological difference between human and porcine placenta [27].

Hypoxia is one of the most ominous fetal conditions, proceeding to fetal demise. The present study suggests the promising use of placental TOI values obtained by the transabdominal application of NIRS to assess the insufficiency of the fetoplacental circulation, as a cause or result of fetal hypoxia. Clinical studies are now under way.

Some caution is required, because fetal IUGR and chorangiosis, as we previously observed in humans, are based on rather long-lasting, chronic changes. In the present study, we examined the acute termination of fetal circulation to the placenta in Clawn miniature pigs. Moreover, the study was carried out by using animals between 102 and 113 days of gestation (114 days full term) due to institutional availability of this animal. These experimental conditions do not exactly mimic chronic changes of fetal IUGR in humans. Therefore, our next aim is to clarify the effect of chronic deterioration of fetal circulation on placental oxygenation.

In conclusion acute occlusion of the whole umbilicus or umbilical vessels significantly increased placental TOI values measured using NIRS in pregnant Clawn miniature pigs. This is the first evidence that a disturbance of the fetal circulation at least partly regulates placental tissue oxygenation.

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Endometrial polyps and their relationship in the pregnancy rates of patients undergoing intrauterine insemination

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Summary
Purpose: To evaluate the effect of the presence of endometrial polyps (EP) on pregnancy rates and how polypectomy could affect pregnancy rates in women scheduled for intrauterine insemination (IUI). Methods: The study included patients who had attended the Second Department of Obstetrics and Gynecology of the University of Athens from April 2003 to October 2008 for infertility treatment and were candidates for IUI. In these women the presence of an endometrial polyp had been already diagnosed during the infertility evaluation. The study group consisted of 86 women who, following the diagnosis of endometrial polyp, had agreed to have the polyps removed hysteroscopically prior to the IUI. The control group consisted of 85 women, who despite the fact that the presence of an endometrial polyp had been previously diagnosed and its removal suggested, elected not to have the polyp removed. We used statistical analysis to check what effect the removal of the polyp had on the total number of pregnancies. Results: There was a statistically significant difference in cumulative pregnancy rates between the two groups. The group that underwent polyp removal had higher pregnancy rates as compared to the one that the polyps were left intact. Conclusions: We propose that hysteroscopic polypectomy of any size appears to improve fertility in women with otherwise unexplained infertility.

Key words: Endometrial polyps; Intrauterine insemination; IVF.

Introduction
Endometrial polyps are common findings during the reproductive years, occurring in up to 24% of women [1-3]. Those structures are benign tumors of the endometrium consisting of glands and endometrial stromal tissue whose blood supply is provided by branches of the endometrial spiral arteries [2]. The etiology for the development of endometrial polyps is not clear, but it seems that they are created by a localized anomaly of hormonal receptivity in certain areas of the endometrium with persistence of estrogen receptors and decrease of progesterone receptors leading to focal proliferation, and growth of the endometrium and the underlying stroma [4]. Disorders associated with prolonged unopposed exposure to estrogen such as oligo-anovulation, luteal phase insufficiency etc. are associated with the development of endometrial polyps [2].

The main presenting symptom related to an endometrial polyp (EP) is abnormal uterine bleeding [4, 5], but the majority are asymptomatic and often discovered during a routine sonographic evaluation or during the process of infertility investigation [6, 7].

The “gold standard” examination for diagnosing EPs is hysteroscopic evaluation of the endometrial cavity [4, 8-12].

The precise relationship of EPs and infertility or recurrent pregnancy loss remains obscure. It seems that the presence of endometrial polyps could cause an adverse effect in embryo implantation [4] although the exact mechanism that regulates implantation and/or other fertility problems is mostly unknown [13-15]. There are several reports indicating that the presence of EPs may adversely affect pregnancy rates in women undergoing IVF-ET [15-17]; thus, when an EP is discovered in the process of infertility evaluation and treatment, the therapeutic suggestion is removal and that rule is almost universally applied in women undergoing IVF [1-6]. To our knowledge, there is no established proof on how EPs affect implantation and furthermore the current literature does not provide a clear answer to the question if a polypectomy could improve pregnancy rates in women undergoing COH and IUI.

The purpose of this study was to evaluate the effect of presence of EPs on pregnancy rates and how a polypectomy could affect pregnancy rates in women scheduled for IUI.

Material and Methods
The study included patients who had attended the Second Department of Obstetrics and Gynecology of the University of Athens from April 2003 to October 2008 for infertility treatment and were candidates for IUI. In these women the presence of an EP had been already diagnosed during the infertility evaluation either by sonographic evaluation (including hysterosonogram) or by hysterosalpingogram (HSG). The study group consisted of 86 women following the diagnosis of an endometrial polyp had agreed to have the polyp removed hysteroscopically prior to the IUI. The control group consisted of 85 women, who despite the fact that the presence of an EP had been previously diagnosed and its removal suggested, elected not to have the polyp removed. Those patients underwent a complete infertility inves-
tigation that included a baseline transvaginal ultrasound (TVS), HSG, a midluteal phase serum progesterone concentration and basic hormonal profile which included a day 2 FSH. Patients with irregular cycles or any suggestion of PCOS had an additional hormonal evaluation which included in addition to FSH, LH estradiol, TSH, prolactin, 17 OH progesterone, Δ4 androstenedione and testosterone (free and total).

Couples were eligible for IUI if the female partner was younger than 38 years of age, day serum FSH was less than 10 mIU/ml and had at least the following: documented tubal patency by HSG or laparoscopy and a semen analysis indicating the absence of severe male factor (sperm count of at least 5 million/ml).

In order to fulfill the criteria of idiopathic infertility, female partners had to have regular menstrual periods, proven spontaneous ovulation either by biphasic basal body temperature charts or by a midluteal serum progesterone concentration of at least 10 ng/ml.

Male partners had to have sperm count of more than 20 x 10^6/ml, more than 50% motile spermatozoa, and more than 14% morphologically normal spermatozoa by Kruger criteria [18].

In couples with male infertility male partners had to have at least two semen evaluations obtained at least three months apart which should have been abnormal, according to WHO criteria [18].

Women in the study group were scheduled to have three cycles of COH combined with IUI three months after the removal of the polyps.

Preparation of sperm and IUI

Semen specimens were produced at the laboratory after 48-72 hours of sexual abstinence. After liquefaction in a 37°C incubator, semen was examined for sperm concentration and motility with the use of a Makler device (Sefi Medical Instruments Ltd., Haifa, Israel). Sperm preparation was carried out as previously described [18] and a final volume of 0.3-0.5 ml after processing was used for the IUI. A small aliquot was retained for a post-wash control and motility analysis.

Intrauterine insemination was performed with a use of Makler device (Sefi Medical Instruments Ltd., Haifa, Israel). Women were requested to remain in bed for approximately 10 minutes after the procedure.

Ovarian stimulation

Superovulation or ovulation induction, where needed, was initiated on the third day of a spontaneous menstrual cycle or the third day after progesterone-induced bleeding in women with ovulatory dysfunction with a fixed dose of recombinant FSH (Gonal F, Serono UK) dose of 50 IU daily. Ovarian response and follicular development were monitored by serial ultrasound and serum estradiol measurements.

When the leading follicle reached a diameter of at least 17 mm, ovulation was triggered by an intramuscular injection of 10000 IU HCG (Pregnyl, Organon). A single IUI was performed 34-36 hours after the injection.

Causes for cancellation included evidence of excessive ovarian response (more than 3 follicles larger than 15 mm), lack of cooperation at the time of IUI, male psychological reasons that caused difficulty of sperm-collection and couple preference.

All patients were asked to have a serum hHCG measured 14 days after the insemination. Clinical pregnancy was confirmed by a transvaginal ultrasound two weeks later which documented gestational sac with fetal pole and present cardial activity.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>31.22</td>
</tr>
<tr>
<td>Mean standard error (age)</td>
<td>.335</td>
</tr>
<tr>
<td>Ovulatory factor (%)</td>
<td>23 (24.7%)</td>
</tr>
<tr>
<td>Cervical factor (%)</td>
<td>10 (11.6%)</td>
</tr>
<tr>
<td>Endometriosis (%)</td>
<td>8 (9.3%)</td>
</tr>
<tr>
<td>Male factor (%)</td>
<td>20 (23.2%)</td>
</tr>
<tr>
<td>Idiopathic (%)</td>
<td>25 (29%)</td>
</tr>
</tbody>
</table>

Table 2. — Polyp size.

<table>
<thead>
<tr>
<th>Polyp</th>
<th>Study group (n = 86)</th>
<th>Control group (n = 85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5 mm</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>5-10 mm</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>11-20 mm</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>&gt; 20 mm</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Mean</td>
<td>13.67</td>
<td>12.01</td>
</tr>
</tbody>
</table>

Table 3. — Pregnancy results according to IUI cycle - cancellation rates - Study group.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Patient number</th>
<th>Cycles</th>
<th>Cancellation</th>
<th>Pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st attempt</td>
<td>86</td>
<td>81</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>2nd attempt</td>
<td>71</td>
<td>61</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>3rd attempt</td>
<td>60</td>
<td>50</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Totals</td>
<td>192</td>
<td>25</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. — Pregnancy results according to IUI cycle - cancellation rates - Control group.

<table>
<thead>
<tr>
<th>Control group</th>
<th>Patient number</th>
<th>Cycles</th>
<th>Cancellation</th>
<th>Pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st attempt</td>
<td>85</td>
<td>78</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>2nd attempt</td>
<td>76</td>
<td>64</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>3rd attempt</td>
<td>70</td>
<td>62</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Totals</td>
<td>204</td>
<td>27</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

Our statistical analysis was performed by the use of a commercially available SPSS program (SPSS Statistics 17.0). The Independent Groups t-test for means calculator tests the means of two independent groups to determine if they are significantly different from one another. We used confidence level: 95%.

The differences were considered to be statistically significant if p < 0.05.

Results

Demographic characteristics of female patients were similar in the two groups (Table 1).

In Table 2 the polyp size in women of our sample is presented. There were no statistically significant differences in polyp sizes.

In women of the first group, there were 25 cancellations of IUI cycles, 192 completed cycles and among those, 35 women managed to conceive (Table 3).

In the second group (which consisted of 85 women each also having 3 cycles of treatment), there were 27 cancellations of IUI cycles, 204 completed cycles, and 19 women managed to conceive (Table 4).

Fifteen women of the 35 women in the first group managed to conceive from the first treatment cycle, 11 women...
Endometrial polyps and their relationship in the pregnancy rates of patients undergoing intrauterine insemination

achieved from the second attempt and nine managed conception after the third cycle (Table 3).

In the control group, nine women managed to conceive from the first treatment cycle, six after the second cycle and four after the third cycle (Table 4).

There was a statistically significant difference in cumulative pregnancy rates between the two groups. The group that underwent polyp removal had higher pregnancy rates as compared to the one where the polyps were left intact.

It should be noted that in the period of three months after removal of the polyps and before the implementation of IUI, there was no pregnancy achieved in the study women.

More specifically, in Table 1 we examine the heterogeneity of the samples. For all variables we found statistically non-significant results. A level frequently quoted is $p < 0.05$ (type-1 error). Thus we could proceed with the analysis. In Table 2 we found statistically non-significant differences in the three categories (<5 mm, 5-10 mm, 11-20 mm) while the fourth result (>20 mm) ($p = 0.045$) was statistically significant. The correlation and t could not be computed because the standard error of the difference was 0, so we could not find a p value for pair 2 and pair 3. The mean comes out statistically non-significant ($p = 0.159$). In Tables 3 and 4 cancellation rates are shown; we found statistically non-significant differences in all three attempts. For cycle variables we found statistically significant results in the third attempt ($p = 0.001$) only. For pregnancy variables we found statistically significant differences in all attempts (1: $p = 0.045$, 2: $p = 0.024$, 3: $p = 0.024$) suggesting that the treatment group achieved more pregnancies and, therefore, treatment is recommended.

Discussion

EPs are a common cause of intrauterine pathology, with a variety of symptoms ranging from unpredictable bleeding to infertility [4-7]. Management of EP is a doubtful decision, particularly because EPs are almost always benign and treatment may be conservative, with follow-up visits every six months to one year being sometimes the approach recommended [19].

Our study examined the effect of hysteroscopic polypectomy to intrauterine insemination success rates.

In a previous study investigators reported that hysteroscopic polypectomy in infertile women possibly increases pregnancy rates by three to four times [20]; the methodological problem is that in that study [20], EP coexisted with submucous myomas, so that the exact impact on infertility is difficult to calculate.

In our study, we found that after the removal of the endometrial polyp, 35 out of 86 women (40.69%) managed to conceive after IUI. On the other hand, in the group of women who did not have a polypectomy, respective results showed a 22.35% (19 out of 85 patients) conception rate.

Other authors have suggested that otherwise asymptomatic polyps, less than 2 cm in diameter, do not interfere with IVF/embryo transfer rates, but may increase risk of spontaneous abortion and, in general, increase pregnancy loss and thus hysteroscopic treatment is needed [15]. Persistent functional EPs, even if small, are likely to impair fertility and thus removal of these lesions tends to improve reproductive performance [4].

In another study it was suggested that hysteroscopic removal of even small polyps improves reproductive outcome and therefore should be recommended to infertile women undergoing assisted reproductive technology procedures [10, 21].

As for time of achieving conception after removal of endometrial polyps, findings of a study by Spiewan-kiewicz et al. reported that 19 out of 25 infertile patients in whom polypectomy was performed conceived in a 12-month-period [17].

In our study, 15 out of 35 women of the study group conceived after the first IUI effort, 11 after the second and nine after the third effort. Those numbers were nine, six, and four for the control group, respectively. Cancellation rates were similar in both groups (25 women cancelled their IUI cycle in the first group, and 27 in the second). Thus, we find an improvement (statistically important) in pregnancy rates of women of all groups.

Hysteroscopy is an effective and safe way to remove EPs because the procedure is done under direct vision (and so problems of blind dilatation and curettage, such as leaving residual tissue or missing even the whole polyp are avoided). Operative hysteroscopy is a reliable technique with low recurrence rates, especially for difficult cases; for example in cases of EPs larger than 2 cm [4]. In our study, we used hysteroscopic scissors or resectoscope to remove the polyps from the underlying endometrial tissue. No major complications (such as uterine rupture or severe blood loss) occurred in our study, a finding consistent with those of other authors [4, 10] who proposed that hysterectomy is a safe and effective diagnostic and therapeutic method.

In conclusion, hysteroscopic polypectomy for any size polyp appears to improve fertility in women with otherwise unexplained infertility. Nonetheless, further studies would be useful to confirm that EPs are not coincidental with infertility, because our study directly implies that EPs may have a causative effect in infertility.

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Assessment of fetomaternal hemorrhage by Kleihauer-Betke test, flow cytometry and α-fetoprotein after invasive obstetric procedures


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Summary

Purpose: The aim of this study was to evaluate the passage of fetal red blood cells to the maternal circulation, after invasive obstetric procedures, through the Kleihauer-Betke test, flow cytometry and by measurement of maternal serum α-fetoprotein level.

Methods: This prospective descriptive study with patients submitted to amniocentesis, cordocentesis, chorionic villus sampling (CVS), amnioreduction and ventriculoamniotic shunt was performed for karyotype analysis, treatment of hydrocephalus and polyhydramnios and to assess fetal lung maturity. Maternal blood samples were collected before and 60 minutes after the invasive obstetric procedure to search for fetal erythrocytes using the Kleihauer-Betke test, flow cytometry and serum α-fetoprotein measurement.

Results: Ten invasive obstetric procedures were performed. The mean age of the patients was 29.2 years and the mean gestational age was 29.6 weeks. The procedures were: five amniocenteses, two cordocenteses, one CVS, one ventriculo-amniotic shunt and one amnioreduction with cephalocentesis. The indications for the procedures were: karyotype analysis in five patients, fetal lung maturity assessment in two patients, amnioreduction in one patient, fetal hydrocephalus shunt in one patient and polyhydramnios related to hydranencephaly in one patient. Regarding the path of puncture, three procedures were accomplished through the placenta and seven apart from it. All punctures were successful at the first attempt. There was no significant increase of fetal erythrocyte quantity in maternal blood samples using the Kleihauer-Betke test. After cordocentesis, a significant increase of fetal erythrocytes was detected by flow cytometry and serum α-fetoprotein measurement.

Conclusion: Invasive obstetric procedures during prenatal care are safe when performed by experienced professionals using adequate techniques, with minimal chance of passage of fetal erythrocytes from the fetal compartment.

Key words: Fetomaternal hemorrhage; Invasive obstetric procedure, Kleihauer-Betke test; Flow cytometry; α-fetoprotein.

Introduction

When the immune system of a Rh negative person comes in touch with Rh positive blood, antibodies that react against the Rh positive cells are produced. If another exposure occurs, preformed antibodies and new antibodies lead to destruction of Rh positive red cells by antigen-antibody reaction [1].

Pregnancy is a risk situation for allogeneic Rh antigen immunization. Structurally, the fetal red blood cells run through maternal-fetal interface in the capillaries present in the chorionic villus, without direct contact with the maternal circulation. Zipurski and Israels [2] related that when a fetomaternal hemorrhage occurs (FMH) with less than 0.1 ml, the risk of isoimmunization demonstrated at six months after delivery is 3%, and if this blood volume is greater than 0.1 ml the risk rises to 14% over the same period. Procedures and complications such as cesarean delivery, manual extraction of placenta, multiple pregnancy, traumtic birth, the external version, invasive procedures and fetal death are FMH facilitating events [3, 4].

In 1957, Kleihauer et al. [5], described the acid-elution method for detection and quantification of fetal red blood cells in maternal circulation [6, 7]. In 1984, Medearis et al. [8] proposed the flow cytometry technique to identify and quantify a small number of cells in large cell populations. The dosage of α-fetoprotein (AFP) was first reported by Abelev et al. [9] in 1963 in rats with liver disease [10]. It was later identified in patients with hepatocellular carcinoma and during pregnancy.

The aim of this short study was to evaluate the passage of fetal red blood cells to the maternal circulation during pregnancy after invasive obstetric procedures.

Materials and Methods

We conducted a prospective descriptive study from January to August 2010 with ten patients undergoing invasive obstetric procedures. This study was approved by the Ethics Committee of the Federal University of Sao Paulo (UNIFESP) (No. 2009/54508-0). All patients who consented to participate voluntarily signed an informed consent form. Invasive procedures were performed at Sao Paulo Hospital, UNIFESP, and consisted of: amniocentesis, cordocentesis, chorionic villus biopsy, ventriculoamniotic shunt (VAS) and cephalocentesis. Exclusion criteria were fetal death, fetal defects of the neural tube, maternal hemoglobinopathy, multiple pregnancies, vaginal bleeding during pregnancy, pregnant women previously sensitized and prior invasive procedures in the current pregnancy. The passage of fetal red blood cells to the maternal circulation was evaluated two ways: directly by the Kleihauer-Betke test and flow cytometry and indirectly by maternal blood α-fetoprotein dosage.
We collected 5 ml of peripheral maternal blood before and 60 minutes after the invasive procedure. Immediately after collection, samples were separated into two EDTA tubes and each tube was dried, lightly homogenized and stored at 4°C for a maximum period of 72 hours.

Kleihauer-Betke test

This technique is based on the fact that fetal hemoglobin is resistant to acid elution, whereas adult hemoglobin is sensitive. The positive control was the mixture of ten parts of adult blood with one part of cord blood for ABO compatible blood types and negative adults only. The technique was based on preparing thin smears of maternal blood samples and controls, leaving them to dry spontaneously, using ethyl alcohol 80% McIlvaine thin smears of maternal blood samples and controls, leaving them to dry spontaneously, using ethyl alcohol 80% McIlvaine buffer, 0.5% B Erytrosin and Harris hematoxylin.

Microscopic analyses (using 40 x magnification) showed fetal red blood cells (RBCs) were intact with pink color while the adult cells were extremely pale. Ten fields were counted and the percentage of fetal cells were calculated in relation to adult cells. The hemoglobin volume in ml was equal to the percentage of fetal cells multiplied by 50 [11]. FMH was considered significant as established by Lachman [12] and taking into account the coefficient of variation of the method, an increase of 40% or more of \( \alpha \)-fetoprotein concentration in the sample was determined by the construction of the standard curve of optical density versus \( \alpha \)-fetoprotein. According to Lachman et al. [12] and considering the coefficient of variation of the method, an increase of 40% or more of \( \alpha \)-fetoprotein concentrations between samples before and after the procedure was considered FMH.

**Results**

We analyzed ten patients before and after the procedure, and ten blood samples collected before and after procedures for each test by Kleihauer-Betke, flow cytometry and dosage of \( \alpha \)-fetoprotein.

Patient ages ranged from 18 to 38 years with a mean age of 29.2 years. The gestational age of the procedures ranged from 13 weeks/3 days to 38 weeks/4 days, with a mean age of 29.6 weeks.

Ten procedures were performed five amniocenteses, two cordocenteses, one chorionic villus biopsy, one ventriculoamniotic shunt and one amniocentesis with chorionic villus biopsy. Of these ten procedures, five were for karyotype analysis, two to assess lung maturity, one for amnioreduction, one for hydrocephalus and one for hydranencephaly with polyhydramnios. According to the placenta localization, three were transplacental and seven did not cross the placenta. All procedures were performed at the first attempt to puncture.

We did not observe a significant increase in fetal red cells in any of the procedures performed by the Kleihauer-Betke test. There was an average increase of 1.8 fetal erythrocytes per procedure, with the largest increase occurring in procedures such as cordocentesis and ventriculoamniotic shunt. Taking into account only the five

### Table 1. — Descriptive analysis of ten invasive obstetric procedures.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Gestational age (week/days)</th>
<th>Procedures</th>
<th>Indication</th>
<th>Transplacental</th>
<th>K-B before (fetal erythrocytes/2000 erythrocytes)</th>
<th>K-B after (fetal erythrocytes/2000 erythrocytes)</th>
<th>FSH (IU/ml)</th>
<th>FC before (%)</th>
<th>FC after (%)</th>
<th>Increase (IU/ml)</th>
<th>AFP before (IU/ml)</th>
<th>AFP after (IU/ml)</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13/3</td>
<td>CVB</td>
<td>Karyotype</td>
<td>Yes</td>
<td>0</td>
<td>2</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
<td>54.3</td>
<td>68.4</td>
<td>25.96</td>
<td></td>
</tr>
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<td>2</td>
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<td>265.7</td>
<td>284.4</td>
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</table>

CVB: chorionic villus biopsy; VAS: ventriculoamniotic shunt; K-B: Kleihauer-Betke test; FMH: fetomaternal hemorrhage; FC: flow cytometry; AFP: \( \alpha \)-fetoprotein.
Assessment of fetomaternal hemorrhage by Kleihauer-Betke test, flow cytometry and α-fetoprotein after invasive obstetric procedures

In an attempt to predict and quantify this risk, several studies are found in the literature attempting to quantify the passage of fetal erythrocytes in maternal blood after invasive procedures. Chitrit et al. [16] in 2007 proved that the greater the passage of fetal red blood cells to the maternal circulation, the greater the risk of fetal death, induction of preterm labor, need for intensive care unit and neonatal transfusion. In the literature there are some articles that evaluated FMH with different methods and none of them was superior. Only the study by Fernandes et al. [17] used flow cytometry. They evaluated 170 chorionic villus biopsies by flow cytometry and Kleihauer-Betke test, and found ten women with fetal cells in maternal peripheral blood in the Kleihauer-Betke test and 26 women in flow cytometry prior to the procedure. Sixty minutes after the procedure, the same patients showed the same cells, respectively, demonstrating good correlation between the two methods. However, flow cytometry was more sensitive and more accurate in determining FMH.

Quantifying the FMH of these procedures is intended to minimize the risks of complications during pregnancy. Among the methods studied, flow cytometry and determination of α-fetoprotein in maternal blood were more accurate in detecting the presence of fetal cells than the Kleihauer-Betke test. The dosage of α-fetoprotein after the procedures seems to be a more sensitive indicator, affordable and accessible to medical practice. Moreover, the result of α-fetoprotein does not have interference from clumping or aggregation of fetal cells.

Conclusion

In summary, we conclude that invasive obstetric procedures are safe to be carried out in fetal medicine when they are followed by standard methods and conducted by trained professionals. Among the invasive obstetric procedures performed, amniocentesis proved to be safe and without risk of passage of fetal erythrocytes to the maternal compartment by any of the three methods. Among

![Figure 1. — Cytology sample before and after cordocentesis. Quadrant Q1: fetal erythrocytes.](image-url)
these methods for detection of fetal red blood cells, flow cytometry and determination of α-fetoprotein were more sensitive in detecting FMH.

References


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Foetal monitoring during labour: practice versus theory in a region-wide analysis

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Summary

Purpose: To evaluate cardiotocography (CTG) alone versus CTG and ST-analysis (STAN) in daily obstetric practice in a complete region. Methods: Prospective registration in the region of Flanders in combination with standard registration of perinatal outcomes. Results: Of 62,606 term deliveries registered, 57,141 (91.3%) were available for complete analysis. In 50,748 (88.8%) CTG alone and in 6,393 (11.6%) CTG + STAN was used. STAN was used significantly more in cases of hypertension, diabetes and induction of labour and was associated both in univariate and multivariate analysis with significantly more secondary caesarean section for suspected foetal distress, instrumental vaginal delivery, low Apgar score and need for neonatal intensive care. There was no difference in perinatal death or asphyxia. Conclusion: ST-analysis versus CTG results in more caesarean sections, instrumental vaginal deliveries and neonatal intensive care. This can not be explained solely by its use in more complicated cases as in multivariate analysis including hypertension, diabetes and induction of labour ST analysis persists as a significant factor. We hypothesise that this could be explained by less well trained users not adhering to STAN-guidelines.

Key words: ST-analysis; Cardiotocography; Foetal monitoring; Labour.

Introduction

Since the year 2000 foetal monitoring with foetal ST-analysis (STAN, Neoventa, Sweden) has been progressively introduced in Europe. From the start of this period, experiences from dedicated single hospitals [1, 2] confirming the results of the larger multicenter randomised trials [3], have been reported. Most users have quickly adopted this new technology, and in the daily practice of busy clinical wards, most users seem to feel confident with ‘the new machine’ [4, 5].

Less is known on what happens with the performance of foetal ST-analysis versus cardiotocography (CTG) alone when this technology is used outside randomised trials and outside dedicated centers, in large and small maternity wards with midwives and gynaecologists having less interest or a lower level of education in foetal electrocardiography and cardiotocography.

To evaluate the performance of CTG alone versus CTG + ST-analysis for foetal monitoring in term deliveries in general obstetric practice, we started a prospective registration of foetal monitoring in the region of Flanders, the northern half of Belgium.

Material and Methods

The Center for Perinatal Epidemiology routinely collects anonymised data on all deliveries in the region of Flanders and covers 100% of hospital deliveries (<1% are home deliveries). From January 1 to December 31, 2009 a prospective registration was added to the routine file, asking for: “foetal monitoring: yes/no; if yes by: STAN, CTG alone, auscultation alone, foetal scalp blood sampling”. For this analysis we included only deliveries from 37 weeks gestational age on (as STAN is not validated before this period); primary caesarean sections were excluded.

Other registered outcomes were: gestational age (in weeks), presence or absence of maternal hypertension (not further specified), diabetes (not further specified), induction of labour, secondary caesarean section, use of forceps or vacuum extractor, Apgar score after 1 and 5 min, need for neonatal reanimation, transfer to a neonatal unit or to the neonatal intensive care unit (NICU), birth weight (in grams), foetal mortality during labour and delivery, early (the first 7 days) and late (until 28 days) neonatal mortality, neonatal asphyxia (as diagnosed by the treating paediatricians).

Outcomes were compared in the two groups: CTG alone versus CTG + ST-analysis.

Statistical analysis was performed with SPSS 17.0. Dichotomous variables were compared using chi square testing and continuous variables with Student’s t-test; significance was accepted at p < 0.05.

After univariate analysis, differences between groups were further evaluated using multiple logistic regression.

Results

In 2009 there were 68 maternity wards in the region of Flanders. Of these, 64 (94%) had at least one STAN-machine available in 2009. In only one of these 68 maternity wards was foetal scalp blood sampling performed in 2009. The total number of deliveries in the region was 68,774 in 2009, of which 62,606 (91%) were at 37 or more weeks. Actually in the group < 37 weeks, 210 deliveries were excluded.

In 5,465 (8.7%) data of these CTG alone was used and in 6,393 (11.2%) CTG and STAN. There were 512 (0.8%) foetal scalp blood samplings, but as these were all performed in a single
hospital they were not further analysed in this regional study.

Table 1 gives an overview of the perinatal outcome in the CTG alone and in the CTG + STAN group.

STAN was significantly more used in pregnancies complicated by hypertension or diabetes and in case of induction of labour. In the STAN group more secondary caesarean sections were performed in general (19.5% vs 6.2%), but specifically caesarean section was more often performed for suspected foetal distress in the STAN group (8.4% of the total group, 43.3% of all caesarean sections) as compared to the CTG alone group (6.2% of the total group and only 19.8% of all caesarean sections).

To discriminate whether the use of foetal ST-analysis was an independent factor determining this rising number of caesarean sections for foetal distress a multiple logistic regression was performed including hypertension, diabetes, induction of labour, maternal age (more or less than 35 years) having had a previous caesarean section, parity, birth weight > 4500 g or < 2500 g and the use of CTG alone or STAN + CTG.

Induction of labour, STAN, low birth weight (< 2500 g), maternal age (> 35 years), primiparity and having had a previous caesarean section were shown to be significant variables (all \( p < 0.001 \)); hypertension (\( p = 0.24 \)), diabetes (\( p = 0.07 \)), and birth weight > 4500 g (\( p = 0.83 \)) were not significant.

Instrumental vaginal delivery was also more frequent in the STAN + CTG group (19% vs 9.8%). Here we also performed a multiple logistic regression including hypertension, diabetes, induction of labour, maternal age (more or less than 35 years), having had a previous caesarean section, parity, birth weight > 4500 g or < 2500 g and the use of CTG alone or STAN + CTG.

In this model diabetes (\( p = 0.001 \)), induction of labour (\( p = 0.005 \)), STAN (\( p < 0.001 \)), birth by secondary caesarean section (\( p < 0.001 \)), instrumental vaginal delivery (\( p < 0.001 \)), birth weight > 4500 g (\( p < 0.01 \)) and maternal age > 35 years (\( p < 0.001 \)) and having undergone a previous caesarean section (\( p < 0.001 \)) were significant variables.

There were significantly more babies born with a low Apgar scores both after 1 and 5 min in the CTG + STAN group, resulting in more cases of neonatal reanimation and more transfers to a neonatal intensive care unit. We performed a multiple regression analysis including as factors: maternal hypertension, diabetes, induction of labour, caesarean section, instrumental vaginal delivery, birth weight < 2500 g or > 4500 g and STAN + CTG versus CTG alone. The significant variables related to transport to a NICU were: diabetes (\( p = 0.001 \)), induction of labour (\( p = 0.005 \)), STAN (\( p < 0.001 \)), birth by secondary caesarean section (\( p < 0.001 \)), instrumental vaginal delivery (\( p < 0.001 \)), birth weight > 4500 g (\( p < 0.01 \)) and maternal age > 35 years (\( p = 0.004 \)). A previous caesarean section (\( p = 0.91 \)), parity (\( p = 0.15 \)), hypertension (\( p = 0.89 \)) and birth weight (\( p = 0.09 \)) were not significant in the model.

No significant difference in intrapartum, and early or late neonatal death were noted. Asphyxia (as a clinical diagnosis by the treating paediatrician) was not different between the STAN and CTG alone group.

**Discussion**

It can be hypothesised that in real life, in less dedicated centers, guidelines concerning cardiotocography and foetal ST-analysis are not as thoroughly followed as in the setting of a randomised trial. Our study is not randomised, and it does not reflect the value of STAN + CTG versus CTG alone in comparable cases but does reflect the clinical scenario of daily practice using STAN and CTG.

STAN was clearly more used in high-risk deliveries including diabetes, hypertension, and in case of induction of labour. In the STAN group slightly more caesarean sections were performed, but if a caesarean is done, this is twice as often due to foetal distress. The same can be said for instrumental vaginal deliveries.

In contradiction to the results from randomised controlled trials showing less or the same frequency of interventions with no change in neonatal outcome [3, 7, 8] in this descriptive analysis, in a real life setting STAN results in more interventions. This difference can not be explained solely by the fact that STAN was more often used in high-risk situations such as hypertension, diabetes and induction of labour, because in multivariate analysis the use of STAN persisted as a significant factor in relation to instrumental delivery, caesarean section and need for neonatal intensive care.

In this region-wide study we have no data on metabolic acidosis as umbilical cord blood gas analysis is not generally performed. Only data on Apgar score and clinical diagnosis of asphyxia and transfer to a neonatal intensive care unit are available. Neither can we comment on
changes in the method of foetal monitoring in Flanders as no previous data are available. Other studies have suggested a relation between a high rate of CTG and ST-analysis and reduction in cord blood acidosis rate [10]. In our study STAN was associated with lower Apgar scores and more transfers to a neonatal intensive care unit, despite, or due to, more interventions (caesarean sections and instrumental vaginal deliveries).

As already mentioned in the multivariate analysis, part of this can be explained by the application of STAN in a selected group of high-risk patients (diabetes, hypertension and induction of labour), but even then the data strongly suggest that either the STAN methodology and guidelines are not correctly followed in clinical practice or the methodology fails in a large general obstetric population when performed by midwives and gynaecologists who are not specifically dedicated to foetal monitoring.

Although with experienced users a high level of interobserver agreement in clinical decision making for CTG + STAN as compared to CTG alone has been reported, nothing is known as to whether this high level of agreement is still present when working in day-to-day busy units with different midwives and doctors [11, 12].

Several authors have mentioned that less foetal blood sampling was necessary in the STAN versus the CTG alone group. We believe the practice of foetal blood sampling has become so rare, as demonstrated in our data, that for a large part of Europe this finding is of no practical value [6, 13].

Different reasons can be given for failure of STAN in daily practice including: lack of continuous training and a high incidence of false-positive ST-events resulting in failure to act when a significant ST-event occurs [14]. It has been demonstrated that outcomes equal to those of the randomised controlled trials can be achieved in busy non-academic district hospitals, but this means continuous evaluation and training which is far more difficult to reach in a complete region including small maternity wards [15]; most maternity units in Flanders have less than 1,000 deliveries a year. We can not exclude a selection in which STAN was used only in the ‘worst cases’.

All this resulted in a finding that is in contrast with all other published reports: in this region-wide survey the use of STAN versus CTG was associated with more intervention, a worse neonatal outcome and more asphyxia in term babies.

It cannot be completely excluded that this outcome is due to the technology itself, but it seems more convincing that our data stress the extreme importance of continuous training and evaluation of the users.

References

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Effect of epidural analgesia on labor times and mode of delivery: a prospective study

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Summary

Purpose: To assess changes in labor times and delivery outcome in low-risk women requesting pain relief and undergoing epidural analgesia, according to the epidural analgesia schemes. Materials and Methods: Prospective observational study of 499 low-risk women with epidural analgesia. Speed of dilatation (SD) (centimeters of dilatation / hours), speed of lowering of the fetal head through maternal pelvis (SL) (centimeters in lowering / hours), time of active phase of labor (TA), cesarean section (CS), vacuum application (VA) were dependent variables in multivariable linear and logistic regressions. Results: Dilution of ropivacain, fentanyl amount, and volume of the first dose of epidural analgesia did not seem to affect labor times. Epidural analgesia with schemes used in this study favored both the dilatation and the fetal head lowering through maternal pelvis. Every five minutes from the first dose of epidural to the last top-up, the odds of an operative vaginal birth (vacuum) increased by about 40% \( (p < 0.001) \). Additionally, every five minutes from the first dose of epidural to the last top-up, SD decreased by about 13% \( (p = 0.002) \), SL decreased by about 14% \( (p < 0.001) \), and TA increased by about 40% \( (p < 0.001) \). Increasing of number of top-ups independently caused a reduction in odds of undergoing CS (odds ratio 0.434; C.I. 95% 0.219 - 0.859, \( p = 0.017 \)), without influencing labor times. Conclusion. Epidural analgesia in patients requesting pain relief favors normal course of labor if it is not discontinued or delayed.

Key words: Epidural analgesia; Labor; Delivery.

Introduction

Epidural analgesia should be offered to women requesting pain relief in labor, as it is effective and overall safe [1]. The clinical obstetric concern that epidural analgesia may affect labor outcome had been discussed, because it was reported that epidural analgesia did not increase cesarean section rate [1, 2], even if it increases the number of operative vaginal births [3, 4] and the duration of labor [5, 6]. In a previous study [7], the authors speculated that epidural analgesia, if not properly performed, may provoke the onset of birth fear during labor. This would lead to a rise of operative vaginal birth rate and to a prolongation of second stage labor. Therefore, first dose epidural schemes and time of top-ups of epidural analgesia should be personalized to avoid birth fear and thereby favor normal course of labor.

To check the hypothesis, the authors performed a new prospective observational study assessing changes in labor times and delivery outcome in women that underwent epidural analgesia, according to the epidural analgesia schemes.

Materials and Methods

Four hundred and ninety-nine low-risk patients undergoing epidural analgesia were enrolled between January and July 2010 at the “Fatebenefratelli Villa San Pietro” Hospital in Rome (Italy). As previously stated by the authors, epidural analgesia is offered on demand in this birth center [7]. Epidural analgesia was administered in a first dose with or without top-ups. The first dose of epidural was ropivacain (from 0.1% to 0.2% of dilution) and fentanyl (50 mg, 75 mg, or 100 mg) within a volume ranging from 15 ml to 20 ml. Top-ups ropivacain (from 0.1% to 0.2% of dilution) was administered in volumes ranging from 10 ml to 20 ml. Timing of top-ups was arbitrarily decided by anesthesiologists.

The authors decided to perform a prospective observational study because it allowed to check various epidural analgesia schemes that cannot be assessed in a controlled trial, in which two or more schemes are compared. These schemes were personalized in relation to pain intensity and labor evolution, as occurs in clinical practice.

Because birth fear during labor randomly occurs, the authors hypothesized that changes in epidural schemes may affect both labor times and labor outcomes, leading to the failure of epidural analgesia, even if pain is controlled [7].

As a policy of “Fatebenefratelli Villa San Pietro” Hospital, laboring women were transferred to the delivery room during the active phase of labor that included three to four cm of cervical dilatation and painful uterine contractions. In clinical practice, some women may be transferred in delivery room before three to four cm in dilatation, while others after. These errors randomly occur and therefore normal distribution of those errors did not affect results of statistical analyses.

Upon admission to the delivery room, midwives and / or physicians scheduled cervical dilatations (cm) and fetal stations (cm from ischial spines) on partograms. Therefore, the authors were able to calculate the SD (centimeters of dilatation / hours) and the SL (centimeters in lowering / hours) in each labor. TA was expressed in minutes from the time in which the patient was transferred in delivery room to the birth. SD, SL, and TA were considered continuous dependent variables in multi-linear regression models. Additionally, CS and VA were considered dependent dichotomous variables in logistic regression models.

The independent variables included: multiparity, amniotomy, oxytocin augmentation, ropivacain dilution of the first epidural...
Effect of epidural analgesia on labor times and mode of delivery: a prospective study

Table 1. — Results of analyses performed.

<table>
<thead>
<tr>
<th></th>
<th>Speed of dilatation (SD) (cm/h)</th>
<th>Speed of lowering of the fetal head through maternal pelvis (SL) (cm/h)</th>
<th>Time of the active phase of labor (TA) (min)</th>
<th>Cesarean section</th>
<th>Vacuum delivery</th>
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<td></td>
<td>R</td>
<td>Sig.</td>
<td>R</td>
<td>Sig.</td>
<td>R</td>
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<td>0.002</td>
<td>0.163</td>
<td>&lt; 0.001</td>
<td>-0.220</td>
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<td>Oxitocyn augmentation</td>
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<td>0.151</td>
<td>-0.055</td>
<td>0.164</td>
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<td>dilution (%)</td>
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<td>First dose fentanyl (mg)</td>
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<td>0.710</td>
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<td>0.887</td>
<td>0.095</td>
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<tr>
<td>First dose volume (ml)</td>
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<td>0.195</td>
<td>-0.144</td>
<td>0.086</td>
<td>0.032</td>
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<tr>
<td>Number of top-ups</td>
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<td>0.677</td>
<td>-0.039</td>
<td>0.644</td>
<td>-0.021</td>
</tr>
<tr>
<td>Time from the first dose to the last top-up (TTU) (min)</td>
<td>-0.134</td>
<td>0.002</td>
<td>-0.144</td>
<td>&lt; 0.001</td>
<td>0.404</td>
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<tr>
<td>Initial dilatation (ID) (cm)</td>
<td>-0.495</td>
<td>&lt; 0.001</td>
<td>0.418</td>
<td>&lt; 0.001</td>
<td>-0.275</td>
</tr>
<tr>
<td>Initial station (IS) (cm from ischial spines)</td>
<td>0.220</td>
<td>&lt; 0.001</td>
<td>-0.027</td>
<td>0.528</td>
<td>-0.068</td>
</tr>
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</table>

Multivariate linear and logistic analyses results. R is the standardized coefficient of correlation from multilinear regressions. Significative results are highlighted in bold letters.

dose (%), first epidural dose of fentanyl (mg), volume of the first epidural dose (ml), number of top-ups, time from the administration of the first epidural dose to the last top-up (TTU) in minutes, dilatation when the first epidural dose is administered (ID), and station when the first epidural dose is administered (IS).

SPSS 16.0 was used for statistical analyses. A p ≤ 0.05 was considered minimally significant.

Results

Of 499 patients, 49 (9.8%) underwent CS and 71 (14.2%) underwent VA. Mean SD was 1.53 (± 0.658) cm/h. Mean SL was 1.25 (± 0.794) cm/h. Mean TA was 185.1 (± 112.29) minutes.

Table 1 shows results of the analyses performed. As expected, multiparity reduced labor times, increased SD and SL, and shortened TA. Moreover multiparas are less likely to undergo CS and VA.

Inverse correlation was found between ID and SD, meaning that SD decreased when the epidural analgesia was performed with advanced dilatations and increased if the epidural analgesia was performed at low values of dilatation. Additionally, direct correlation was found between ID and SL, meaning that SL increased with increasing of ID when epidural analgesia was performed. Such behavior suggests that epidural analgesia with schemes used in the present study favors both the dilatation and the fetal head lowering through maternal pelvis. Moreover, the more advanced the ID when epidural analgesia was performed, the shorter the TA became.

Furthermore, the lower the IS was when epidural analgesia was performed, the faster the SD became.

As expected, if the ID was advanced and the IS was low, the odds of a CS were reduced, without observing a rise in VA.

Interestingly, amniotomy and oxytocin augmentation do not seem useful in shortening labors with epidural analgesia. Amniotomy increased the SL, while oxytocin augmentation increased the TA.

Dilution of ropivacain, fentanyl amount, and volume of the first dose epidural analgesia do not seem to affect labor times. It should be mentioned that the dilution of first dose ropivacain fails to reach the significance in relationship with SL. However, it seems that the greater the concentration of the ropivacain was, the slower was the SL. This may explain the rise in odds of having a CS with greater concentration of ropivacain, even if the statistical model overfits.

TTU strongly affected labor times and delivery outcome. Every five minutes from the first dose of epidural to the last top-up, SD decreased by about 13% (p = 0.002), SL decreased by about 14% (p < 0.001), and TA increased by about 40% (p < 0.001). Additionally, every five minutes from the first dose of epidural to the last top-up, the odds of an operative vaginal birth (vacuum) increased by 0.7% (p < 0.001).

Increasing of number of top-ups independently caused a reduction in odds of undergoing CS (odds ratio 0.434; C.I. 95% 0.219 - 0.859, p = 0.017), without influencing labor times.
Discussion

The current study aimed to demonstrate that various epidural schemes diversely affect labor times and delivery outcome. The authors did not find a relationship between various first dose epidural schemes and changes in labor times. A greater concentration of ropivacain administered in the first epidural dose seemed to decrease the SL. This behavior may be addressed to motor block, since proprioceptive perception of pelvis muscles are needed to coordinate pushing. However, the effect would have been very mild with epidural drugs schemes used in this study; therefore, results did not reach statistical significance.

The higher concentration of the first dose ropivacain seemed to lead to a rise in odds for CS, suggesting that proprioceptive perceptions are needed for favoring fetal head adaptation to the maternal pelvis. However, the results are overall inconclusive according to the authors’ opinion, because of model overfitting.

The more effective predictor of a successful labor and delivery was TTU. It seems that a policy in shortening the time intervals of top-ups may have favored spontaneous delivery and may have shortened the labor times, acting both on cervical dilatation and on fetal lowering through pelvis. Additionally, increasing number of top-ups reduced the odds of undergoing CS. These findings are in accordance with our previous results [7].

Some studies assessing labor times and outcome in relationship with epidural analgesia reported that epidural analgesia may increase labor times [5, 6, 8–11]. In retrospective studies, some other authors did not report that labor times were influenced by epidural analgesia [12, 13]. It should be mentioned that in clinical practice, many factors influence labor times and birth outcome.

Rohrbach et al. [14] reported that women selected for intrapartal epidural analgesia already represented a population with an increased risk of an unfavorable course of labor. Hess et al. [15] reported that patients with epidural analgesia may undergo CS in relation to the worst control of labor pains. Therefore, labor course should be assessed in relation to labor pain.

As the goal of epidural analgesia is to alleviate pain, one would suppose that the advantage of epidural analgesia for obstetricians is the overall reduction of birth fear and anxiety related to pain [16], rather than avoiding any pain perception. There are some new perspectives [17] in neurosciences that link some discomforting visceral perceptions to anxiety and mood perturbation. This kind of nociceptive stimuli from uterus and pelvis may reach the brain crossing the sympathetic chains in laboring women. Under this condition, some women asking for epidural analgesia could experience anxiety before complaining of pain, and overall could experience birth fear. Therefore first dose epidural analgesia and timing of top-ups may be very useful in blocking such kind of stimuli, with prevention of birth fear. Such behavior is suggested by the results of Leo et al. [18]. These authors proved that automated mandatory bolus infusion was better than basal infusion in patient-controlled epidural analgesia, reducing the analgesic consumption as well. Interestingly, Lavand’homme et al. [19] reported that adding 150 mg oral pregabalin to epidural analgesia performed during medical termination of pregnancy improves pain control and satisfaction. The authors also suggested that this behavior may be related to central sensitizations provoked by visceral stimuli and successfully controlled by pregabalin. Moreover, pregabalin reduced the need of more anesthetic and anesthetic restore. The present study demonstrated that SD increased with low values of ID, when uterine contractions were less painful and when birth fear was structured from visceral nociceptive stimuli. Therefore, it may appear that birth fear compromises the initial phases of cervical dilatation, leading to more operative deliveries, as reported by Laursen et al. [20].

It should be speculated that low concentration of first dose ropivacain could block overall nociceptive stimuli responsible of fear. Moreover, birth fear should increase more rapidly when epidural is discontinued or delayed in top-ups administering, independently from number of top-ups. Finally, oxytocin augmentation increased painful contractions [21] and anxiety, leading to the failure of epidural analgesia. Labor times could therefore be conditioned by fear and anxiety rather than by epidural analgesia schemes.

It is necessary to quantify birth fear during labor. Further research should address how therapies and tools for reducing pain [22–24] overall reduce birth fear, obtaining patient collaboration, and reducing operative birth.

This study had a major limitation because it did not control the characteristics of top-ups (volume and dilution). Theoretically, changes in volumes and ropivacain dilution of the top-ups may have affected labor behavior. The authors decided not to control these variables because top-ups characteristics did not vary that much and because volume and dilution of the first dose did not affect labor times and mode of delivery, suggesting that top-ups volumes did not condition labor behavior as well. Moreover, adding other indifferent variables to calculations would cause inconclusive multivariable models.

In conclusion, the study demonstrated that in labouring patients requesting epidural analgesia, the most effective epidural analgesia should be provided as soon as possible and continuously, while administering top-ups in a short time from the first dose. This policy allows to reduce labor time and operative vaginal birth, without increasing CS rate.

References

Effect of epidural analgesia on labor times and mode of delivery: a prospective study


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Effect of combined oral contraceptive use on platelet volume in women at reproductive age

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Summary

Investigation: Combined oral contraceptives use is associated with an increased risk of developing venous and arterial thromboembolic events. Platelet size, measured as mean platelet volume (MPV), is associated with platelet reactivity. Methods: Ninety-five women using oral contraceptives for contraception were investigated retrospectively. The patients' blood pressure, pulse and hematological values at application and at the sixth month were evaluated retrospectively. Results: There was no difference between the values of blood pressure (systolic and diastolic), pulse, hematological values (which contain leukocytes, platelets and mean platelet volume) at application and at the sixth month. Conclusion: We determined that using oral contraceptives for contraception did not change MPV values in young women.

Key words: Combined oral contraceptives; Mean platelet volume.

Introduction

Combined oral contraceptives (COC) represent the most extensively used method for birth control with about 100 million users worldwide [1]. However, COC use is associated with an increased risk of developing venous and arterial thromboembolic events.

Epidemiological studies indicate that COC use increases the absolute risk of venous thrombosis (VT) [2] and all cardiovascular arterial diseases such as myocardial infarction and ischemic stroke [3]. Platelet size, measured as mean platelet volume (MPV), is associated with platelet reactivity. MPV increases in acute myocardial infarction, and this has been identified as an independent risk factor for future myocardial infarction and stroke. An increasing MPV was identified as a predictor for venous thromboembolism (VTE) [4]. High MPV is associated with a variety of established risk factors, cardio- and cerebrovascular disorders, and low-grade inflammatory conditions prone to arterial and venous thromboses [5].

However, MPV values have never been studied in reproductive age women who were using oral contraceptives. Therefore, we aimed to investigate the effect of oral contraceptive use on MPV in reproductive age women.

Material and Method

Ninety-five reproductive age women who were admitted to the university hospital or were referred to their family physician in Konuralp/Duzce region from June 2010 to December 2010 and used oral contraceptives were investigated retrospectively. Exclusion criteria were known diseases that could affect MPV:

- Exclusion criteria were known diseases that could affect MPV:
  - venous and arterial thromboembolic events.
  - Clinical and demographic data before COC administration and at the sixth month visit including blood pressure, pulse, and hematological values were obtained from medical files and recorded. These data were evaluated retrospectively. The ethical committee of the medical faculty of Duzce University approved the study protocol.
  - Biochemical measurements:
    - Blood samples were drawn after a fasting period of 12 h. Glucose, creatinine, alanine aminotransferase and lipid profile was determined by standard methods. We measured MPV and platelet count in a blood sample collected in citrate (1:4 v/v) in order to avoid platelet swelling induced by EDTA. A Cell-Dyn 3500 (Abbott) was used for whole blood counts. MPV (fL) was measured directly. The expected values for MPV in our laboratory ranged from 7.0-11 fL.
    - Statistical analysis:
      - Statistical analyses were performed using the statistical package SPSS 13.0 for Windows. Normally distributed continuous variables among groups were compared with the paired t-test. The chi-square test was used to compare categorical variables. MPV values were compared with the Student’s t-test in smokers and non-smokers. A p value < 0.05 was considered statistically significant.

Results

The mean age of the individuals was 29 ± 6 (minimum: 16, maximum: 45) and mean body mass index (BMI) was 25 ± 3 (minimum: 19, maximum: 29). Mean follow-up time was 6 ± 1.5 months. No women had any episode of thromboembolism during the study period. Twenty-nine women (31%) were smokers. Forty of them (42%) were using 0.15 mg levonorgestrel and 0.03 mg ethinylestradiol, 50 (53%) were using 3 mg drosiprenone and 0.03 mg ethinylestradiol, and five (5%) were using 0.1 mg lev...
Effect of combined oral contraceptive use on platelet volume in women at reproductive age

Table 1. — General characteristics of individuals.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Before COC Use</th>
<th>Follow-up Use</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow up time (months)</td>
<td>6 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>92 ± 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>167 ± 32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>49 ± 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>95 ± 54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>07 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>21 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>19 ± 26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index.

Table 2. — The values before and after COC use.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Before COC Use</th>
<th>Follow-up Use</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>113 ± 13</td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74 ± 6</td>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td>Heart rate (beat/minute)</td>
<td>73 ± 6</td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.6 ± 1.3</td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td>White Blood Cell (n/ml)</td>
<td>7.7 ± 2</td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>Platelet counts (10⁹)</td>
<td>264 ± 65</td>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td>Mean platelet volume (fl)</td>
<td>8.7 ± 1.6</td>
<td></td>
<td>0.75</td>
</tr>
</tbody>
</table>

BP: Blood pressure.

Among current COC users, there was a 2.5 relative increased risk of adverse cardiovascular events, including cardiovascular death, nonfatal MI, and stroke. The increase in cardiovascular deaths and nonfatal MI and stroke in current users was believed to be associated with the prothrombotic effects, and seven of ten adverse cardiovascular events occurred in current cigarette smokers [16]. It has been demonstrated that the risk of VT is the highest in the first year of pill use [17, 18], particularly during the first three months of COC use [19]. Stopping COCs was associated with a decline in the risk for adverse cardiovascular events, suggestive of reversal of the COC prothrombotic effects with cessation of use; however, other mechanisms such as an antiatherosclerotic effect could also be contributory [20].

Orcestral and 0.02 mg ethinylestradiol. The main characteristics of the study population are reported in Table 1. There was no difference between the values of blood pressure (systolic and diastolic) (p = 0.45 and p = 0.90, respectively), pulse, hematological values (which contain leukocytes, platelets and mean platelet volume) (p = 0.36, p = 0.63, p = 0.75, respectively) at application and at the sixth month (Table 2). Mean MPV did not differ between smokers and non-smokers.

Discussion

We determined that using oral contraceptives for contraception did not change MPV values in reproductive age women. Also MPV did not significantly differ in smokers and their non-smoker counterparts. This is concordant with the data published by Butskiewich et al. They showed that smoking had no effect on mean platelet volume, percentage of large platelets, concentration of thrombopoietin, absolute count of reticulated platelet and concentration of betal-thromboglobulin in women [6].

Mean platelet volume is associated with increased platelet reactivity, shortened bleeding time, [8] and increased platelet aggregation ex vivo [9]. Large platelets have higher thrombotic potential [10] and express higher levels of P-selectin [11] and glycoprotein IIb-IIIa [12] than small platelets. It has been reported that elevated values of MPV are associated with cardiovascular disease [13]. It also increases in acute myocardial infarction, acute ischemic stroke and venous thromboembolism [4, 14, 15].

References


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Modified repeated intracyclic clomiphene citrate therapy after conventional clomiphene therapy

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Department of Obstetrics and Gynecology, Kohseichuo General Hospital, Tokyo (Japan)

Summary

Purpose of investigation: We compared modified repeated intracyclic clomiphene citrate therapy (RICCT) to gonadotropin therapy to determine whether this modified regimen was an effective alternative after conventional clomiphene therapy. Methods: Patients with ovulation disorder received treatment with modified RICCT and gonadotropin, and ovulation, pregnancy, total drug cost, and adverse effects were compared. Results: Among a total of 16 patients, 14 successfully ovulated after modified RICCT and 11 ovulated after gonadotropin therapy; two did not respond to either therapy. The total drug cost was US $36.3 ± 17.9 for modified RICCT, which was significantly lower than the cost of gonadotropin therapy, US $213.9 ± 100.4 (p = 0.0001). Conclusions: Because modified RICCT does not require the discomfort of daily injection and has excellent ovulation-inducing effects, it is a useful treatment after conventional clomiphene therapy.

Key words: Clomiphene citrate; Gonadotropin; Infertility; Ovulation disorder; Multiple pregnancy; Polycystic ovary syndrome.

Introduction

Ovulation induction agents are used in treating patients with ovulation disorders due to abnormalities of the pituitary or hypothalamus, polycystic ovary syndrome (PCOS), or infertility. Clomiphene citrate is usually selected as first-line treatment [1, 2], and gonadotropin therapy is typically evaluated as the next step [3]. However, nonresponders to clomiphene citrate might require a high dose of human menopausal gonadotropin (hMG) until ovulation while undergoing gonadotropin therapy, which can lead to adverse effects such as multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). There has been no intermediate alternative between these two therapies.

During clomiphene citrate therapy, the drug is usually initially administered at a dose of 50 mg/day for five days. The dose is increased to 100 mg/day in patients who have a poor response in the cycle after withdrawal bleeding. If there is still no response, the daily dose is increased to 150 mg [3, 4]. The effectiveness of this regimen soon reaches a limit, however, and its ovulation-inducing effect is lower than that of gonadotropin therapy. In a previous study, we developed a new method of administering clomiphene citrate and obtained an ovulation-inducing effect comparable to that of hMG therapy [5]. In the present study, we compared modified repeated intracyclic clomiphene citrate therapy (RICCT), a new clomiphene citrate regimen, with gonadotropin therapy with regard to ovulation rate as well as drug cost, psychological burden, time required, and adverse effects.

Materials and Methods

The subjects were chosen from infertility patients who visited our hospital and a hospital which cooperates with our hospital from 2000 through 2010. Modified RICCT and conventional gonadotropin therapy were explained to the patients, and the treatments were given to those from whom we obtained consent. Modified RICCT was given first, and gonadotropic therapy was performed after observing one or two episodes of withdrawal bleeding. In the modified RICCT regimen, clomiphene citrate was given for five days, starting five days after withdrawal bleeding, and the second course was started after an interval of five to ten days. The dose of the second course was basically identical to that of the first course [5]. In gonadotropin therapy, the daily dose was 150 or 300 IU, in principle, and urinary hMG was used in all patients. No patient performed self-injection. Variables such as total dose, total drug cost, number of days of administration, number of ovarian follicles, presence or absence of adverse effects, and pregnancy status were compared between the two therapies.

Statistical analysis was performed using the paired t-test. A p value less than 0.05 was considered to indicate statistical significance. Drug prices were calculated using an exchange rate of US $1 = ¥82 resulting that the cost of the drugs in Japan was ¥113 ($1.38) per tablet of clomiphene citrate and ¥1,896 ($23.12) for 75 IU, ¥2,236 ($27.27) for 150 IU, and ¥2,916 ($3.56) for 300 IU of hMG.

Results

Eleven of a total of 16 patients responded to both therapies, and the total dose of clomiphene citrate and hMG was 1,295.5 ± 638.0 mg and 1,509.1 ± 911.9 IU, respectively. Total drug cost was $36.3 ± 17.9 and $213.9 ± 100.4, respectively; modified RICCT was significantly cheaper (p = 0.0001). Three patients responded to modified RICCT but not to gonadotropin therapy, and two did not respond to either therapy (Table 1).

The number of ovarian follicles, number of hospital visits, occurrence of OHSS, and pregnancy status were compared between patients receiving the two therapies.
The numbers of mature and immature follicles and hospital visits were significantly lower with modified RICCT ($p = 0.01$, $p = 0.005$, $p = 0.0005$, respectively). No OHSS was observed during administration of modified RICCT, but moderate and mild OHSS were observed in one and three patients, respectively, during gonadotropin therapy. The only patient who became pregnant through modified RICCT had a miscarriage. Three patients became pregnant while receiving gonadotropin therapy; pregnancy is ongoing in two and was aborted in one.

Patients in whom follicular development could not be expected even with continued treatment, those in whom only immature follicles increased, and those who were not given human chorionic gonadotropin (so as to avoid the risk of OHSS) and consequently did not ovulate were classified as poor responders.

### Table 1. — Comparison of dosing conditions between modified RICCT and gonadotropin therapy.

<table>
<thead>
<tr>
<th>Patients</th>
<th>RICCT</th>
<th>Gonadotropin (hMG) therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose</td>
<td>Duration</td>
</tr>
<tr>
<td></td>
<td>(mg/day)</td>
<td>of dosing periods</td>
</tr>
<tr>
<td>1</td>
<td>Y.S.</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>R.A.</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>M.S.</td>
<td>150</td>
</tr>
<tr>
<td>4</td>
<td>A.T.</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>A.T.</td>
<td>150</td>
</tr>
<tr>
<td>6</td>
<td>K.T.</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>M.T.</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>K.W.</td>
<td>150</td>
</tr>
<tr>
<td>9</td>
<td>A.T.</td>
<td>150</td>
</tr>
<tr>
<td>10</td>
<td>M.M.</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>S.Y.</td>
<td>50</td>
</tr>
</tbody>
</table>

| Mean | 1295.5 | 36.3* | Mean | 1509.1 | 213.9** |
| SD   | 638.0  | 17.9  | SD   | 911.9  | 100.4  |

The 11 patients shown above achieved ovulation with both therapies, and the five shown below showed no ovulation with either or both therapies.

*Significant at $p = 0.005$.

### Table 2. — Comparison of the results of modified RICCT and gonadotropin therapy.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mature*</th>
<th>Immature**</th>
<th>No. of hospital visits***</th>
<th>OHSS</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>hMG</td>
<td>CC</td>
<td>hMG</td>
<td>CC</td>
<td>hMG</td>
</tr>
<tr>
<td>1</td>
<td>Y.S.</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>R.A.</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>M.S.</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>A.T.</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>A.T.</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>K.T.</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>M.T.</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>K.W.</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>A.T.</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>M.M.</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>11</td>
<td>S.Y.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

| Mean | 1.36 | 2.82 | 0.36 | 10.36 | 2.91 | 8.00 |
| SD  | 0.48 | 1.40 | 0.48 | 8.25  | 0.29 | 2.63 |

*Significant at $p = 0.01$; **Significant at $p = 0.005$; ***Significant at $p = 0.0005$.

### Discussion

Clomiphene citrate therapy is selected as the first-line treatment in many patients with ovulation disorders or infertility, and gonadotropin therapy is available as second-line therapy. While 10-day [6, 7] and 8-day [8] regimens have been reported for clomiphene citrate therapy, conventional clomiphene citrate therapy is usually limited to five days [3, 4]. The effect of the drug is increased simply by raising the daily dose, but a limit is soon reached. It has also been reported that gonadotropin therapy is not usually effective in poor responders to clomiphene citrate because of interference between endogenous and exogenous gonadotropins [1]. In gonadotropin therapy, both the ovulation-inducing effect and frequencies of adverse effects such as multiple preg-
nancies (number of ovulated ova) and OHSS are greater than in clomiphene citrate therapy, and these therapies differ greatly.

Modified RICCT is a new treatment that might prove more effective than gonadotropin therapy [5]. In the present study, as compared with patients receiving gonadotropin therapy, the pregnancy rate was lower, but the number of patients who ovulated was higher and the incidence of OHSS was lower among those receiving RICCT. The small number of mature follicles is likely to have affected the pregnancy rate, but the therapy appears to suppress the occurrence of multiple pregnancies and OHSS. Thus, we believe that modified RICCT should be considered before gonadotropin therapy is selected. Two methods resembling RICCT have been reported, one for disorders of the hypothalamus [1] and the other for PCOS [9]. In both reports, clomiphene was first administered for five days, followed by another 5-day administration if there was no withdrawal bleeding. This regimen was reported to sensitize the pituitary-hypothalamus-ovary system, which resulted in recovery of menstruation, as we suggested previously [5]. These therapies conform to our modified RICCT, in that 5-day administration was repeated during the same menstrual cycle, although the dose was increased for the second course.

Our modified RICCT showed more favorable results as compared with gonadotropin therapy with regard to the analyzed variables other than pregnancy and miscarriage. Because the numbers of mature and immature follicles were lower, and the ovulation rate was higher, the therapy had a sufficient ovulation-inducing effect even when the dose of the second course was identical to that of the first course. Therefore, a dose increase in the second course might lead to over-responsiveness. Our experience with modified RICC suggested that the dose could even be reduced for the second or third course.

In comparison with gonadotropin therapy, drug cost was clearly lower for modified RICCT, giving it an overwhelming economic advantage. The smaller number of injections results in less physical, psychological, and temporal burdens and makes the therapy more acceptable, particularly among patients with a limited desire for children. Recently, recombinant follicle-stimulating hormone (FSH) has become widely available, but it is more expensive than conventional urinary hMG despite the superior effectiveness and side-effect profile of hMG. Currently, the efficacy of FSH does not differ markedly from that of urinary hMG [10, 11]; therefore, the relative benefits of modified RICCT would remain, even if the present study were done using recombinant FSH.

Although modified RICCT may be superior to gonadotropin therapy in many respects, it was inferior with regard to pregnancy rate and miscarriage. Poor penetration of sperm due to the inferior characteristics of cervical mucus is a side effect of clomiphene citrate therapy [12, 13]. Indeed, when we examined patients who became pregnant during modified RICCT, artificial insemination was needed when clomiphene citrate was given at a daily dose other than 50 mg/day (unpublished observation). The necessity for artificial insemination is likely to increase further if the dose of clomiphene citrate is increased or the treatment period is prolonged. Combinations of clomiphene therapy with hMG have been reported to enhance the advantages of both agents [14, 15], and combinations of modified RICCT with hMG will be indispensable in the development of clomiphene citrate therapy.

It has been reported that patients with PCOS are likely to develop diabetes or dyslipidemia and that long or irregular menstrual cycles increase the risk of diabetes [16]. The use of combined estrogen/progesterone preparation to induce withdrawal bleeding is insufficient for treating women with disorders of the hypothalamus, and functional improvements from clomiphene citrate administration are necessary [2]. From this perspective, methods of ovulation induction that are advantageous in preventing conditions such as OHSS should be recommended, even if the pregnancy rate is lower.

Conclusion

Clomiphene citrate therapy has been used as first-line treatment because of its favorable side-effect profile, but gonadotropin therapy poses many problems as a second-line therapy. Modified RICCT might prove superior to gonadotropin therapy and is a second-line treatment that can be given to a wide range of patients after clomiphene citrate therapy.

References


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The role of hypertension, body mass index, and serum leptin levels in patients with endometrial hyperplasia during premenopausal period

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Introduction

Endometrial diseases in premenopausal women with abnormal bleeding is not common. The incidence of endometrial hyperplasia in premenopausal women is reported to be 2% to 10% [1, 2], but endometrial hyperplasia incidence with and without atypia peaks during early postmenopausal years and in early 1960s women, often in association with abnormal uterine bleeding (AUB) [3].

Endometrial hyperplasia has been classified into three main types: 1) simple hyperplasia, characterized by minimal endometrial glandular crowding and with low risk of progression to endometrial carcinoma; 2) complex hyperplasia, characterized by greater endometrial glandular crowding and intermediate risk of progression; and 3) atypical hyperplasia, characterized by an endometrium with complex glandular crowding and / or cytologic atypia, and the greatest risk of endometrial carcinoma progression [4, 5].

The diagnoses of endometrial hyperplasia are only made in women who have had endometrial sampling. Due to the invasive nature of endometrial sampling, very few studies have performed routine endometrial biopsies on asymptomatic women [6, 7]. These researches show that among women with normal bleeding patterns, the prevalence of simple and complex hyperplasia is 0.5% to 5% and the prevalence of atypical endometrial hyperplasia or carcinoma is less than 1%.

Rapid lifestyle changes in modern society have produced an epidemic of obesity implicated in diabetes and hypertension. All three conditions are included among risk factors of endometrial cancer as established by many studies [8, 9].

Although the risk factors for endometrial hyperplasia in postmenopausal women have been well-established [10], they do not apply to premenopausal women. A retrospective study report of 46 premenopausal women found that body weight, age, infertility, family history of colonic carcinoma, and nulliparity were risk factors for endometrial hyperplasia [11].

The purpose of this study was to analyze possible associations between simple endometrial hyperplasia and adipose tissue distribution: evaluating BMI, hip and waist circumferences, waist to hip ratio (WHR), leptin concentration, hypertension (HTN), diabetes, and life style.

Materials and Methods

All premenopausal women, who had menstrual irregularity for one or two years associated with hot flashes or night sweats, ranged from 40 to 55 years (mean 48.2 years) going to the

Summary

Objectives: to investigate whether body mass index (BMI), hypertension (HTN), diabetes, age, and physical activity can be considered risk factors for endometrial simple hyperplasia in premenopausal women. Furthermore this study was undertaken to determine whether serum concentration of leptin in patients with BMI $\geq 30$ Kg / m² with endometrial hyperplasia deviate from values in patients with normal endometrium. Materials and Methods: The authors enrolled 167 hyperplasia cases and 282 controls. Demographic characteristics and data on age, diabetes, hypertension, BMI, physical activity, and anthropometric parameters were collected. Leptin concentration in serum was measured with immunoenzymatic test kit from IBL. Univariable and multivariable analysis were performed to verify the association among age, HTN, BMI, physical activity, diabetes, and the presence of uterine hyperplasia. Furthermore the authors evaluated the correlation between BMI and leptin level (with Pearson’s linear correlation) in women with simple hyperplasia and in controls. Results: The prevalence of hyperplasia found was 34.4%. The following factors were independently associated with increased risk of endometrial hyperplasia: HTN (odds ratio 3.19, 95% confidence interval 1.20 - 8.48, $p < 0.020$) and BMI $\geq 30$ Kg / m² (odds ratio 6.43, 95% confidence interval 3.92 - 10.53, $p < 0.000$). Mean leptin concentration in serum was higher in patients who had endometrial hyperplasia than in controls ($p < 0.005$) and the leptin levels depended on BMI. Conclusions: The following are risk factors for endometrial hyperplasia in premenopausal women: BMI $\geq 30$ Kg / m² and HTN (blood pressure $\geq 130 / 85$ or in therapy). Leptin appears to participate in proliferative processes of the endometrium, depending on BMI. Current guidelines may need to be reconsidered.

Key words: Diabetes; BMI; Hypertension; Endometrial hyperplasia; Leptin.
A successful treatment: Incidence of multiple infectious diseases in the general population.

In a study conducted by the Department of General Practice, a total of 639 patients were referred to the Department for evaluation of abnormal uterine bleeding or endometrial abnormalities disclosed at sonography (dishomogeneous echo pattern, absence of central echo or presence of focal lesions). One hundred fifty-six patients (24.4%) had abnormal bleeding and 483 (75.6%) patients were asymptomatic.

Exclusion criteria were as follows: (1) amenorrhea ≥ one year; (2) gynaecological organic pathologies, such as diagnosis of endometrial cancer or history of endometritis or annexial flogosis; (3) patients receiving hormonal therapy or exposed previously to exogenous estrogens. Patients with an intrauterine device (IUD), or who had received hormonal treatment in the previous three months, or who had already undergone dilatation and curettage (D&C) or diagnostic or operative hysteroscopy were excluded from the analysis. Inclusion criteria were as follows: (1) premenopausal women between 40 and 55 years old who complained of abnormal uterine bleeding > three months (heavy menstrual bleeding, irregular or intermenstrual bleeding) regardless of cycle; (3) abnormal ultrasonographic pattern (endometrial hyperechogenic spots, irregular endometrial line, suggestion of uterine septa).

The authors invited participants to describe AUB symptoms using the specified simple list of four terms with three choices of descriptive words for each term. The four keys were: cycle regularity (specified as irregular, regular or absent), frequency of menstruation (specified as frequent, normal or infrequent), duration (specified as prolonged, normal or shortened) and volume of menstrual flow (specified as heavy, normal or light) [12].

Past obstetric and medical history, as well as ongoing pharmacological therapy, were recorded. The authors used ultrasongraphy as first step in all patients (n = 639) to evaluate possible abnormal patterns. Ultrasonography was performed by gynaecologists independently of the phase of the cycle. The ultrasonic finding was considered abnormal when the technician visualized a lesion inside the cavity or when the maximum endometrial thickness measured in the sagittal plane according to the technique of Ozdemir et al. was ≥ 8 mm [13]. Doubtful sonograms with findings neither definitively negative nor positive, due to poor visualization and / or difficult interpretation were considered abnormal. The gynaecologist found 97 (15.2%) submucous myoma (diagnosed at ultrasonography in the presence of a nodular formation with well-defined margins, heterogeneous structure, and varying echogenicity, which displaced the endometrial lining) and 57 (8.9%) endometrial polyps. The authors treated these patients by running the diagnostic hysteroscopy and later surgery as needed, but have not included them in the study. The authors found 282 (44.1%) patients with normal sonography pattern and 203 (31.8%) with abnormal pattern.

All women were submitted to hysteroscopy to assess abnormal uterine bleeding or abnormal sonographic patterns. The inclusion of both symptomatic and asymptomatic patients, provided a better estimation of endometrial hyperplasia prevalence within the general population. Hysteroscopy was always carried out in sterile conditions after cleansing of external genitalia, vagina, and cervix with a povidone iodine antiseptic solution. The investigation was postponed if an acute cervico-vaginal infection was present. Diagnostic hysteroscopy was performed by a gynaecologist, with a 5 mm Storz hysteroscope (Karl Storz, Tuttlingen, Germany).

Ultrasonography

- 639 enrolled (n = 156 with AUB; n = 483 asymptomatic)

Hysteroscopy

- 167 simple hyperplasia
- 2 endometrial cancer
- 5 atypical hyperplasia
- 29 complex hyperplasia

Figure 1. — Patient allocation according to histological results.

(without speculum) was used to avoid patient discomfort or pain not directly related to uterine examination. The investigation was postponed if an acute cervico-vaginal infection was present. Only in women with a history of previous pelvic inflammatory disease was a single prophylactic two gram dose of cefoxitin injected i.m. A minimum of both hysteroscopy, Endometrial hyperplasia was defined, macroscopically, as thick, hyper-vascular, friable mucosa that was mamillated or polypoid. At the end of the procedure, an intrauterine biopsy was obtained with a small cutting curette [14]. The diagnosis of uterine hyperplasia was histologically made by the pathologist of the Department of Pathology of St. Anna and St. Sebastiano Hospitals.

Histologic diagnosis of the endometrium distinguished between normal findings (n = 282), with abnormal findings (n = 203): 167 (34.4%) cases were simple hyperplasia, two (0.4%) endometrial carcinoma, five (1%) hyperplasia with atypia, and 29 (6%) were complex hyperplasia. The samples of carcinoma, complex hyperplasia, and atypic hyperplasia were not included in this study. Depending on the results of histology, patients were allocated into one of two groups: group I (n = 167) simple hyperplasia and group II (n = 282) control patients with normal endometrium (endometrium in secretory stage, endometrium in proliferative stage) (Figure 1).

A general physical including a gynecological examination were performed. Anthropometric measurements and other data were recorded. The authors evaluated: body weight, height, BMI, leptin levels, waist and hip circumferences, WHR, fasting glucose, blood pressure, and the quality of physical activity.

Body weight was measured, in underwear, to the nearest kilogram with a balance scale. Height was measured to the nearest centimeter. The authors used a Seca 200 scale (Seca, Hamburg, Germany) with a balance scale. BMI was calculated as weight / height² (kg / m²). Leptin concentrations in serum were measured with immunoenzymatic test kit from immuno-biological laboratories (IBL). Waist circumference was measured, using a steel tape measure, to the nearest centimeter at a level midway between the lower rib margin and iliac crest. Hip circumference was measured at the widest point between hips and buttocks. Measurements were taken in the upright position. WHR was calculated as waist circumference in centimeters divided by hip circumference in centimeters. WHR > 0.8 was considered abnormal in women [15]. Fasting glucose was measured in all patients (n = 485) and assays for glucose was performed in the hospital’s chemistry laboratory.
A diabetes diagnosis was assigned to the following diabetes registration criteria [16]: fasting plasma glucose (FPG) ≥ 126 mg/dl (7.0 mmol/l) and insulin is defined as no caloric intake for at least eight hours. In the absence of unequivocal hyperglycemia, the authors confirmed diagnosis by repeat testing of evaluation ≥ 200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test (OGTT).

Diagnosis of HTN was assigned to those who met the following criteria: greater than or equal to two blood pressure measurements greater than 130/85 mmHg in accordance with the rules to perform an accurate pressure measurement [17] or measurements greater than 130/85 mmHg in accordance with the rules to perform an accurate pressure measurement [17] or greater than or equal to one prescription for an anti-HTN drug.

Patients were further subdivided according to BMI values of < 30 kg/m² and ≥ 30 kg/m². A positive correlation was noted between the concentration of leptin in serum and BMI; the concentration of leptin in group I patients with BMI ≥ 30 was 7.8 ng/ml as opposed to 3.2 ng/ml in the respective controls. For BMI ≥ 30 leptin concentrations in patients with or without endometrial hyperplasia were 13.5 ng/ml versus 7.8 ng/ml (p < 0.005). Mean plasma leptin level was 8.6 ± 4.8 ng/ml and the range was 1.7 to 29.6 ng/ml (Table 1).

The median age of women with hyperplasia was 49.8 years; in those with normal endometrium, the median age was 50.5 years (range 40 - 55 years). 98 (58.7%) patients with hyperplasia had HTN. In the group of patients that had hyperplasia, 25 (15.0%) were diabetic; 227 (81.5%) patients with hyperplasia practiced low physical activity (Table 2).

BMI and weight were higher in hyperplasia cases than in controls (82.5 vs 63.9) and waist and hip circumference and WHR was higher too (Table 3).

The age of patients ranged from 40 to 55 years (mean 48.2 years). From a total of 485 eligible women, 203 (41.8%) patients had an abnormal sonography pattern; in 167 (34.4%) the samples obtained with biopsy during hysteroscopy showed simple hyperplasia; two (0.4%) were endometrial carcinoma; five (1%) were hyperplasia with atypia and 29 (6%) were complex hyperplasia. Excluding the samples of carcinoma, complex and atypical hyperplasia, and depending on the results of histology, patients were allocated to one of two groups: group I (n = 167) simple hyperplasia and group II (n = 282) control patients with normal endometrium.

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The role of hypertension, body mass index, and serum leptin levels in patients with endometrial hyperplasia during etc.

### Table 1. — Leptin concentration in serum depending on BMI in groups I and II.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I</th>
<th>Group II</th>
<th>p (I vs II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>7.8</td>
<td>13.5</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>BMI &lt; 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI ≥ 30</td>
<td>3.2</td>
<td>7.8</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. — Independent sample test: evaluation of anthropometric parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>p (I vs II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>82.5</td>
<td>63.9</td>
<td>0.000</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.1</td>
<td>164.7</td>
<td>0.503</td>
</tr>
<tr>
<td>BMI</td>
<td>30.2</td>
<td>23.49</td>
<td>0.000</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>111.4</td>
<td>78.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>118.5</td>
<td>103.2</td>
<td>0.000</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94</td>
<td>0.76</td>
<td>0.000</td>
</tr>
</tbody>
</table>

### Table 3. — Independent sample test: evaluation of age, BMI, HTN, diabetes, and low physical exercise in hyperplasia cases and in controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>I n = 167</th>
<th>II n = 282</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, sd)</td>
<td>49.8 (3.0)</td>
<td>50.5 (3.2)</td>
<td>0.022</td>
</tr>
<tr>
<td>BMI (mean, sd)</td>
<td>30.2 (3.8)</td>
<td>23.49 (1.6)</td>
<td>0.000</td>
</tr>
<tr>
<td>HTN (n, %)</td>
<td>98 (58.7%)</td>
<td>59 (20.9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>25 (15.0%)</td>
<td>17 (6.0%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Low physical exercise (n, %)</td>
<td>227 (81.5%)</td>
<td>45 (26.9%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### Table 4. — Independent risk factors for adenomatous hyperplasia (n = 449).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>p value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.949</td>
<td>0.816</td>
<td>1.098</td>
<td>0.467</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>6.432</td>
<td>3.927</td>
<td>10.536</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td>3.192</td>
<td>1.201</td>
<td>8.489</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.275</td>
<td>0.004</td>
<td>19.680</td>
<td>0.553</td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>1.377</td>
<td>0.503</td>
<td>3.574</td>
<td>0.533</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

This study has reported a rate for endometrial hyperplasia in premenopausal women with abnormal menstrual bleeding sufficient to warrant endometrial sampling. In the present study the incidence of adenomatous hyperplasia decreased with increasing age. Adenomatous hyperplasia was most frequent before 55 years of age, preceding the peak incidence of endometrial carcinoma by more than 10 years.

In this study, endometrial hyperplasia prevalence was approximately 34% of patients investigated by biopsy. It unusual to find 34% of premenopausal women with AUB or with abnormal sonography patterns diagnosed with endometrial hyperplasia. This is significantly higher than other previous estimates even if authors as Anastasiadis PG et al. disclose an endometrial hyperplasia prevalence of approximately 20% of patients investigated by biopsy for abnormal bleeding [18]. Taddei GI et al. [19, 20] found in a study with 1,075 patients with AUB, a prevalence of endometrial hyperplasia of 20.2%, while 9.2% of patients were diagnosed with carcinoma. In the same study, taking into account only postmenopausal patients, hyperplasia represented 23.4%, with the prevalence of endometrial carcinoma increased to 12.3%. The analysis of the 203 cases of endometrial hyperplasia showed a clear predominance of cases with hyperplasia without atypia (167 cases), compared with cases with complex hyperplasia with (five cases) and without atypia (29 cases).

The present data showed that body weight, BMI, and WHR were higher in perimenopausal women with endometrial hyperplasia than in population controls. In particular the authors evaluated obesity, not only using weight, but also measuring waist circumference, hip circumference, WHR, and BMI. The obesity was present in a large percentage of the examined patients in this study. Obesity could lead to the development of endometrial hyperplasia by increasing the concentration of circulating estrogens and thus stimulation of growth of the endometrium. This could occur in several ways: by decreasing levels of circulating sex hormone-binding globulin [21] or by increasing the conversion of androstenedione to estrone that occurs with increased adipose tissue [22]. Premenopausal women who are obese could be at additional risk, since they are more likely to have periods of anovulation and therefore lower progesterone levels [23, 24], which increases their risk of endometrial proliferation and inadequate menstrual shedding of the endometrium. It is an emerging disease, characterized by increased peripheral aromatization of androgens to estrogens in adipose tissue and seems to be associated with an estrogenic state. However, the association between adenomatous hyperplasia and WHR disappeared after adjustment for BMI. This indicates that the quantity, but not the location of body fat is a risk factor for adenomatous hyperplasia. Other studies suggested that higher BMI is associated with endometrial hyperplasia as compared to women with lower BMI [20, 25].

Systemic HTN was present in 98 (58.7%) cases and in 59 (20.9%) controls of the examined patients of this study. Hypertension, especially associated with obesity, appears to be an important factor that may play a role in the pathogenesis of endometrial hyperplasia, Vorgias et al. found that hyperplasia with or without atypia occurs in approximately 50% of hypertensive women [26]. Moreover, it is known that systolic pressure and the prevalence of HTN increase dramatically with age.

Diabetes was present in 25 (15.0%) cases and in 17 (6.0%) controls of the patients included in this study. Although diabetes seems to be linked to endometrial hyperplasia even if there is only a study on rats [27], but BMI and nulliparity seem to be directly linked to endometrial hyperplasia [28].

In this study when performing multivariable logistic regression, only two independent variables had statistical significance. These data are in accordance with the international literature that indicates that the likelihood of hyperplasia is related to age and hormonal status [28]. These data could also be explained by the strict association between aging and climactery. Indeed, during the perimenopausal years, which are characterized by the endometrium’s prolonged exposure to both estrogens and low progesterone levels, the unopposed estrogens may contribute to the pathogenesis of endometrial hyperplasia.

Furthermore the authors found that serum concentration of lepin in simple endometrial hyperplasia was higher than in controls with normal endometrium. The authors evaluated a positive correlation between lepin levels in serum and BMI. Other studies showed this correlation, but they analyzed the serum concentration of lepin in patients with endometrial cancer. Petridou et al. studied 84 women with endometrial cancer and 84 controls and they noted higher lepin levels in cancer patients and their correlation with BMI [29]. Cymbaluk et al. found similar data in their study, in particular they analyzed lepin levels in patients with endometrial cancer and with endometrial hyperplasia with atypia [30].

In conclusion, this study leads the authors to reconsider the influence of age, diabetes, HTN, BMI, physical activity on the development of endometrial hyperplasia, and highlights the need to find a clear relationship between experimental and epidemiologic features on the growth of endometrium.

The authors have also added evidence that lepin may be involved in hyperplasia as in other proliferative processes of the endometrium. Yuan et al. showed the expression of lepin receptors in endometrial cancer cells and found higher level of lepin in serum [31]. It is higher in obese women and the authors found that the risk factors statistically significant in this study for endometrial hyperplasia were BMI ≥ 30 kg / m^2 and HTN.

Increased level of lepin in obesity can be also considered as a marker of developing insulin resistance [32] by reducing tissue sensitivity to insulin, it is responsible for hyperinsulinemia, and it is the cause of elevated levels of free fatty acids. Leptin is also associated to high blood
pressure and high level of triglycerides [33]. The role of leptin should be studied as a possible independent risk factor for adenomatous hyperplasia.

A limit of this study is that the distribution of hormone-related characteristics in such a group almost certainly would not reflect that of women in general. Another limit of this study is that BMI, glucose levels, and blood pressure were examined as dichotomous variables. Future prospective studies, considering such risk factors in a continuous model, may more powerfully identify age as the key factor in hyperplasia development and definitely exclude a relationship between other variables and endometrium growth.

References

Effect of uterine artery blood flow on recurrent pregnancy loss

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Summary

We investigated the effect of uterine artery blood flow on recurrent pregnancy loss. One hundred and twelve patients admitted to our clinic were included in the prospective study. The study group consisted of 28 cases with a history of three miscarriages before the 20th gestational week, and the control group consisted of cases with at least one prior live birth without any history of miscarriage or poor obstetric outcome. The mean pulsatility index (PI), resistance index (RI) and systolic/diastolic ratio (S/D ratio) values of the uterine artery were measured between the 18th and 23rd days of the menstrual cycle via transvaginal Doppler ultrasonography. No statistically significant difference could be detected regarding uterine artery PI (p = 0.703), RI (p = 0.333), and S/D (p = 0.403) values between the study group and the control group (p > 0.05). In order to clearly determine etiologic causes of recurrent pregnancy loss, new randomized and controlled clinical trials with large patient populations are needed.

Key words: Recurrent pregnancy loss; Uterine artery; Doppler ultrasonography.

Introduction

Recurrent pregnancy loss is defined as three or more miscarriages before 20 weeks of gestation. Cases with no live birth are referred to as primary and those with live birth as secondary recurrent pregnancy loss. More than 80% of the abortions occur within the first 12 weeks and this rate rapidly decreases thereafter [1]. More than half of the abortions are caused by chromosomal anomalies. The incidence decreases as gestational age increases. The risk of spontaneous abortion increases with increasing age of parents [2].

The frequency of recurrent pregnancy loss in a fertile population is 1-2% when those with three or more pregnancy losses are taken into consideration. This rate is reported to be 5% when the cases with two or more pregnancy losses are taken into consideration [3]. Using current diagnostic methods, the underlying cause can be determined only in half the cases with recurrent pregnancy loss [4].

Detailed visualization of female internal genital organs has been possible particularly with the use of high resolution transvaginal ultrasound probes [5]. These devices allow close examination of small details since they are placed in close proximity to the organs to be inspected [6]. Hemodynamics of pelvic and genital organs can also be evaluated by Doppler ultrasound (US). Taylor et al. was the first to perform oварian and uterine artery blood flow measurements during the menstrual cycle [7]. Pelvic genital organs can be evaluated by continuous wave, pulsed wave, and color flow mapping methods [8].

In this study, we evaluated the relationship between recurrent pregnancy loss and uterine artery Doppler flow.

Materials and Methods

Between 2008 and 2010, 112 patients admitted to the 4th Department of Obstetrics and Gynecology at Göztepe Training and Research Hospital were included in this prospective and controlled study. The study group consisted of 28 patients between 18 and 40 years of age who had at least three abortions before 20 weeks of gestation, regular menstrual cycles in the last three months, who did not use hormonal contraception or intrauterine devices, and who were not pregnant at the time of the study.

Four patients with irregular menstrual cycles, three patients who were using hormonal contraception, four patients who were using intrauterine devices, and one patient who was pregnant at the time of the study were excluded from the study. One case with diabetes mellitus, two cases with hypothyroidism and three cases that were found to have consanguineous marriages were also excluded from the study. Two cases had abnormal karyotypes, two cases had IgG anticardiolipin antibodies, six cases had heterozygous and one case had homozygous factor V Leiden mutation, 21 cases had heterozygous and two cases had homozygous MTHFR mutation. These cases were all excluded from the study. One case was excluded because of a previous oophorectomy operation due to an ovarian cyst. Three cases could not be reached during the study.

Using a simple random sampling method, 28 cases were selected as the control group among 128 cases who were admitted to our outpatient clinic for family planning counselling and who had at least one live birth and who no history of abortion or poor obstetric history.

Patient age, age of spouse, number of abortions, abortion week, consanguineous marriage, systemic disorders (diabetes mellitus, thyroid disease, chronic liver, kidney or heart disease), and history of venous thrombosis were all recorded; prolactin levels were measured and karyotype analysis was performed. IgG and IgM anticardiolipin antibodies and lupus anticoagulant were measured. Factor V Leiden (G1691A) mutation, prothrombin G20210A mutation, and MTHFR C677T mutation were investigated in peripheral blood obtained from the patients.
All cases were evaluated during the secretory phase of the menstrual cycle between days 18 and 23. Measurements were performed using a Logiq P5 (General Electric, CA, USA) Doppler US device and 7.5 MHz endovaginal probe after the patients were placed in the lithotomy position on a gynecologic examination table. US scanning gel was applied to the probe which was then covered with a sterile condom. After applying lubricating gel, the probe was gently inserted into the vagina. The probe was positioned as to obtain sagittal images in which caudal structures are displayed at the right, and cranial structures are displayed at the left side of the monitor. After repositioning the probe by rotating 90 degrees, a coronal view was acquired in which the right side of the patient was displayed on the right side and the left side of the patient was displayed on the left side of the monitor. After orientation to the pelvis minor and determination of anatomic landmarks (cervix, ovary, pelvic wall), the US probe was moved into the lateral fornix of vagina. Examination was performed with minimal possible pressure of the probe. Doppler signal was recorded from the uterine artery at the isthmus level. After locating the artery on real-time imaging, Doppler wave and gate were adjusted as to detect Doppler shift. The mean pulsatility index (PI), resistance index (RI), and systolic/diastolic ratio (S/D) ratio values for bilateral uterine arteries were automatically calculated by the computer. All measurements were performed twice by the same investigator and average values were used in the analysis.

NCSS (Number Cruncher Statistical System) 2007 & PASS 2008 Statistical Software (Utah, USA) was used in statistical analyses of the data. Along with descriptive statistics, the Student’s test was used in comparison of variables with normal distribution. The level of significance was set at 0.05.

**Results**

Age of the patients ranged between 20 and 39 years; mean age was 31.42 ± 3.72 years in the study group and 31.10 ± 3.75 years in the control group. There was no significant difference between the groups with respect to age. Mean age of the spouses was 33.85 ± 3.48 years in the study group and 34.14 ± 3.00 years in the control group; no significant difference was found between the groups. Age characteristics of the groups are shown in Table 1.

**Discussion**

Abortion is the most common complication occurring during pregnancy and it constitutes the major reason for bleeding during the first and second trimester. The probability of abortion decreases as gestational age increases. The risk of abortion is 11.5% where the gestational sac is visualized but not the embryo itself; the risk is 5% when a fetal heart beat is detectable and the risk is below 3% at the 11th week of gestation [9]. The risk of spontaneous pregnancy loss increases with age. In our study, both patients in the study and control groups were under 40 years of age and no statistically significant difference was found between the groups with respect to age.

Among all the factors investigated, genetic, anatomic, and immunological factors are considered definitive causes of recurrent pregnancy loss. Alloimmunopathology, hereditary thrombophilia, endocrinopathies, infections, and environmental factors are still investigated. After a detailed investigation, definitive cause of recurrent pregnancy loss could not be explained in about more than half of the couples. In our study, 3.5% of the cases had a systemic disorder, 38% had anti-phospholipid syndrome and hereditary thrombophilia, and 2.3% had abnormal karyotype. None of the cases had a history of exposure to radiation, chemicals or drugs.

The ovary and uterus are two organs in which marked neoangiogenesis occurs under physiological conditions in adults. Blood flow curves of the uterine arteries reflect the vessel anatomy of uterine vasculature and furthermore provide clues about functional status of the same arteries. Color and pulsed transvaginal Doppler US is a simple, fast, and reproducible examination method. Compared to B- and M-mode US, color Doppler and other methods used in blood flow measurements cause higher exposure to US waves. Measurement of uterine perfusion is an invasive method to examine the intrauterine environment but it can also provide additional information about pathophysiology of recurrent pregnancy loss and endometrial implantation. Rhythmic changes in uterine blood supply during the menstrual period could be related with blood progesterone/estrogen ratio. The higher estrogen to progesterone ratio, the higher is the quantity of blood flow through the uterine vascular bed. Proges-

---

### Table 1. — Age characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Study group (n = 28)</th>
<th>Control group (n = 28)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age</td>
<td>31.42 ± 3.72</td>
<td>31.10 ± 3.75</td>
<td>0.805</td>
</tr>
<tr>
<td>Spouse age</td>
<td>33.85 ± 3.48</td>
<td>34.14 ± 3.00</td>
<td>0.754</td>
</tr>
</tbody>
</table>

* p < 0.05 was considered significant.

### Table 2. — PI, RI values and S/D ratios between the study and control group.

<table>
<thead>
<tr>
<th></th>
<th>Study group (n = 28)</th>
<th>Control group (n = 28)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsatility index</td>
<td>2.28 ± 0.35</td>
<td>2.32 ± 0.39</td>
<td>0.703</td>
</tr>
<tr>
<td>Resistance index</td>
<td>0.85 ± 0.03</td>
<td>0.84 ± 0.04</td>
<td>0.333</td>
</tr>
<tr>
<td>Systolic/diastolic ratio</td>
<td>7.12 ± 1.78</td>
<td>6.73 ± 1.72</td>
<td>0.403</td>
</tr>
</tbody>
</table>

* p < 0.05 was considered significant.
terone antagonizes uterine vasodilator effects of estrogen and the degree of inhibition depends on ratio of these two steroids [10, 11].

Kurjak et al. performed transvaginal colour Doppler US in 100 infertile patients and 150 control patients to determine changes in blood flow of the uterine and ovarian arteries during the menstrual cycle. They found a statistically significant difference between the study and control groups with respect to mean RI of the uterine artery [12]. Zaidi et al. investigated whether or not pregnancy and implantation success rates could be predicted by the assessment of blood flow in the uterine artery on the day hCG was administered to patients undergoing IVF treatment. They suggested that the success of the implantation could be predicted by measuring the uterine artery PI [13]. Yokota et al. studied 63 infertile patients and investigated PI values of the uterine and ovarian arteries on the day of ovulation using transvaginal color Doppler US. They noted that measurement of uterine artery PI by transvaginal color Doppler US could be of benefit in patients with infertility of unknown etiology [14].

Hoozemans et al. assessed 83 patients undergoing IVF-ET treatment by serial Doppler US examinations performed at different times of the cycle and reported that uterine artery Doppler US cannot predict which women could conceive and which of the pregnancies would end in miscarriage [15]. Wakeman et al. studied 192 women in the follicular phase of the menstrual cycle and they compared uterine and ovarian blood flows using transvaginal Doppler US between patients who conceived in a natural menstrual cycle with those who did not. Transvaginal US was performed between the 3rd and 12th day of the cycle. They did not find any statistically significant difference between pregnant and non-pregnant cycles in terms of utero-ovarian blood flow parameters [16].

In this study, we did not find statistically significant difference in terms of PI, RI values, and S/D ratio between the control and study group with a history of recurrent pregnancy loss. Some studies in the literature suggest that no significant relation exists between recurrent pregnancy loss and PI, RI values, and S/D ratio measured by transvaginal Doppler US during the luteal phase of the menstrual cycle but there are also studies which assert the contrary. Ferreira et al. compared 43 women with recurrent pregnancy loss and 43 women with no history of abortion and at least one child born at term. The uterine artery PI and flow velocity wave (FVW) patterns were investigated, and a higher PI, incidence of FVW of the A and uterine artery impedance were found among women with recurrent pregnancy loss [17]. Nakatsuka et al. examined uterine artery blood flow in 104 pregnant women between the 4th and 5th weeks of gestation using transvaginal Doppler US. Uterine artery PI value was found to be significantly higher in the study group with recurrent pregnancy loss compared to the control group. They suggested that uterine artery PI value could be an independent indicator in cases with recurrent pregnancy loss and that it could be used to detect patients with recurrent pregnancy loss caused by the impairment in uterine perfusion [18]. Lazzarin and colleagues compared uterine artery PI values between a control group consisting of 50 subjects who did not have a history of miscarriage and who had at least one live birth with a study group consisting of 230 cases with recurrent pregnancy loss with unknown etiology. They used transvaginal US in mid-luteal phase. They found significantly higher uterine artery PI values in patients with recurrent pregnancy loss (2.60 ± 0.7) compared to the control group (2.08 ± 0.47). They emphasized that increased uterine artery blood flow resistance is an independent risk factor in cases with recurrent pregnancy loss [19]. By using uterine artery Doppler US in the mid-luteal phase, El Mashad et al. compared patients who had three or more recurrent pregnancy losses with a control group. They found significantly higher uterine artery PI values in the study group (2.71 ± 0.259) compared to the control group (2.06 ± 0.194) (p < 0.01). As a result, they suggested that the uterine artery PI value could be used to determine patients with recurrent pregnancy loss caused by impairment in uterine perfusion [20].

Frates et al. examined 96 cases with a history of recurrent pregnancy loss during the first trimester of their pregnancies and found that uterine artery RI values decreased significantly from the 6th week until the 13th week of gestation. They noted that uterine artery RI value in the first trimester is of no use in predicting pregnancy outcomes in cases with recurrent pregnancy loss [21]. However in the English literature the number of studies that have similar results with Frates et al. and our study on uterine artery Doppler flow and recurrent pregnancy loss is limited.

Conclusion

Early pregnancy loss is a distressing condition for the entire family. Most families have the fear that this problem will reoccur. Early pregnancy loss is also an important issue for physicians dealing with reproductive health, because its etiology is unknown and diagnostic methods and treatment options are limited. In this study, we found no statistically significant difference between women with and without a history of recurrent pregnancy loss in terms of uterine artery PI, RI values, and S/D ratio. However, randomized and controlled studies on wider patient populations are needed to elicit etiological factors of recurrent pregnancy loss and to propose standardized treatment protocols.

References

Effect of uterine artery blood flow on recurrent pregnancy loss


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Vulvodynia: a case series of a poorly recognized entity


"2nd Department of Dermatology & Venerology, 1st Department of Obstetrics & Gynecology, Aristotle University School of Medicine, Papageorgiou Hospital, Thessaloniki (Greece)"

Summary

Vulvodynia remains a poorly recognized entity with unclear pathogenesis. In a case series of six patients with vulvodynia over a five-year period in a tertiary university hospital, we describe the clinical features, the diagnostic procedures, the impact on each patient’s emotional status and discuss the necessity and efficacy of the chosen treatment options in accordance with the current therapeutic guidelines.

Key words: Vulvodynia; Chronic vulvar pain.

Introduction

Vulvodynia is an uncommon and poorly recognized entity with unclear pathogenesis. In a small case series of six patients with vulvodynia over a five-year period at a tertiary university hospital we describe the clinical features, the diagnostic procedures, family and professional parameters, the impact on each patient’s emotional status and discuss the necessity and efficacy of the chosen treatment options in accordance with the current therapeutic guidelines.

Case Series

Case 1

A 48-year-old patient, married, para 0, gravida 0, presented with dysesthesia, dyspareunia and burning sensation of the vulva. The symptoms had exacerbation and remission of various duration depending on stress-associated factors. She was examined by a number of different physicians and was thoroughly investigated with no pathological results. Topical antifungals, topical steroids and tranquillizers were administered resulting in no relief of the symptoms. By searching her history, it was revealed that the patient had a neurotic and hypochondriac personality long before the vulvodynia signs presented. On clinical examination, there was slight erythema of the vulva, while the vagina and cervix were normal. When pressing the vestibulus with a cotton swab (Q-tip test), hypersensitivity and a burning sensation were induced. Having found no etiological relations, the diagnosis of the vulvodynia was set and an explanation of the unclarified nature of this entity was given to the patient. One year later, symptoms were totally absent and after a five-year follow-up, the patient remains symptom-free. Meanwhile a severe family issue, probably the major cause of the patient’s stressed condition, had been resolved.

Case 2

A 52-year-old patient, married, gravida 1, para 1, presented with tense dyspareunia, dysuria and typical burning sensation of the vulva. Symptoms were so severe for more than three years, that her sexual life and her emotional world were heavily disturbed. The patient had visited many different physicians, undergone a number of laboratory examinations and was treated with various regimens. On clinical grounds, there was no lesion on the vulva, vagina or cervix. Having a long talk with the patient, no preceding chronic stress causative facts in the family or at her work environment were revealed. The Q-tip test was positive. Biopsies from three different sites of the afflicted area were taken showing findings of a non specific chronic inflammation. After a thorough explanation of the nature of vulvodynia, in the absence of other treatment modalities, laser ablation was applied in order to reduce the number of sensory nerve fibres. Ten months later the patient presented in the same condition.

Case 3

A 38-year-old patient, married, gravida 1, para 1, presented with a three-month duration of intense burning and pain sensation on the clitoris area. The Q-tip test was positive, although the symptoms were present with or without any pressure. On clinical examination the area was normal, with no erythema or edema. A number of physicians had already examined her resulting in no diagnosis. Laser ablation below the clitoris area was then performed with no response. This discomforting entity began to influence her personality, resulting in depression. She was treated with gabapentin and duloxetine per os and with topical application of xylocaine, with signs of improvement after a three-month follow-up.

Case 4

A 47-year-patient, gravida 3, para 2, presented with a burning and painful sensation on the vulva with dyspareunia for the previous eight months. Clinically there were no findings of inflammation. The Q-tip test was positive, though. Many physicians proposed various examinations without setting a diagnosis. The patient was treated with fluoxetine per os and came for the follow-up visit after four months, showing slight improvement. She was to continue with the same regimen but she did not appear at her next follow-up visit.

Case 5

A 59-year-old patient, gravida 2, para 2, presented with intense pain on the vulva. She had suffered for three years and despite the various treatments from many different physicians,
Vulvodynia: a case series of a poorly recognized entity

There was a continuing deterioration of the burning sensation ending in her not tolerating her own undergarments. All investigations were negative. Application of topical steroids and topical anesthetics for a long period did not achieve relief of the symptoms. On clinical examination, which was extremely difficult due to the reaction of the patient to even a minor touch, there was mild erythema and slight atrophy of the minor labia mucosa and of the vulva. The patient reported an increase of her body weight of over 30 kg, the last few years. After the long lasting application of steroids, and to avoid the risk of tachyphylaxis, only neutral anaplastic creams in combination with systemic anti-depressants and low calorie diet were proposed. At a follow-up visit one year later, the patient had lost twenty 25 kg of body weight and she was relieved from symptoms.

**Case 6**

A 33-year-old patient, gravida 0, para 0, presented for clinical evaluation as she had felt intense pain at the vulva and her clitoris for almost 15 months. These symptoms started after long treatment with econazole vaginal suppositories for a non established “candida vaginitis”. The patient reported dyspareunia and pain with tight clothing. She had been examined by a number of physicians and had undergone urine and vaginal excretion examination, as well as a biopsy, with no diagnostic findings. On clinical examination there was slight erythema of the vulva, probably due to the chronic application of topical xylocaine gel and topical steroids. The Q-tip test was positive. Treatment with gabapentin at a dose of 1200 mg per day for three months showed some efficacy at first but was discontinued by the patient herself due to persistent dizziness and sleepiness.

**Discussion**

For many decades the literature has hosted many different names for chronic vulvar pain (essential vulvodynia, dyesthesic vulvodynia, vulvar vestibulitis syndrome, vulvar dyesthesias, provoked vulvar dyesthesias, spontaneous vulvar dyesthesias, vestibulodynia, burning vulva, and clitororodynia). The classification of chronic vulvar pain to either vulvar pain related to a specific disorder or vulvodynia was proposed from the International Society for the Study of Vulvovaginal Disease in 2003 and offered a clear definition of terms [1].

Vulvodynia is today considered to be vulvar discomfort, most often described as burning pain, occurring in the absence of a relevant specific infectious, inflammatory, neoplastic or neurologic disorder. Localized or generalized, provoked or spontaneous, vulvodynia is rather a diagnosis of exclusion, reached commonly after a multidisciplinary approach and after a significant number of laboratory investigations. A great number of patients with vulvodynia seem to have a hidden or underecognized emotional instability. Vulvodynia has a severe impact on the patients’ quality of life and its management remains a challenge.

According to the recently published evidence-based treatment guidelines, by the British Society for the Study of Vulval Diseases Guideline Group, an initial step of utmost importance is to explain the condition to the patient, allaying any fears and reassuring her that the condition is not infectious or related to cancer [2].

Initially, the importance of gentle genital hygiene should be emphasized, as well as elimination of excessive cleansing habits. Patients should also be encouraged to wash themselves with plain warm water and with a mild hypoallergenic and fragrance free nonsoap bar or cleanser, using hands only and not wash clothes or sponges [3].

From the topically applied agents, a trial of local anesthetics may be considered in all vulvodynia subsets. Systemically, tricyclic antidepressants (TCAs), e.g. amitriptyline or nortriptyline, are an appropriate initial treatment mainly for unprovoked vulvodynia. In addition to a TCA, gabapentin and pregabalin may be considered. Surgical excision of the vestibule or techniques to desensitize the pelvic floor muscles may be beneficial to a minority of patients with provoked pain. Selective serotonin reuptake inhibitors (SSRIs), like fluoxetine and serotonin norepinephrine reuptake inhibitors (SNRIs), such as venlafaxine and duloxetine, have also been used.

**Table 1. — Diagnostic procedures, disease duration, emotional status, treatment and outcome in all patients.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Number of medical specialties involved</th>
<th>Laboratory investigations</th>
<th>Symptom duration</th>
<th>Emotional disorders</th>
<th>Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>48</td>
<td>5</td>
<td>Blood, urine, vaginal excretion, biopsy</td>
<td>2 years</td>
<td>Neurotic &amp; hypochondriac personality</td>
<td>Psychological support</td>
<td>Free of symptoms after one year and at a 5-year follow-up</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>4</td>
<td>Blood, urine, vaginal excretion, three biopsies</td>
<td>&gt; 3 years</td>
<td>Signs of depression</td>
<td>Laser ablation</td>
<td>Stable at 10 months</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>4</td>
<td>Urine, vaginal excretion</td>
<td>3 months</td>
<td>Depression</td>
<td>Laser ablation</td>
<td>Improvement</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>4</td>
<td>Urine, vaginal excretion</td>
<td>8 months</td>
<td>None</td>
<td>Fluoxetine</td>
<td>Slight improvement after 4 months</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>4</td>
<td>Urine, vaginal excretion</td>
<td>3 years</td>
<td>None</td>
<td>Antidepressants, low calorie diet, anaplastic creams</td>
<td>Free of symptoms after one year</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>2</td>
<td>Urine, vaginal excretion, biopsy</td>
<td>15 months</td>
<td>None</td>
<td>Gabapentin</td>
<td>Improvement</td>
</tr>
</tbody>
</table>
to treat vulvodynia, but only in small trials. Intradermal steroid or botox injections may be considered in some cases [2, 3].

Few placebo-controlled studies have been conducted on medical treatments for vulvodynia. In the study by Bornstein et al., the true effectiveness of topical medications for the treatment of vulvodynia is questioned. The main finding of this study was that for low and high concentrations of topical nifedipine, as well as placebo ointment, mean pain intensity, assessed by the Q-tip test, speculum insertion and reports of sexual intercourse, was reduced at post-treatment compared with pre-treatment. What was really the benefit of the use of the topical medication? It was rather a placebo effect [4].

When psychological distress is expressed as a physical symptom and results in vulvodynia (somatoform hypothesis), then the necessity of treatment should be discussed. This vulvar pain may result in a conditioned avoidance response with spasm of the pelvic floor muscles. Depression and anxiety may contribute to a cycle of pain and patients may worry that sexual activity will be painful or vulvodynia may develop secondarily [5].

In our case series, all patients had undergone a number of laboratory examinations and many uneffective topical therapies (Table 1). When patients finally received a diagnosis of vulvodynia and were provided with clear and reliable information they expressed some relief. From the suggested regimens, only systemic antidepressants seemed to reduce the severity of symptoms. A possible explanation is that half of the patients had signs of depression. Was an underlying depression the cause of vulvodynia or did the vulvodynia cause anxiety and depression?

A crucial problem with vulvodynia patients remains the lack of awareness of this entity and the number of unnecessary investigations which lead to a delay in diagnosis and management. A recent survey demonstrated that there is lack of basic knowledge on chronic vulvar pain and vulvodynia among junior gynecologists who are being trained in tertiary units where most women with vulval pain conditions usually are referred to [6].

**Conclusion**

Vulvodynia remains a poorly recognized entity. Awareness and a multidisciplinary collaboration on vulvodynia are of great importance for early recognition and the appropriate management, as well as teaching seminars on this entity for all residents in dermatology and gynecology.

**References**


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Clinical importance of detection of bacterial vaginosis, trichomonas vaginalis, candida albicans and actinomyces in Papanicolaou smears

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Summary

Objective: The aim of this study was to determine the role of Papanicolaou (pap) smears in the diagnosis of lower genital tract infections. Materials and Methods: A retrospective study was planned by reviewing charts of patients for trichomonas vaginalis, bacterial vaginosis, candida albicans, actinomyces and nonspecific vaginitis. Results: Charts of 9,080 patients were reviewed and 1,733 women had a diagnosis of lower genital tract infection in the pap smear or had had a clinically treated lower genital tract infection. Only 33.5%, 30.4%, 43.3%, and 0% of patients with bacterial vaginosis, trichomonas vaginalis, candida and actinomyces, respectively on pap smears were diagnosed and treated clinically. Postmenopausal patients had a higher rate of trichomonas vaginalis infection and a lower rate of candida infection when compared to women of the reproductive age group. Patients using an intrauterine device for contraception had a statistically significantly increased rate of trichomonas vaginalis and candida infection when compared to women using other contraceptive methods or those who were not using any contraception. Conclusions: Finding trichomonas vaginalis, bacterial vaginosis and actinomyces infections in pap smears might be considered an indication for treatment without performing other diagnostic tests. Treatment of asymptomatic infections can prevent complications in selected patients. Candida can be a commensal bacteria in the vagina, therefore asymptomatic patients may not require treatment. Detection of a higher rate of trichomonas vaginalis and candida infection in IUD users can increase the risk of vaginal infections and associated complications.

Key words: Bacterial vaginosis; Trichomonas vaginalis; Candida albicans; Actinomyces; Pap smear.

Introduction

The Papanicolaou (pap) smear is a simple screening test used routinely for detection of cervical cancer and precancerous lesions of the cervix. Although the main aim of the pathologist is to focus on the detection of cervical intraepithelial neoplasia, it is common practice to report genital tract infections as trichomonas vaginalis, bacterial vaginosis, candida albicans, actinomyces and nonspecific infections within the pap smear results whenever detected. The Bethesda System also supports making a diagnosis of microorganisms. It seems logical to use a test performed periodically for cervical cancer screening to detect asymptomatic genital infections or to confirm a clinically diagnosed infection. Trichomonas vaginalis is a sexually transmitted infection [1] and bacterial vaginosis increases the risk of obstetric and gynecological morbidity including pelvic inflammatory disease, postoperative infections, cervicitis, endometritis, chorioamnionitis, preterm birth [2], HIV acquisition [3] and cervical intraepithelial neoplasia [4].

There is no national cancer screening program in Turkey, but in our gynecology clinic smear tests are performed periodically. The objective of this study was to determine the role of pap smears in the diagnosis of lower genital tract infections.

Materials and Methods

This is a retrospective study carried out by searching charts of patients who attended the gynecology clinic at Avrupa Hospital of Bilim University between 2002 and 2010. Every woman attending our clinic receives a yearly cervical smear. Samples were collected by a cytobrush by rotating the brush 360°. The collected material was spread on a glass slide and fixed with spray. The samples were stained by the conventional Papanicolaou technique. All smears were evaluated by pathologists (not by cytotechnicians) according to Bethesda system criteria. Exclusion criteria consisted of the following: pregnancy, presence of vaginal bleeding, use of vaginally applied medications three days prior to the sample collection and sexual intercourse or vaginal douching within 24 hours of the sample collection. Cases with nonspecific vaginal infections, bacterial vaginosis, trichomonas vaginalis, candida albicans and actinomyces on pap smear reports were identified. Also patients that had been treated for vaginitis but showed no signs of vaginal infection on the pap smear report were recorded. The study protocol was within the ethical guidelines of the Declaration of Helsinki.

Results

Results of 9,080 women were suitable for analysis. Of these, 1,733 women had a clinically diagnosed lower genital tract infection or had a normal clinical examination but had had a microorganism reported within the pap smear results. Mean age at the time of smear test was 37.9 ± 11.4 (range 17-79). Mean gravida was 2.17 ± 2.32 (range 0-16), mean abortus was 0.11 ± 0.41 (range 0-4), and mean termination of pregnancy on demand was 1 ± 1.6 (range 0-14) (Table 1). Of the patients included in this

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study (1,566/1,733), 90.4% were in the reproductive age group. A lower genital tract infection was diagnosed in 13.6% of the women in pap smears. In this study 11.4% (197/1733), 3.3% (56/1733), 8.1% (141/1733), 0.1% (2/1733) and 48.5% (840/1733) of pap smears were positive for bacterial vaginosis, trichomonas vaginalis, candidiasis, actinomycosis and nonspecific vaginitis, respectively. Infections detected in pap smears were clinically diagnosed and treated in only 33.5%, 30.4% and 43.3% of bacterial vaginosis, trichomonas vaginalis and candida infections, respectively. When patients were stratified by age, detection rate of trichomonas in pap smears after 40 years of age increased statistically significantly (Table 2). Stratification of patients into age groups statistically significantly increased the detection rate of trichomonas infection in pap smears in patients older than 40 years, but this did not increase the clinical diagnosis and appropriate treatment rates. In contrast patients younger than 40 years received appropriate treatment for bacterial vaginosis and candida infection before the detection of infection in pap smears when compared to patients older than 40 years, p = 0.001 and p = 0.019, respectively (Table 2). Patients older than 50 years had an increased rate of diagnosis of nonspecific infection in pap smear (Table 2).

When the menopausal status of patients were taken into consideration the rate of trichomonas vaginalis infection in pap smears was significantly less common in the reproductive age group when compared to the postmenopausal group, 45/1,566 (2.9%) and 11/167 (6.6%), respectively, p < 0.01 (Table 3). In contrast the rate of candida infection in pap smears was more common in the reproductive age group when compared to the postmenopausal group, 135/1,566 (8.6%) and 6/167 (3.6%) respectively, p = 0.02. Similarly nonspecific infection was more common in the reproductive age group when compared to the postmenopausal group, 465/1,566 (29.7%) and 29/167 (17.4%), respectively.

As a contraceptive method 222 patients were using an intrauterine device (IUD) and 12.4%, 5.5%, 11.9%, 0.009% had bacterial vaginosis, trichomonas vaginalis, candida and actinomycosis infection detected with a pap smear, respectively (Table 4). In women who were not using a contraceptive method or using contraceptive methods other than an IUD, the infection rates detected with pap smears were lower, and 11.6%, 2.7%, 7.8%, 0% had bacterial vaginosis, trichomonas vaginalis, candida and actinomycosis infection, respectively. Patients using an intrauterine device for contraception had statistically significantly more trichomonas and candida infection detected in pap smears when compared to patients using other contraceptive methods or no contraception, 12/222 (5.4%) and 44/1511 (2.9%), p = 0.05 for trichomonas and 27/222 (12.2%) and 114/1511 (7.5%), p = 0.019 for candidiasis.

Smears showing ASCUS (atypical squamous cells of undetermined significance) and LGSIL (low grade squamous intraepithelial lesion) constituted 1.7% (30/1733) of smears. Similarly 1.7% (29/1733) of this population had genital warts. Patients presenting with high-grade lesions were excluded.

### Table 1. — Demographic characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37.9 ± 2.32</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>2.17 ± 2.32</td>
</tr>
<tr>
<td>No. of deliveries</td>
<td>1.1 ± 1.13</td>
</tr>
<tr>
<td>No. of abortus</td>
<td>0.11 ± 0.41</td>
</tr>
</tbody>
</table>

### Table 2. — Results of pap smears after stratification of patients according to age groups.

<table>
<thead>
<tr>
<th>Age</th>
<th>Trichomonas vaginalis</th>
<th>Bacterial vaginosis</th>
<th>Candida</th>
<th>Nonspecific infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>1.8%</td>
<td>44.2%</td>
<td>8.4%</td>
<td>46.8%</td>
</tr>
<tr>
<td>30-39</td>
<td>2.6%</td>
<td>50%</td>
<td>7.9%</td>
<td>48.2%</td>
</tr>
<tr>
<td>40-49</td>
<td>4.9%</td>
<td>23.6%</td>
<td>9.9%</td>
<td>41.6%</td>
</tr>
<tr>
<td>50-59</td>
<td>3.8%</td>
<td>14.3%</td>
<td>5%</td>
<td>63.6%</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>5.6%</td>
<td>33.3%</td>
<td>5.6%</td>
<td>61.1%</td>
</tr>
</tbody>
</table>

Mantel-Haenszel Test.

### Table 3. — Comparison of patients in the postmenopausal group and reproductive age group.

<table>
<thead>
<tr>
<th>Pap smear result</th>
<th>Reproductive age group</th>
<th>Postmenopausal group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial vaginosis (+)</td>
<td>11.6% (181/1566)</td>
<td>9.6% (16/167)</td>
<td>0.44</td>
</tr>
<tr>
<td>Trichomonas vaginalis (+)</td>
<td>2.9% (45/1566)</td>
<td>6.6% (11/167)</td>
<td>0.01</td>
</tr>
<tr>
<td>Candida (+)</td>
<td>6.6% (137/1566)</td>
<td>3.6% (6/167)</td>
<td>0.02</td>
</tr>
<tr>
<td>Nonspecific</td>
<td>29.7 (465/1566)</td>
<td>17.4% (29/167)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Chi-square test.

### Table 4. — Comparison of patients using an intrauterine device (IUD) and other methods.

<table>
<thead>
<tr>
<th>Pap smear result</th>
<th>IUD (+)</th>
<th>IUD (-)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial vaginosis (+)</td>
<td>13.1% (29/222)</td>
<td>11.1% (168/1511)</td>
<td>0.394</td>
</tr>
<tr>
<td>Trichomonas vaginalis (+)</td>
<td>5.4% (12/222)</td>
<td>2.9% (44/1511)</td>
<td>0.05</td>
</tr>
<tr>
<td>Candida (+)</td>
<td>12.2% (27/222)</td>
<td>7.5% (114/1511)</td>
<td>0.019</td>
</tr>
<tr>
<td>Nonspecific</td>
<td>23.4% (527/222)</td>
<td>29.3% (442/1511)</td>
<td>0.072</td>
</tr>
</tbody>
</table>

Chi-square test.

### Discussion

Bacterial vaginosis is characterized by decreased concentration of lactobacilli and an overgrowth of mixed anaerobic flora [5]. In gynecology clinics the prevalence of bacterial vaginosis ranges from 9-38% [6-8]. Clinically bacterial vaginosis is diagnosed by using Amsel’s criteria [9] or by gram-stained vaginal smears [10]. Platz-Christensen reported the sensitivity and specificity of pap smears as accurate as Amsel’s criteria [9] or by gram-stained vaginal smears [10]. Platz-Christensen reported the sensitivity and specificity of pap smears as 90% and 97%, respectively and showed that pap smears could be used for clinical diagnosis of bacterial vaginosis as accurate as Amsel’s criteria [4] and as gram-stain [11]. Later other studies supported the high sensitivity and specificity of pap smears in the detection of bacterial vaginosis [12-15] and other studies showed contradictory results [8]. Tokyol et al. reported that the sensitivity of pap smears is not enough to screen for bacterial vaginosis but that it can be used as a diagnostic test when positive because of the high specificity [16]. Considering the results we obtained from this study we suggest that pap smears can be used to detect asymptomatic and clinically missed cases of bacterial vaginosis.
Lamont et al. reported that sensitivity and specificity of the smear for the diagnosis of bacterial vaginosis increased from 81% to 87% and 91% to 97%, respectively when smears were evaluated by cytotechnicians and cytopathologists [17]. In Turkey we have no cytotechnicians, so all smears are evaluated by pathologists. This may be the reason for the increased detection of clinically undiagnosed infections in pap smears.

A survey conducted in the United Kingdom revealed that all four of Amsel’s criteria are used only by 30% of clinics [18]. This may decrease the detection rate of bacterial vaginosis. On the other hand there are reports that using only two of Amsel's criteria has enough sensitivity and specificity for the detection of bacterial vaginosis [19, 20]. For some clinics it can be time consuming to be expensive to use all of Amsel’s criteria and gram-stain, or they may not have adequate equipment. In this case it is a money saving procedure to use the routine pap smear for the diagnosis of lower genital tract infections. Half of the cases [21], can be detected and further diagnostic work-up can be planned. Some propose that it can be time consuming to look for signs of vaginal infection in the smear test and that it can direct the cytologist away from the main purpose, diagnosing cervical intraepithelial neoplasia. If the hypothesis proposing cervical intraepithelial neoplasia as a complication of bacterial vaginosis is established then diagnosis and treatment of bacterial vaginosis will gain more importance.

Trichomonas vaginalis is a sexually transmitted protozoal infection [1]. Fifty percent of trichomonias infections are asymptomatic [22]. In this case asymptomatic patients may act as a reservoir and transmit the protozoa to their partners. This may lead to pelvic inflammatory disease, premature rupture of the membranes, preterm delivery and abortion [23]. Wet mount was reported to be less sensitive than culture and pap smear [24]. Burja et al. reported that 68% of asymptomatic cases presenting for a gynecological examination were left untreated, in 61% of cases infection was detected only in pap smears and in 28% clinically detected infections were confirmed by pap smears [25]; our results present similar findings. Loo et al. reported sensitivity and specificity of pap smears in detection of trichomonias vaginalis as 96% and 98%, respectively [26]. Nearly 70% of cases detected by pap smears are clinically missed, therefore we agree with previous reports that the pap smear is a valuable test in detecting and treating trichomonias vaginalis infections [27, 28].

In previous studies an increased rate of trichomonias vaginalis infection was diagnosed in women using an IUD when compared to women using other contraceptive methods [29, 30]. In this study also an increased rate of trichomonias vaginalis infection was detected in pap smears of IUD users (p < 0.05). When the asymptomatic nature of the infection is considered, pap smears can be used to treat trichomoniasis and to decrease complications such as pelvic inflammatory disease, which increases both in IUD users and trichomoniasis.

Spinillo et al. performed a study in symptomatic vaginitis patients and found an increased prevalence of trichomonas vaginitis in postmenopausal women when compared to women of childbearing age, 10.8% and 1.92%, respectively [31]. In our study 6.6% of postmenopausal women and 2.9% of the reproductive age group had trichomoniasis. This may be the result of changes in vaginal cytology in the postmenopausal period that makes the diagnosis of trichomonas vaginalis easier or may be the result of increased parabasal cells in estrogen deficient women that could be diagnosed as trichomonas trophozoites by mistake. Also in postmenopausal patients there is an increased rate of nonspecific infection, which can be.

Seventy-five percent of sexually active women will experience at least one episode of vulvovaginal candidiasis in their lifetime [32]. Sixty-six percent of women with candida detected in pap smears were reported to be asymptomatic [33]. As confirmed with this study, in postmenopausal women candida rates decrease when compared to women of childbearing age. Our results are very similar to the report of Shurbaji et al., where the candida detection rate with pap smears in clinically asymptomatic women was 66% [34], Audisio et al. found the specificity of pap smear for candida infection to be 98.5% [27]. Candida can be a commensal bacteria in the vagina and is not associated with any serious gynecologic or obstetric complications, so detection of candida in pap smears may not require any treatment at all.

Gupta et al. reported an increased presence of actinomyces infection in women with IUDs [35]. Although for actinomyces infection presence of an IUD is not a must, in our study population no actinomyces was detected in women without an IUD. This may be the result of increased attention of the cytologist when the presence of an IUD has been reported by the clinician. Actinomyces was positive in 11% of women with IUDs in a previous study [36], but in our study group this was only 0.9%. Although this presents a small group of patients, clinically none of them were recognized.

In conclusion finding trichomonas vaginalis, bacterial vaginosis and actinomyces infections in pap smears might be considered an indication for treatment without performing other diagnostic tests. Treatment of asymptomatic infections can prevent complications associated with these infections. Candida can be a commensal bacteria in the vagina, therefore asymptomatic patients may not require treatment. Detection of a higher rate of trichomonas vaginalis and candida infection in IUD users shows that IUDs can increase the risk of vaginal infections and associated complications.

References


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The impact of six-month tibolone postmenopausal treatment on cell adhesion molecules levels

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Summary

Purpose: The aim of the present study was to evaluate the effects of tibolone on inter-cellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), inter-cellular adhesion molecule-2 (ICAM-2) and P-selectin levels in healthy postmenopausal women. Methods: This prospective study included 25 postmenopausal women, complaining of hot flashes, assigned in two groups. Fifteen women received tibolone (dosage of 2.5 mg per day for six months) and ten women did not receive any therapy, according to their personal preference. Basal control included complete medical history, anthropometrics, clinical examination, and blood sampling to perform hormonal, biochemical, hematological testing and ICAM-1, ICAM-2, VCAM-1 and P-selectin measurements. Evaluation was repeated in three and six months. Results: There was no significant difference in ICAM-1, VCAM-1, ICAM-2, P-selectin, homocysteine, total cholesterol, HDL, LDL, and triglyceride concentrations between the women of the two groups after either three or six months of treatment. However, a significant reduction in the frequency and intensity of hot flashes was noted in both groups. Conclusions: Tibolone does not have any adverse effects on cell adhesion molecule levels which primarily affect atherosclerotic processes or on triglyceride and homocysteine concentrations. These results may support the view that tibolone could be considered a safe treatment, regarding its impact on the endothelium, in healthy postmenopausal women.

Key words: Tibolone; Menopause; Cell adhesion molecules; ICAM-1; ICAM-2; VCAM-1; P-selectin.

Introduction

Atherosclerosis is considered a dynamic and progressive process triggered by endothelial dysfunction and inflammation and leading to cardiovascular disease [1]. Women present an age-dependent and more precisely, an estrogen-dependent cardiovascular disease risk pattern [2]. Estrogens exert a widely favorable effect on endothelium function, as already known [2]. This is reflected on the apparent growth in the number of cardiovascular events after menopause [3]. In these terms, conventional hormone therapy (HT) was thought to reduce the higher cardiovascular risk by replacing the intrinsic lack of estrogen in postmenopausal women [4]. However, the value of hormone replacement therapy as a means of cardiovascular disease risk reduction has been seriously debated since the publication of the first results of the Women’s Health Initiative (WHI) study [5].

Tibolone is a synthetic steroid with estrogenic, progestagenic and androgenic effects. It has been used as an alternative treatment for menopausal symptoms and gained popularity after the announcement of the negative impact of conventional HT on the cardiovascular system [5-7]. The positive effects of tibolone on the lipidemic profile are widely studied, in contrast to the direct effects on the endothelium or the indirect effects via independent biomarkers.

An initial step in the atherosclerotic process is the adhesion of white blood cells (WBCs) to endothelium. WBC adhesion is mediated by cell-cell adhesion molecules (CAMs) which are produced by the endothelium and include five superfamilies (the immunoglobulin superfamily, integrins, cadherins, transmembrane proteoglycans, and selectins) [8, 9]. The immunoglobulin superfamily includes more than 70 members, among them ICAM-1, ICAM-2, ICAM-3, VCAM-1 and VCAM-2 which adhere to leucocute surfaces via integrins. Selectins include L-selectin which is expressed on WBCs, E-selectin expressed on activated endothelial cells and P-selectin expressed on both activated platelets and endothelial cells [10]. Selectins mediate the calcium-dependent cell-extracellular matrix binding in blood and, thus, they promote WBCs binding to endothelium as part of an inflammatory response.

In case of endothelial dysfunction, there is derangement of endothelial homeostasis and accumulation of WBCs in the arterial intima, mediated by increase in ICAM, VCAM and especially P-selectin expression. Indeed, several CAMs have been found elevated in patients with established atherosclerosis or with high risk for cardiovascular disease [11]. On the other hand, HT interferes with endothelial dysfunction and ameliorates many cardiovascular risk markers [12]. Tibolone effects on cardiovascular risk markers have been also investigated in human cells cultures [13-15], primates [16] and postmenopausal women [17-20]. However, opinions on the effects of tibolone on endothelium remain divergent as the results of both clinical and in vitro studies are not unanimous. In these terms, the aim of the present study was the evaluation of tibolone effects on ICAM-1, VCAM-1, ICAM-2 and P-selectin in healthy postmenopausal women compared with controls.
Materials and Methods

From January 2006 to December 2007, 25 healthy postmenopausal women, referred to the Menopause Infirmary of the First Department of Obstetrics and Gynecology because of severe hot flashes that affected their quality of life, were enrolled voluntarily in the present study. The protocol and procedures were approved by the Institutional Ethics Committee. None of the authors had any conflict of interest.

The main inclusion criterion was the presence of severe hot flashes affecting the patient’s quality of life. All women had had their last menstrual episode at least one year before in cases of natural menopause and four months before in cases of surgical menopause, and presented serum FSH levels higher than 40 IU/ml. Furthermore, all participants had similar lifestyles and body mass index (BMI). Exclusion criteria included: history of thromboembolism, arterial hypertension, cardiovascular disease, liver or kidney disease, thyroid dysfunction, diabetes mellitus, coagulation disturbance, estrogen-dependent tumor, prior hormone replacement therapy or tibolone intake and excessive smoking.

All 25 women completed the protocol. It should be noted that the women were also selected based on similar lifestyles and were advised to continue the same lifestyle throughout the study period. The 25 participants were divided in two groups. Group A included 15 women who received tibolone (Livial, Organon, Holland) at a dosage of 2.5 mg per day for six months early in the morning. Group B included ten women who did not receive any therapy. All subjects were assigned to the groups according to their personal preference, after being fully informed.

Complete medical history, anthropometrics, clinical examination and Pap smear test, ultrasound of the internal genitalia, mammography, and dual energy X-ray absorptiometry (DEXA) were carried out. Blood samples were collected for evaluation of ICAM-1, ICAM-2, VCAM-1 and P-selectin concentrations, and furthermore for hormonal FSH levels, biochemical levels (glucose, urea, creatinine, uric acid, K, Na, SGOT, SGPT, GT, LDH, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, homocysteine, bilirubin, total proteins, PT, ePTT, and fibrinogen) and hematological tests. The evaluation was repeated after three and six months.

Blood samples were collected in noncitrated tubes between 8 and 9 a.m. after an overnight fast. To assess CAM concentrations, blood samples were centrifuged at 3000 rpm for 10 min. The serum was aspirated and kept in special aliquots at -22°C. All samples were assayed together.

ICAM-1, ICAM-2 and VCAM-1 were measured by sandwich ELISA (Diaclone, France). P-selectin was also measured by sandwich ELISA (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer’s instructions. The sensitivity of the ICAM-1 assay was lower than 0.1 ng/ml. Intra- and inter-assay variation coefficients (CVs) were 3.56% and 8.76%, respectively. The sensitivity of the ICAM-2 assay was lower than 0.2 U/ml. Intra- and inter-assay CVs were 3.20% and 7.15%, respectively. The sensitivity of the VCAM-1 assay was lower than 0.6 ng/ml. Intra- and inter-assay CVs were 3.34% and 5.94%, respectively. The sensitivity of the P-selectin assay was lower than 0.5 ng/ml. Intra- and inter-assay CVs were 5.80% and 8.90%, respectively.

SPSS software v.16.0 (SPSS inc, Chicago, IL) was used for data analysis. Normality of the distribution was checked by the Kolmogorov-Smirnov test. T-test and a general linear model for repeated measurements were used for the assessment of differences in significance between the mean levels of all study parameters of the two groups for changes in time and between groups. All tests were 2-tailed at a significance level of 0.05.

Results

At baseline (time-t = 0), there was no significant difference in the mean values of age, BMI and time since menopause between the women of the two groups. Similarly, there was no significant difference in the mean values of HDL, LDL and triglycerides. Also, the mean homocysteine, ICAM-1, VCAM-1, ICAM-2 and P-selectin levels were not different between the two study groups. Only, the mean total cholesterol concentration was significantly higher in women of group B (p = 0.004) (Table 1). After completion of three (t = 3 months) and six months (t = 6 months) of treatment, all measurements were repeated. There was no significant difference between the two groups regarding the mean concentrations of total cholesterol, HDL, LDL, triglycerides, homocysteine, ICAM-1, VCAM-1, ICAM-2, and P-selectin after either three or six months of treatment (Table 1). Regarding hot flashes, all women reported improvement in frequency as well as intensity both at three and six months after initiation of the study.

The general linear model analysis indicated a non significant main effect of time on total cholesterol (F = 1.632, p = 0.209), HDL (F = 2.048, p = 0.151), LDL (F = 0.207, p = 0.763), triglycerides (F = 2.364, p = 0.110), homocysteine (F = 0.627, p = 0.494), ICAM-1 (F = 0.701, p = 0.483), VCAM-1 (F = 0.369, p = 0.649), ICAM-2 (F = 1.708, p = 0.197) and P-selectin (F = 1.566, p = 0.221) levels. Furthermore, the pattern of the main effect of time was not significantly different between the two groups with regards to the mean values of total cholesterol (F = 0.450, p = 0.622), HDL (F = 1.699, p = 0.201), LDL (F = 0.680, p = 0.510), triglycerides (F = 2.683, p = 0.084), homocysteine (F = 0.426, p = 0.597), ICAM-1 (F = 0.170, p = 0.815), VCAM-1 (F = 0.208, p = 0.766), ICAM-2 (F = 1.308, p = 0.279) and P-selectin (F = 1.291, p = 0.284) over the whole study period.

Discussion

This study demonstrates that tibolone intake for a period of six months had no impact on ICAM-1, VCAM-1, ICAM-2, P-selectin, total cholesterol, HDL, LDL, triglycerides and homocysteine levels in the serum of healthy postmenopausal women complaining of hot flashes. As described above, women receiving one tablet (2.5 mg) of tibolone per day did not present any difference from controls who did not receive any therapy throughout the whole study period. Importantly, all women included in this study were outpatients complaining of hot flashes, without any prior history of cardiovascular diseases, in an effort to ensure a homogeneous sample and compensate for the small sample size. Additionally, all participants were of similar age and BMI, as these parameters exert a direct or even indirect influence on the cardiovascular system. All women had the same nationality and similar lifestyle.

A limitation point is the small number of women included in the study as well as the lack of randomization.
However, replacement therapy is seen under the prism of profound scepticism on the part of women and even the gynecologists in Greece. Prescription of replacement therapy is significantly lower in comparison to other European countries, especially after the publication of WHI and HERS [5-7] Greek women often exclude the possibility of receiving therapy for menopausal symptoms rendering, thus, the performance of a blind study very difficult, as already stated by other researchers [20].

The main findings of the study are the almost stable impact of tibolone or other replacement therapy in postmenopausal women. VCAM-1 has been found to be reduced after hormone therapy in a clinical [19] and an in vitro study [13]. However, our results do not confirm the latter study probably because of the small number of patients or the different study time length. Regarding the levels of P-selectin, there is only one recent publication referring to non significant alterations of P-selectin serum levels after eight weeks of oral tibolone intake [18].

The non significant changes in homocysteine levels in the present study are supportive of the lack of negative impact of this drug on the endothelium. Homocysteine serum levels have also been found to be unaffected in previous studies of tibolone administration during either three or 18 months [24, 25]. Although inflammatory activation of the endothelium has been shown during concurrent transient hyperhomocysteinaemia, no correlation between homocysteine and CAM or P-selectin levels has ever been reported [26].

As for the impact of tibolone on the lipid profile, a reducing trend in total cholesterol, triglycerides, LDL and also HDL is generally indicated [20, 27-31]. In the present study, no significant difference in any of the lipids was found. This discrepancy could be attributed to the sample size, though comparable with some previous studies, or even to genetic variability and dietary habits. The fact that the treatment did not reduce HDL levels could be regarded as positively for the use of the drug, although there was no difference in total cholesterol, LDL, and triglycerides.

All women enrolled in this study initially complained of hot flashes but afterwards they all referred improvement in the frequency and intensity of hot flashes and, furthermore, general amelioration of mood and quality of life. Although this has been a subjective judgment that was not systemically evaluated, it is an important case in point that should be considered for the treatment choice, at least for this population of patients, as already pointed out [32, 33].

In conclusion, the six-month tibolone treatment did not influence cell adhesion molecule levels. However, the fact that CAM levels remained stable, as well as lipid and

---

### Table 1. — Anthropometrics and serum lipids and CAM levels in the women of the two study groups at baseline (t=0), 3 and 6 months.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A</th>
<th>Group B</th>
<th>p values</th>
<th>Group A</th>
<th>Group B</th>
<th>p values</th>
<th>Group A</th>
<th>Group B</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.40 ± 0.73</td>
<td>49.50 ± 0.67</td>
<td>0.403</td>
<td>205.00 ± 6.21</td>
<td>224.30 ± 7.60</td>
<td>0.062</td>
<td>202.80 ± 6.15</td>
<td>219.70 ± 7.53</td>
<td>0.096</td>
</tr>
<tr>
<td>TSM (months)</td>
<td>10.86 ± 2.03</td>
<td>15.30 ± 2.58</td>
<td>0.188</td>
<td>50.40 ± 3.53</td>
<td>53.40 ± 4.37</td>
<td>0.726</td>
<td>50.40 ± 3.47</td>
<td>51.20 ± 4.25</td>
<td>0.885</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>25.56 ± 0.50</td>
<td>24.41 ± 0.62</td>
<td>0.166</td>
<td>144.66 ± 7.35</td>
<td>152.30 ± 9.03</td>
<td>0.518</td>
<td>149.40 ± 8.40</td>
<td>149.40 ± 8.40</td>
<td>0.348</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>207.20 ± 5.20</td>
<td>233.3 ± 6.41</td>
<td>0.004</td>
<td>107.69 ± 11.62</td>
<td>0.862</td>
<td>125.06 ± 9.25</td>
<td>124.92 ± 11.33</td>
<td>0.612</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>50.40 ± 3.86</td>
<td>55.90 ± 4.72</td>
<td>0.400</td>
<td>125.06 ± 9.25</td>
<td>124.92 ± 11.33</td>
<td>0.612</td>
<td>631.99 ± 51.02</td>
<td>657.65 ± 62.49</td>
<td>0.225</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>130.00 ± 6.65</td>
<td>155.60 ± 8.14</td>
<td>0.052</td>
<td>125.06 ± 9.25</td>
<td>124.92 ± 11.33</td>
<td>0.612</td>
<td>631.99 ± 51.02</td>
<td>657.65 ± 62.49</td>
<td>0.225</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>102.73 ± 10.14</td>
<td>95.60 ± 12.43</td>
<td>0.661</td>
<td>87.26 ± 10.22</td>
<td>0.862</td>
<td>125.06 ± 9.25</td>
<td>124.92 ± 11.33</td>
<td>0.612</td>
<td></td>
</tr>
<tr>
<td>Homocysteine (mmol/l)</td>
<td>10.03 ± 0.62</td>
<td>10.33 ± 0.76</td>
<td>0.764</td>
<td>10.06 ± 0.55</td>
<td>10.53 ± 0.68</td>
<td>0.598</td>
<td>10.04 ± 0.54</td>
<td>10.30 ± 0.66</td>
<td>0.769</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>690.99 ± 34.93</td>
<td>665.37 ± 42.78</td>
<td>0.520</td>
<td>692.98 ± 43.42</td>
<td>701.54 ± 53.18</td>
<td>0.952</td>
<td>631.99 ± 51.02</td>
<td>657.65 ± 62.49</td>
<td>0.225</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>915.06 ± 84.56</td>
<td>899.44 ± 103.57</td>
<td>0.092</td>
<td>920.63 ± 84.56</td>
<td>899.44 ± 103.57</td>
<td>0.092</td>
<td>920.63 ± 84.56</td>
<td>899.44 ± 103.57</td>
<td>0.092</td>
</tr>
<tr>
<td>ICAM-2 (U/ml)</td>
<td>462.61 ± 23.41</td>
<td>429.08 ± 39.70</td>
<td>0.647</td>
<td>466.44 ± 40.00</td>
<td>0.862</td>
<td>125.06 ± 9.25</td>
<td>124.92 ± 11.33</td>
<td>0.612</td>
<td></td>
</tr>
<tr>
<td>P-selectin (ng/ml)</td>
<td>134.32 ± 9.48</td>
<td>107.69 ± 11.62</td>
<td>0.862</td>
<td>125.06 ± 9.25</td>
<td>124.92 ± 11.33</td>
<td>0.612</td>
<td>111.17 ± 8.63</td>
<td>108.04 ± 10.54</td>
<td>0.922</td>
</tr>
</tbody>
</table>

TSM: time since menopause; BMI: body mass index; *Statistical difference (p = 0.004) at baseline mean levels between the two groups.
References


Are alanine, cysteine, glycine and valine amino acids the cause of non-immune hydrops fetalis?

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Summary

Our objective was to measure amniotic fluid amino acid concentrations in pregnant women diagnosed as having fetuses with non immune hydrops fetalis in the second trimester of pregnancy. Twenty-three pregnant women who had fetuses with non immune hydrops fetalis detected by ultrasonography (non immune hydrops fetalis group) in the second trimester and 19 women who had healthy fetuses (control group) were enrolled in the study. Amniotic fluid was obtained by amniocentesis. The chromosomal analysis of the study and control groups was normal. Levels of free amino acids were measured in amniotic fluid samples using EZ: fast kits (EZ: fast GC/FID free (physiological) amino acid kit) by gas chromatography (Focus GC AI 3000 Thermo Finnigan analyzer).

The mean levels of alanine, cysteine, glycine and valine amino acids were found to be significantly higher in fetuses with non immune hydrops fetalis than in the control group (p < 0.05). The detection of significantly higher amino acid concentrations in the amniotic fluid of fetuses with a non immune hydrops fetalis in healthy fetuses suggests loss of amino acids from the fetus through capillary permeability or/and the lymphatic system through the amniotic fluid may contribute to the etiology of non-immune hydrops fetalis.

Key words: Non-immune hydrops fetalis; Amniocentesis.

Introduction

Hydrops fetalis is a medical condition characterized by an accumulation of fluid, or edema, in at least two fetal compartments. Immune and non-immune are two types of hydrops fetalis [1]. Hydrops is classified as non-immune hydrops fetalis (NIHF) when it occurs without evidence of isoimmunization. NIHF is an uncommon but serious disorder associated with an overall poor prognosis. There are more than 100 causes of NIHF, but cardiac and functional anomalies are a common cause. However the success rate of prenatally diagnosed NIHF in finding an underlying cause may be as low as 40% [2]. The aim of our study was to determine the amino acid concentrations of NIHF cases in amniotic fluid in the second trimester of pregnancy. We hypothesized that concentrations of amino acids may be a cause of the result of the other causes of NIHF.

Material and Methods

The study was performed at the Prenatal Diagnosis Unit of Dicle University Hospital and Adana Numune Research Hospital between January 2009 and June 2011. The study was approved by the Institutional Review Board and Ethics Committee of the university hospital, and written informed consent was obtained from all participants. All pregnant women who had a fetus with NIHF (n = 23, study group) in the second trimester were included in the study. Nineteen (n = 19, control group) women who attended our clinic and had abnormal triple screens indicating an increased risk for Down’s syndrome were included in the study as the control group. Detailed ultrasound (US) examination, fetal karyotyping, investigations for fetal cardiac malformations infections and genetic diseases should be performed for all cases.

Mean maternal age was 28.2 ± 1.0 years for the NIHF group and 29.0 ± 1.1 years for the control group. The mean gestational age at sampling was 19.1 ± 1.3 weeks for the NIHF group and 18.9 ± 1.0 weeks for the control group. Maternal body mass index was 24.3 ± 1.0 kg/m² in the NIHF group and 25.2 ± 1.0 kg/m² in the control group. Obese patients and those with any systemic or endocrine disorder were excluded from the study. All pregnancies were accurately dated by the last menstrual period and by first-trimester US investigation. Amniotic fluid samples were obtained by routine transabdominal amniocentesis and collected into 10-ml dry tubes. All amniotic fluid samples were free of blood contamination. All samples were immediately centrifuged at 3000 g for 10 min and stored at -20°C until assayed. Levels of free amino acids (histidine, leucine, isoleucine, methionine, phenylalanine, tryptophan, and valine, alanine, asparagine, aspartic acid, cystathionine, cysteine, glutamine, glycine, tyrosine) were measured in plasma and amniotic fluid samples using EZ: fast kits (EZ: fast GC/FID free (physiological) amino acid kit) by gas chromatography (Focus GC AI 3000 Thermo Finnigan analyzer, Milan, Italy; injection: Split 1:15 at 250°C; 2.5 μ; carrier gas: helium 1.5 ml/min (60 kPa) at 110°C; pressure rise: 6 kPa/min; oven program: 30°C/min from 110°C to 320°C, hold at 320°C for 1 min; Detector: FID at 320°C; intrvariability: 2.4%; intervariability: 3.2%). The results are reported as means ± SD. A t-test was performed for statistical analysis. The statistical relationship between the two variables was checked by Pearson correlation coefficients; a p value of less than 0.05 was considered to be statistically significant.

Results

Twenty-three women who had fetuses with NIHF were included in the study (non-immune hydrops fetalis group). NIHF was diagnosed by US and confirmed after delivery. Detailed US examination, fetal karyotyping, and investigations for fetal cardiac malformation infections...
were performed for all cases. All investigations were normal. Pregnancy was terminated in the NIHF group. The 19 control group women were submitted to amniocentesis because of abnormal triple screens indicating an increased risk for Down’s syndrome. None of the control group fetuses showed structural abnormalities in US at the time of amniocentesis and none had chromosome abnormalities. All patients in the control group gave birth to a healthy child. The rates of nulliparity, the mean maternal and gestational ages and body mass index at the time of amniocentesis did not differ significantly between the two groups ($p < 0.05$).

The mean concentrations of alanine (424.9 ± 290.7 vs 392.5 ± 83.1), cysteine (30.3 ± 19.1 vs 14.6 ± 5.5), glycine (322.1 ± 242.3 vs 209.0 ± 82.0) and valine (239.1 ± 69.8 versus 199.5 ± 109.8) amino acids were significantly higher in the NIHF group than in the control group ($p < 0.05$).

Discussion

The pathogenesis of NIHF remains unclear and depends on the underlying disorder. Reduction in osmotic pressure due to liver disease or nephropathy may be a cause. The common etiologies of NIHF include cardiogenic and chromosomal anomalies, viral infections, and fetal anemia [3].

The cardiovascular group includes structural abnormalities, arrhythmias, and myocardiopathies. The pathophysiology of structural cardiac anomalies leading to hydrops is high right atrial pressure, or volume overload, and right heart congestion resulting in increased central venous pressure, and heart failure leads to edema [4].

Hyproproteinemia and liver dysfunction can result in a low osmotic pressure causing leakage of fluid into the interstitium due to lower osmotic pressure of the intravascular compartment in NIHF cases [5].

The underlying mechanism for hydrops is still not clear. The link between a cause and mechanism that generates hydrops is not always clear. The fetus is at risk of interstitial fluid accumulation owing to great capillary permeability and lymphatic disorders [6].

We found that amino acid levels of alanine, cysteine, glycine, and valine amino acids in amniotic fluid were significantly higher in the NIHF group than in the control group suggesting that loss of amino acids from the fetus through capillary permeability or/and lymphatic system through the amniotic fluid may contribute to the etiology of NIHF. The causes of cardiogenic and chromosomal anomalies, viral infections and fetal anemia may be complicated by alanine, cysteine, glycine, and valine amino acid loss which might partly explain fetal morbidity and mortality.

This is a preliminary study on amniotic fluid amino acid concentrations conducted on a small patient series. We think that it would be beneficial to conduct further studies with larger groups to determine the amino acid levels of fetuses with non-immune hydrops fetalis.

References


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Screening of cervical cancer: 27 years experience in six Republics of Panama

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Summary

Between the years of 1983 and 2010, a program of screening of patients was conducted in six of the Republics of Panama, applying cytology, colposcopy, and directed biopsy when required. In the community of Pocri de Los Santos, a tour of 33 rural areas was included in 27 years of consecutive coverage. This allowed to confirm that application that screening was successful, resulting in initial, evolutionary, or final diagnoses. These were of extreme importance because they indicated the disease and its response to conservative treatments applied and to the fact that the cancer evolution was nil.

Key words: Cervical precancerous lesions; Colposcopy; Early diagnosis.

Introduction

The rural preventive tours for uterine cancer commenced in December 1983 on Isla Grande (Colon). Fifty-four women were examined and showed an oncogenic risk (O.R.) in 17% of the cases. Successively, 14 communities of the other six provinces of the Republic of Panama were covered and 2,998 women underwent 5,472 colposcopies, and cytology - histologic exams when needed.

The analysis of the group of patients considered as O.R. in an integral form was found in 47.8% of the cases, in comparison with 33.2% of the ones found in the community of Pocri de Los Santos, and the national average of Panama, which was 53.5% [1, 2].

The results were based on the evidence of the follow-ups included until 2010 and showed a success rate of 87% achieved with the treatments, which were predominantly conservative and localized.

The author considered the need to divulge at both a national and at an international level the statistics obtained in the present scientific study. The results included a low incidence of cervical uterine cancer risk and an evolution of zero for cancer in the annual monitoring completed with a disciplined assistance of the patients over the 27-year period.

Materials and Methods

During the 27 years of rural medical touring, 71 visits were made and a total of 2,998 women were evaluated, and 5,472 colposcopic studies were performed. Special attention was given to the population of Pocri de Los Santos. In 33 tours conducted until 2010, 898 women were examined. As expected, almost all the patients were objectively studied. Follow up was carried out annually and on occasion, bi-annually. Both colposcopy and cytology were contemporarily performed and supplied immediate information concerning the extent of lesion, where applicable. Biopsy was taken and analyzed when diagnostically suggested. All the results obtained were studied and gave initial information regarding initial, evolutionary, or final diagnoses until 2011 [1, 2].

Results

In Pocri de Los Santos, 298 (33%) patients were found with O.R. On the contrary, the results in the other groups of Panama, the O.R. was higher (53.5%). These findings reflected a low incidence of cancer risk in the Pocri population [3]. The results of the preventive methods applied are reported in Table 1.

Cytology confirmed that most common pathologies was HPV pure infection without dysplasic or neoplasic lesion in 36.5%, while the false negatives (negatives + inflammatory lesion) reached 57% (Table 2).

The atypical transformation zone (ATZ) was the most frequent, followed by the atypical re-epithelization zone (ARZ), leukoplakia (L), and condyloma (HPV), in 33.6%, 26%, 18%, and 17% respectively. The false negatives reached 21.5% (Table 3).

Table 1. — Diagnosis and O.R. according to the different lesions in the community of Pocri de Los Santos.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No</th>
<th>% O.R.</th>
<th>Percentage distribution in the population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical O.R.</td>
<td>62</td>
<td>21%</td>
<td>6.9%</td>
</tr>
<tr>
<td>Pure or alone HPV</td>
<td>189</td>
<td>63.4%</td>
<td>21%</td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>4</td>
<td>1.3%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Mild dysplasia / HPV</td>
<td>29</td>
<td>9.7%</td>
<td>3.2%</td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td>2</td>
<td>0.7%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Moderate dysplasia / HPV</td>
<td>5</td>
<td>1.7%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>1</td>
<td>0.3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Severe dysplasia / HPV</td>
<td>1</td>
<td>0.3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>CA in situ / HPV</td>
<td>3</td>
<td>1%</td>
<td>0.3%</td>
</tr>
<tr>
<td>CA invasive / HPV</td>
<td>1</td>
<td>0.3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1</td>
<td>0.3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Total</td>
<td>298</td>
<td>100%</td>
<td>32.9%</td>
</tr>
</tbody>
</table>
The biopsies performed were a total of 131. The most common pathology was pure HPV, followed by mild dysplasias associated with HPV, in 42.7% and 16% respectively. Here the false negatives reached 32.4% (Table 4).

Of the patients considered with cancer risk, the evolution in 82 cases is unknown, although most were treated with cryotherapy or cauterization. Twenty-three cases were pending review and 185 cases were followed up. Many of these cases were reviewed annually, reaching up to 29 check-ups. The achievements confirmed the elimination of the high incidence of the effect produced by the most important inducing agent, which was the papilloma virus, and its most frequent picture included was ATZ. Sixty-nine percent of treated cases corresponded to pure papilloma, 15% to clinical lesion, 14% to dysplasias, and 1% to cancers.

The treatments were conservative with cryotherapy, cauterization and/or topical application of medicaments [4]. Surgery was recommended in the cases that required it and were referred to their physicians who accepted the indication. Two cases of cancer in situ and one invasive cancer were referred to the Oncology Institute, whose diagnosis was made during one of the tours.

At the end of the study in 2010, healing was achieved in 87% of the patients, 6.5% showed persistence, 3.7% showed improvement, 1% showed recurrence, and 2% showed progression. It should be mentioned, that progression only occurred in the minor grades and the evolution to invasive cancer was zero. Regarding the cured cases, 35% of these were result spontaneous, as have also been reported by other researchers in other latitudes [5].

### Discussion

Although there are many opinions regarding an incurable HPV infection and its effects, this study differs considering both the progress reached in a systematic form and by the annual follow up of the same patients in 27 years of study.

The author of the present study did not vary the dynamics of care at any given time and did not hesitate to repeat treatments to achieve eradication of the lesion found in the following years. Also, the perseverance in personal attention allowed the author to confirm that this population remains as low risk of cancer, compared with the numbers found in the Republic of Panama, in all categories of pre-cancerous lesion, including HPV, mild, moderate, and severe dysplasia, associated or not with HPV, and cancer alone [6].

The local conservative treatments were characterized by the ease in the application, contained expense, reduced waiting time, mainly due carrying out the recommended follow-up every three months until healing was achieved [7].

A decrease of cervical cancer was seen in this decade and although the incidence of Papilloma Virus is maintained and has doubled its association with dysplasia, the response to cryotherapy has been a resounding success [8].

### Conclusions

The present study shows that local conservative treatment triggers the immunologic system and therefore, the antigen-antibody response, achieving the resistance to the papilloma virus infection; the acquired immunological response determines the relation between dysplasia and cancer [9]. The natural immunological response was found in 35% of the cases, and can only accepted as such, without considering the parameters required for a thorough investigation [10].

The epidemiological methodology is valuable considering the simultaneous application of colposcopy and cytology, as it reduces diagnosis time and treatment, and the follow up is carried out in a simple and disciplined form. Furthermore, being able to performed statistics demonstrates that the conservative local treatments are more than sufficient to eliminate the precursory lesions that
lead to cancer of the cervix, vagina, and vulva, whose costs are really insignificant, as they avoid the invading cancer which is more expensive [11, 12].

In conclusion, the author reaffirms that the perseverance, education and the will of paramedic and medical staff to perform studies, such as the present, with volunteers committed to this work, will maintain the achievement: "evolution to cancer: no case".

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Female genital mutilation in Greece

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Summary
The number of migrants and refugees with a female genital mutilation (FGM) living in Greece is rising. This study explores the characteristics and psychosexual issues of women with FGM who were examined in the 2nd Department of Obstetrics and Gynecology, University of Athens Medical School, Greece during the year 2009. The women were asked to fill out an anonymous questionnaire asking for demographic data, obstetric history, current complaints, and psychosexual problems. The results are presented and discussed, as FGM is a new reality for Greece. Healthcare providers have to familiarize themselves with issues related to FGM and improve their skills in transcultural care, so as to manage and support women with FGM adequately.

Key words: Female genital mutilation; FGM; Migration; Infibulation; Greece; Migrants.

Introduction
Female genital mutilation (FGM) is a procedure involving partial or total removal of the external female genitalia, as well as injury of the female genital organs for non-medical reasons [1]. FGM is practiced in about 28 countries in Africa and the Middle East by Muslims, Animists, Atheists, Catholics, Protestants, Orthodox Copts and others [2]. Of all girls and women 80 to 90% have undergone FGM in Egypt, Ethiopia, Eritrea, Gambia, Mali, Sierra Leone, Somalia and Sudan [3]. According to the World Health Organization (WHO), it is estimated that 100 to 140 million girls and women worldwide are presently living with FGM. The United Nations Children Fund (UNICEF) raises this number to over 150 million. It is also estimated that three million girls are at risk of FGM every year [4].

The first ever reference of FGM appears in an old illustration from ancient Egypt [5, 6]. Although the ritual is older than Christianity and Islam, the Koran does not refer to it. However, a number of “Hadith” (statements by the prophet Mohammed) make reference to FGM [7].

The increasing numbers of migrants and refugee resettlements in Europe mean that the respective national societies are becoming more culturally diverse. Much of this recent adjustment is related to migration of sub-Saharan African populations. Greece, similarly to other Mediterranean countries is the final destination or an ordinary route for African migrants to Europe. Thus more and more Greek gynecologists face cases with FGM due to the progressively increasing rates of migration.

The aim of this prospective study was to explore and analyze the effects and consequences of FGM in women that visited our department and address relevant issues. It is expected that the management of these cases by healthcare providers, and above all gynecologists, will be improved as they will start better understanding better this group of women and their needs.

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Methods
This is a prospective study that was undertaken from January 2009 to December 2009 by the 2nd Department of Obstetrics and Gynecology at Aretaion Hospital in Athens. An anonymous questionnaire was given to women with FGM who attended the family planning clinic.

The participants were informed of the objectives of the study and filled the questionnaire on a voluntary basis. The questionnaire collected information regarding sociodemographic data, extent of knowledge concerning the FGM procedure and type of mutilation, obstetric history, and long-term emerging problems such as sexual dysfunction, psychological and other problems currently being faced.

Moreover, all patients underwent a routine gynecological examination. The questionnaires and the gynaecological exam findings were transcribed and analysed with the help of Excel software (Microsoft Office Excel 2007 1.0).

Results
The sociodemographic characteristics of women with FGM are presented in Table 1 and the psychosexual issues and complications in Table 2. Seven out of 11 women with FGM that visited our gynecology outpatient department accepted to be included in the study and completed the anonymous questionnaire. The median age of the patients was 24.7 years old (19-31 years old), while the median age that FGM was performed was 4.9 years old (3-8 years old). The majority of women were married (86%) and were examined by an obstetrician for first time during their pregnancy. One, four and two women from the study group had undergone FGM type 1, 2 and 3 respectively. None ever experienced orgasm during sexual intercourse. Additionally all of them face psychological problems as shown in Table 2.

For the theoretical question if they would ask for rebuilding in case of vaginal delivery, three out of seven responded positively and four negatively. Additionally all women stated that they had difficulty in accessing the family planning clinic as relevant information was not readily available to them.
Table 1. — Sociodemographic characteristics of women with FGM.

<table>
<thead>
<tr>
<th>Woman</th>
<th>Nationality</th>
<th>Religion</th>
<th>Age (years)</th>
<th>Type of FGM</th>
<th>Age at first sexual intercourse</th>
<th>Pregnancy/mode of delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Ethiopian</td>
<td>Muslim</td>
<td>19</td>
<td>3</td>
<td>19</td>
<td>No/No</td>
</tr>
<tr>
<td>B</td>
<td>Ethiopian</td>
<td>Muslim</td>
<td>24</td>
<td>5</td>
<td>23</td>
<td>Yes/CS</td>
</tr>
<tr>
<td>C</td>
<td>Egyptian</td>
<td>Christian</td>
<td>31</td>
<td>6</td>
<td>28</td>
<td>Yes/CS</td>
</tr>
<tr>
<td>D</td>
<td>Egyptian</td>
<td>Muslim</td>
<td>26</td>
<td>5</td>
<td>25</td>
<td>Yes/CS</td>
</tr>
<tr>
<td>E</td>
<td>Eritrean</td>
<td>Muslim</td>
<td>25</td>
<td>3</td>
<td>25</td>
<td>Yes/CS</td>
</tr>
<tr>
<td>F</td>
<td>Ethiopian</td>
<td>Muslim</td>
<td>25</td>
<td>8</td>
<td>24</td>
<td>Yes/CS</td>
</tr>
<tr>
<td>G</td>
<td>Somali</td>
<td>Muslim</td>
<td>23</td>
<td>4</td>
<td>23</td>
<td>Yes/CS</td>
</tr>
</tbody>
</table>

Table 2. — Complications and psychosexual issues related to FGM.

<table>
<thead>
<tr>
<th>Woman</th>
<th>Complications</th>
<th>Psychological problems</th>
<th>Organs experienced?</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Dysuria, pyelonephritis, dyspareunia</td>
<td>Nightmares</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>Dysuria, dyspareunia</td>
<td>Depression</td>
<td>No</td>
</tr>
<tr>
<td>C</td>
<td>Dysuria</td>
<td>Fear of the future</td>
<td>No</td>
</tr>
<tr>
<td>D</td>
<td>No Depression</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>No Depression</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Dyspareunia</td>
<td>Fear of the future</td>
<td>No</td>
</tr>
<tr>
<td>G</td>
<td>Dyspareunia</td>
<td>Fear of the society</td>
<td>No</td>
</tr>
</tbody>
</table>

The women were also asked to make free comments at the conclusion of the questionnaire concerning the procedure and its effects on their lives. Woman A referred to the procedure as: “Until today, I have nightmares of the old woman and the cutting”. Woman B described: “I can never forget the bleeding and being left alone outside my village for ten days”. Woman C said: “I will never give my consent to perform such a cutting on my daughters”. Woman D: “This is the most horrible moment of my life, I have no sexual pleasure, I feel humiliated”. In contrast, two women with type 2 FGM had different opinions as shown on their free comments. In particular, woman E stated: “I could not manage to have a good marriage without the cutting; it is a tradition in my country”. Woman F said: “The cutting is promoted by our religion, as it is believed that the clitoris is controlled by the devil”.

According to the vaginal examinations that took place during their visit to the family planning clinic, the classification of the FGM type agreed with the type stated by each woman.

Discussion

According to our knowledge this is the first study from Greece exploring FGM from the patient’s perspective. FGM is carried out as a part of a tradition in girls aged between birth (7 days) and pre-adolescence (10-12 years), always before the first menstruation and marriage [8]. The practice of FGM is spread through the generations by mothers and older women who prepare their daughters for adulthood and marriage. The causes of FGM include among others tradition, cultural ideals of femininity and modesty, aesthetic and hygienic reasons, proper sexual behavior, premarital virginity, protection of the family honor, marital fidelity, increase of fertility, increase of male sexual satisfaction and even fear of sudden newborn death caused by contact of the fetus with the clitoris at the time of birth [1, 8].

According to the current classification by the WHO (World Health Organization), FGM is classified into four types as shown in Figure 1 [1, 8-10]. The removal of the clitoris and the labia minora (type 2) is the most frequent form, amounting to 80% of all cases. The most extreme form, about 15% of all cases, is infibulation (type 3). Furthermore, defibulation describes the opening of an infibulation, whereas refibulation describes the close (restitching) of a previously opened infibulation at the time of vaginal birth.

FGM can lead to direct medical or psychological complications [11]. The complications in this group are analogous to those described in the literature, with two patients referring severe hemorrhage from the site of operation that made them feel weak, dizzy, and holding their breath for weeks. Other immediate complications of the study group include shock and severe pain, particularly if FGM was carried without analgesia (Figure 2). Moreover in our study three women complained of dysuria that had not resolved yet. Other chronic long-term consequences described in the literature include cysts, citoral neura, recurrent bladder and urinary tract infections, renal calculus, kidney damage, incontinence, uterus and oviduct infections, painful menstruation (caused by partially obstructed blood flow), infertility and abdominal cavity infection [10].

Sexual intercourse may be painful throughout life and orgasm may be difficult to achieve. None of the women in our study reported any experience of orgasm and four out of seven women had dyspareunia. The pelvic examination is also difficult for a woman who has undergone FGM, particularly for types 2 and 3. The use of a speculum is determined by the size of the introitus, and in many cases a pediatric speculum is preferable. In a group of 137 women who had undergone different types of FGM there were significant differences between the study group and an equivalent group of controls in desire, arousal, orgasm and satisfaction [12].

During birth, most inflitulated women need to be cut open (defibulation). Problems during labor and birth are prolonged second stage of labor, increased perinatal mortality, difficulties with vaginal assessment for progress of labor, bladder catheterization, perineal lesions, fistulas, and post-partum hemorrhage [10, 13-15]. Although no difference in cesarean section rates have been reported for women with FGM in many countries [16], women with FGM living in Greece have an increased risk for cesarean section due to obstetricians’ unawareness of the condition and the fear of handling women with FGM. The increased cesarean section rate for cases with FGM in Greece is in accordance with rates reported in Germany and Norway [17, 18]. Some women get stitched (closed) again after vaginal birth in their home countries.
In this study three out of seven women responded positively to the relevant hypothetical question. However, there are guidelines such as those of the Royal College of Obstetricians and Gynecologists (RCOG) stating that women with FGM should not be refertilated after delivery [19]. The procedure itself (refertilation) is often described by many women as traumatic. Emotional effects include anxiety, fear, depression, sleeping and eating disorders, mood disorders, impaired cognition, inferiority feelings, panic attacks, and post-traumatic stress disorder [11]. Women with FGM report chronic irritability and nightmares. They have a higher risk for psychiatric and psychosomatic diseases [11].

To date there are no published data or evidence that FGM is practiced in Greece. Some countries have criminalized FGM with new laws and legislation. The European Council and the European Parliament have specifically condemned FGM and demanded the commitment of the member states to eradicate this practice as FGM violates the human right to physical integrity, health and equality [20, 21]. The legislation in Greece does not specifically focus on FGM practice by gynecologists or other health professionals. However a doctor in Greece would not practice FGM or refertilation, even if asked, as he would be punished under the relevant Greek penal law that prevents corporal injuries [22].

The increasing number of immigrants or refugees from countries practicing FGM raises some concerns in the countries of the European community, as these women constitute a relatively new maternity client group. In Greece, the first women with FGM were recorded in 2003, as reported by healthcare professionals [23].
Female genital mutilation in Greece

A recent study showed that there is a lack of knowledge among gynecologists about FGM in Western societies including its classification, the provision of care and legislation [24]. Only 58% of healthcare professionals in a University teaching hospital in the United Kingdom were able to list the categories of FGM; 47% of them incorrectly thought that cesarean section was the best way to manage the delivery of patients with FGM. Lack of relevant experience and appropriate training for healthcare providers can lead to inadequate treatment, for instance to an unnecessary cesarean section, due to ignorance of the defibulation technique [25]. Healthcare professionals should be able to provide reasonable medical and psychological support, as well as an understanding of the motives and attitudes of women towards FGM. They also need to know the medical facts and treatment possibilities as well as the legal background.

To this end regular education and counselling sessions should be organized and supported by local scientific societies. Greek gynecologists should be educated further and change their attitude towards this group of women. There is a need for a transcultural approach which is closely linked to the identity of the migrants or refugees. The moral commitment of healthcare professionals should be to avoid these traditional practices which imply discriminatory, violent, degrading and painful treatment towards women. Patients with FGM should be encouraged to seek gynecological counselling in family planning clinics. Specialized pediatric and adolescent gynecologists and pediatricians should be made aware of the problems and participate when necessary. As there are no guidelines for FMG in Greece (concerning gynecology and obstetrics), the care of women is based on the gynecologist’s experience as to what might constitute the best practice for the case. However the gynecologist in charge may lack experience. Other European countries have issued guidelines and the procedures are standardized [26, 27]. Similar guidelines should be issued in Greece, after the necessary adaptation.

Furthermore there is a need to change and intensify laws in Greece in order to limit any FGM procedures in the future. New legislation should be introduced aiming to prevent FGM. According to the findings of this study, it is important that the education of Greek healthcare professionals provides understanding of FGM and knowledge of the existing Greek law. Till now, no FGM courses are scheduled for doctors, midwives or medical students in Greece. The Greek Family Planning Association and the Advanced life Support in Obstetrics Greek group are going to motivate and promote a dialogue with health professionals and families.

Conclusion

In recent years there has been a growing concern about the problems related to FMG in the Greek society. FGM refers to a spectrum of actions from very minor cutting to more significant procedures. A team approach to affected women and families by obstetricians and gynecologists, pediatricians, midwives and psychologists with specialist training in FGM-related issues are necessary. The adoption of clinical guidelines is recommended. Actions from the Greek state and Europe should aim to put an end to...
this brutal tradition, especially to the second generation migrants of the FGM practicing communities in Greece. Improved access to the services for women and families, information leaflets, and help groups will create additional opportunities for change.

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References


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The effect of sorafenib in postoperative adhesion formation in a rat uterine horn model

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Summary

Objective: Postoperative adhesions are a serious problem. In this study, we aimed to observe the effects of sorafenib in postoperative adhesions and, to examine the effects of sorafenib on tissue levels of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Material and Methods: Twenty female Wistar albino rats were randomized into two equal groups; sorafenib group (sorafenib treated) and control group; then all rats underwent laparotomy. Adhesions were developed by scalping on the anti-mesenteric surfaces of the right uterine horns. After 14 days, adhesions were investigated by using macroscopic, histopathological and immunohistochemical (for VEGF and PDGF) methods. Results: The sorafenib group had lower scores of total adhesions [1 (0-2.5) vs 1.5 (1-4); p: 0.037], staining of VEGF [1 (0-1) vs 1 (1-3); p: 0.029] and PDGF [1 (0-2) vs 2 (1-3); p: 0.006], and vascular proliferation [1 (0-2) vs 2 (1-3); p: 0.038] than the control group. Conclusion: The findings of the present study show that sorafenib, a tyrosine kinase inhibitor, significantly reduced postoperative adhesion formation. This effect may be explained by inhibition of VEGF, PDGF, and thus vascular proliferation.

Key words: Rat; Uterus; Adhesion; Sorafenib; VEGF; PDGF; Angiogenesis.

Introduction

Intraabdominal adhesions after surgery lead to substantial problems. Infertility can be added to these in women, unlike men. It was reported that early formation of adhesions is associated with size of angiogenesis [1]. Regulation of new vessel formation provided by growth factors and angiogenesis inhibitors is the basis of angiogenesis. Fortunately infertility due to adhesions can be solved with assisted reproductive techniques today, but abdominal pain and bowel obstruction still continue to be a problem.

Sorafenib, known as a tyrosine kinase inhibitor, inhibits a wide range of kinase targets in addition to the vascular endothelial growth factor receptors (VEGFR-1, -2, and -3) [2]. The VEGF family consists of five proteins (VEGF-A, -B, -C, -D, and placental growth factor) and signaling is mediated by the binding of these growth factors to three receptors. The binding with receptor affects some biological functions such as proliferation, migration, and morphogenesis of endothelial cells [3, 4].

After sorafenib (30 mg/kg) administration, expression of VEGF significantly decreased on experimental choroidal neovascularization in the rat [5]. The role of VEGF has been demonstrated in wound repair and tissue remodeling through its effect on fibroblast function [6]. Most adhesions containing unwanted fibrous bands occurred between the two deperitonealized surfaces, as wound healing. The effect of sorafenib is not confined only to a potent inhibition of angiogenesis, but also to tyrosine kinase inhibition which could have multiple effects on several crucial mechanisms in adhesion formation. The aim of this study was to evaluate the effects of sorafenib on adhesions in a uterine horn model.

Materials and Methods

Twenty female Wistar albino rats (10-12 weeks old; weight 230-290 g) were used. They were housed five animals per cage with the appropriate diet and water ad libitum. All rats were observed for several days to ascertain health before operations. All procedures were approved by the local animal care and use committee and performed under their guidelines. One day prior to surgery, the rats were randomly treated with sorafenib (30 mg/kg in 0.5 ml saline) or 0.5 ml saline only. The dose of sorafenib was selected based on a previous study [5]. Rats were anaesthetized by intraperitoneal administration of 60 mg/kg ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert Iac Sanayi, Levent, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloric acid (Rompun, Bayer Sisli, Istanbul, Turkey). Before surgery, the abdominal skin was shaved and antisepsis was obtained with 10% povidone iodine solution. Using a sterile technique, a 3 cm ventral vertical incision was made to expose the reproductive organs. Puncture serosal hemorrage was generated by scraping with a scalpel blade (No: 15) until petechial bleeding emerged at the abdominal sidewall and antimesenteric surface of the right uterine horn to create adhesions (Figure 1A). This model was based on a previous postoperative adhesion formation of a rat uterine horn study [7]. The midline incision was closed after completion of the procedure. Both groups were treated daily by orogastric gavage at the same dose for ten days. On postoperative day 14 animals were sacrificed by a high dose of anesthesia. A transverse subcostal incision was made above the cephaled extent of the midline laporo-

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The effect of sorafenib: Incidence of multiple pregnancy score of 3 in the control group.

The extent and severity of adhesions in the operation site for each uterine horn were evaluated according to the criteria of Linsky et al. [8]. The extent of adhesions was graded as follows: 0; no adhesion, 1; 25% of traumatized area, 2; 50% of traumatized area, 3; total involvement. Scores were recorded by an investigator blinded to the treatment group. The severity of adhesions was graded as follows: 0; no resistance to separation, 0.5; some resistance (moderate force required), 1; sharp dissection needed. Total adhesion score (TAS) was recorded as arithmetic sum of severity and extent of adhesions. The investigators scoring the adhesions were blinded to which group the rats belonged.

Figure 1. — A) Petechial bleeding of uterine horn to create adhesions. B) Adhesion free rat in the sorafenib group. C) Total adhesion score of 3 in the control group.

Table 1. — Histological adhesion score [23].

<table>
<thead>
<tr>
<th>Group</th>
<th>Severity score</th>
<th>Extent score</th>
<th>Total adhesion score</th>
<th>PDGF staining score</th>
<th>VEGF staining score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>0.5 (0-1)</td>
<td>1 (1-3)</td>
<td>1.5 (1-4)</td>
<td>2 (1-3)</td>
<td>1 (1-3)</td>
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<tr>
<td>Sorafenib (n = 10)</td>
<td>0 (0-0.5)</td>
<td>1 (0-2)</td>
<td>1 (0-2.5)</td>
<td>1 (0-2)</td>
<td>1 (0-1)</td>
</tr>
<tr>
<td>p*</td>
<td>0.054</td>
<td>0.055</td>
<td>0.037</td>
<td>0.006</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Table 2. — Adhesion scores and staining with VEGF and PDGF of control versus sorafenib treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Severity score</th>
<th>Extent score</th>
<th>Total adhesion score</th>
<th>PDGF staining score</th>
<th>VEGF staining score</th>
</tr>
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<tbody>
<tr>
<td>Control (n = 10)</td>
<td>1 (1-1)</td>
<td>1 (0-3)</td>
<td>0 (0-2)</td>
<td>0 (0-1)</td>
<td>2 (1-3)</td>
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<tr>
<td>Sorafenib (n = 10)</td>
<td>1 (0-1)</td>
<td>1 (0-3)</td>
<td>0 (0-2)</td>
<td>0 (0-2)</td>
<td>1 (0-2)</td>
</tr>
<tr>
<td>p*</td>
<td>0.146</td>
<td>0.813</td>
<td>0.687</td>
<td>0.861</td>
<td>0.308</td>
</tr>
</tbody>
</table>

Table 3. — Histological scores of control versus sorafenib treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Severity score</th>
<th>Extent score</th>
<th>Total adhesion score</th>
<th>PDGF staining score</th>
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<td>0 (0-2)</td>
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</table>

Phosphate-buffered saline was used for rinsing between each step and finally all sections were counterstained with Mayer’s hematoxylin.

For VEGF and PDGF antibodies, staining density and severity were evaluated in the areas where the stained cells were mostly found in both adhesion positive and normal tissues. Results were evaluated as follows: 0 = no staining, 1 = mild, 2 = moderate and 3 = strongly positive (Figure 2C-F).

Statistical analysis

Data were presented as median (min-max). Mann-Whitney U test was used to compare scores of adhesion extent, adhesion severity, total adhesions, inflammation, fibroblastic activity, foreign body reaction, collagen formation, vascular proliferation, and staining of VEGF and PDGF between groups. Spearman’s test was used for correlation analyses; a p value < 0.05 was considered as significant.
The effect of sorafenib in postoperative adhesion formation in a rat uterine horn model

Results

The adhesion model was performed on 20 rats. There was no intraoperative or postoperative mortality. Wound infection developed in one of the control group animals. Two of ten (20%) rats in the sorafenib group were completely adhesion-free (Figure 1B). All control rats developed intraabdominal adhesions (Figure 1C). There was no significant difference between the two groups for adhesion severity score and extent score. However total...
adhesion score was statistically significantly lower in the sorafenib group than in the control group (p < 0.05) (Table 2). Staining with VEGF and PDGF of the sorafenib treated group was significantly lower than the control group (p < 0.05) (Table 2).

When inflammation, fibroblastic activity, foreign body reaction and collagen formation scores of the controls were compared to the sorafenib group, no significant difference was found between groups (Table 3). However the vascular proliferation score was statistically significantly lower in the sorafenib-treated group than in the control group (p < 0.05) (Table 2 and Figure 2A,B).

Remarkable results of the correlation analysis are mentioned below. There was a highly significant and positive correlation between vascular proliferation and total adhesion scores in both the control group and sorafenib group (r: 0.789; p: 0.007, r: 0.805; p: 0.005, respectively). There was a highly significant and positive correlation between vascular proliferation and staining of VEGF and PDGF in the sorafenib group (r: 0.791; p: 0.006, r: 0.750; p: 0.012). There was a highly significant and positive correlation between VEGF staining and PDGF staining in the sorafenib group (r: 0.791; p: 0.006).

Discussion

In this randomized prospective double-blind experimental study, the effects of sorafenib on the formation of intraperitoneal adhesions were assessed in a rat uterine horn model by both visual scores and histological analyses of adhesion. Findings of the study have shown that sorafenib is effective in preventing adhesion formation in an animal model.

In the past 30 years, after the discovery of vascular proliferation factors, angiogenesis has become one of the most intensively studied fields. Inflammation, fibrolysis, angiogenesis and tissue remodeling are central to peritoneal wound repair and adhesion formation. Growth factors have a major role in adhesion formation and angiogenesis [10]. Peritoneal sclerosis rat models studied by an immunohistochemical method showed that VEGF is increased in thickened peritoneum [11]. Similar to this study, Arıtaş et al. demonstrated that VEGF staining in normal areas was significantly low compared with the adhesion-positive areas in the control and sham groups of a cecal abrasion model study [12]. Park et al. found that after sorafenib (30 mg/kg) administration, expression of VEGF was significantly decreased, however, they could not find any statistically significant difference in PDGF levels between treated groups and controls in a choroidal neovascularization study [5]. However VEGFR and PDGF receptors (PDGFR) are major targets of sorafenib [13]. On the other hand, in our study both VEGF and PDGF were significantly less stained in the sorafenib group. In addition a highly significant and positive correlation was demonstrated between VEGF staining and PDGF staining.

The PDGF family (PDGFA, -B, -C, and -D) bind to two different receptors, known as PDGFR-a and -b [14]. PDGFs are important for maturation and stability of the vasculature [15]. In our study, we also found a positive correlation between vascular proliferation and staining of VEGF and PDGF in the sorafenib group. The small-molecule tyrosine kinase inhibitors sunitinib and sorafenib target the VEGFR and PDGFR (primarily VEGFR), and have shown clinical efficacy in diverse cancer types [13, 16, 17].

Jonathan et al. found that the mean microvessel density was slightly lower in the sunitinib-treated animals compared with the control animals, although this difference was not significant [18]. We did not study microvessel density, but the vascular proliferation score was significantly lower in the sorafenib-treated group than in the control group. There were statistically significant fewer adhesions in the sorafenib group than the control group in our study. The results of our study are similar to the study of Jonathan et al. [18] and Kim et al. [19], where they studied the effect of sunitinib on postoperative adhesions in rabbits and mice, respectively.

Bevacizumab is the first angiogenesis inhibitor that was clinically approved. In experimental studies that investigated the effect of bevacizumab on intraperitoneal adhesions, it was demonstrated that intraabdominal administration of bevacizumab diminished intra-peritoneal adhesions [20-22]. In addition, significantly lower staining with VEGF was demonstrated by studies [20, 21]. The results of our study are similar to these studies.

In conclusion, our results demonstrated that sorafenib had a positive effect on prevention of postoperative adhesions. This effect could be through inhibition of vascular proliferation via VEGF and PDGF.

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The effect of sorafenib in postoperative adhesion formation in a rat uterine horn model


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The efficacy of dinoprostone vaginal insert for active management of premature rupture of membranes at term: a randomized controlled trial

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Summary

**Purpose:** To evaluate the efficacy and safety of the vaginal insertion of dinoprostone in terms of achieving cervical ripening, shortening the length of labor, and lowering the cesarean delivery rate for term pregnancies complicated with premature rupture of membranes. **Methods:** A prospective, randomized, controlled trial enrolled 100 women with term pregnancies complicated with premature rupture of membranes. Each had a normal non stress test, unscarred uterus, a singleton pregnancy with cephalic presentation, and a Bishop score of less than 4. Patients were randomized to receive a 10 mg dinoprostone vaginal insert single dose or no medication. After cervical ripening, oxytocin induction was performed during labor for both the study and control group. Cervical ripening in the 12th hour, total delivery time and delivery mode were compared between the two groups. **Results:** More often cervical ripening was obtained in the study group women who used dinoprostone vaginal inserts compared to the control group (p: 0.001). Latent phase of labor and total delivery time was shorter in the study group women than the control group (p: 0.022 and p: 0.026). There was no difference in terms of delivery mode and indication of section between study and control groups. **Conclusion:** The use of dinoprostone vaginal inserts in patients with term pregnancy of premature rupture of membranes reduced both the latent phase of labor and total delivery time without increasing the rate of cesarean section.

**Key words:** Premature rupture of membranes; Dinoprostone vaginal insert; Cervical ripening; Labor induction.

Introduction

Premature rupture of membranes (PROM) is defined as rupture of membranes that occurs at term before the onset of labor. It occurs in 8-10% of pregnancies. The most significant risk of PROM is intrauterine infection, which increases with duration of rupture. The optimum management of term pregnancies with PROM and an unfavorable cervix is controversial. Management options are induction of labor or expectant care. Several reports showed that expectant management increased maternal and neonatal morbidity [1, 2]. Active management yields to a shorter interval from PROM to delivery, reducing the risk of maternal and neonatal infection, without changing the rate of cesarean delivery [1].

The dinoprostone vaginal insert has been shown to be safe and efficacious in promoting cervical ripening in women with term pregnancies and low Bishop scores. However, data related to the efficacy and safety of dinoprostone in term pregnancies complicated with PROM are scarce.

Therefore, the purpose of the study was to compare the efficacy and safety of vaginal insertion of dinoprostone and expectant management in terms of achieving cervical ripening, shortening the length of labor, and lowering the cesarean delivery rate in term pregnancies complicated with PROM.

Material and Methods

This prospective randomized controlled study was carried out at Izmir Ataturk Training and Research Hospital, 1st Gynecology and Obstetrics Department, from May 2009 to December 2009.

Women with prelabor rupture of amniotic membranes with the clinical decision to induce labor were asked to participate. The inclusion criteria were: a live singleton fetus at term (37-42 weeks of gestation), cephalic presentation, a reactive non stress test (NST), presenting with PROM and Bishop score of 4 or less. Women in active labor or with abnormal fetal heart rate, malpresentation, estimated fetal weight above 4,500 g, multiple pregnancies, cephalopelvic disproportion, more than four previous term pregnancies, with previous uterine surgery, antepartum hemorrhage, chorioamnionitis, and contraindication to prostaglandin use (bronchial asthma, glaucoma) were excluded from the study.

A detailed history was taken for all women followed. A dry aseptic speculum examination was performed to confirm that the membranes had ruptured. Then a sterile digital examination was carried out and Bishop score was calculated.

Randomization sequence was generated by a computerized random number generator in blocks of 8 and prepared by an investigator. The treatment allocation was placed into numbered, sealed envelopes each of which contained a piece of paper bearing the legend ‘dinoprostone pessary’ or ‘no treatment’.

The dinoprostone vaginal insert is a preparation of PGE$_2$ packaged in a hydrogel polymer matrix and designed for intravaginal release of 10 mg of dinoprostone at a rate of 0.3 mg/h over 12 hours. It was inserted into the posterior fornix of the study group women. The control women were followed spontaneously until effective uterine contractions began. Continuous cardiotocogram monitoring was maintained throughout the induction and labor. After cervical ripening was provided, oxy-
tocin infusion was started for both the study and control group women. The dinoprostone pessary was removed after a maximum of 12 hours. In the event of uterine hyperstimulation or a non reassuring cardiotocogram, cervical ripening or effective uterine contraction was achieved, the pessary was removed earlier.

Hyperstimulation was defined as more than five contractions per ten minutes or a contraction lasting at least two minutes. Cervical ripening was defined as Bishop score more than 9. Oxytocin infusion (5 mU oxytocin in 500 ml of Ringer’s lactate solution) was started at a rate of 2 mU/min and increased by 2 mU/min every 20 minutes. The contractions were considered effective if they reached a frequency of four per ten minutes for two consecutive 10-minute periods; the oxytocin dose was not increased further.

Antibiotic prophylaxis against Group B streptococcal infection was routinely administered in case PROM lasted for 18 hours or more; 2 g of ampicillin IV was started as a standard prophylactic regimen followed by 1 g ampicillin IV every four hours until delivery.

Primary outcomes were the duration of latent and active phase of labor and total delivery time, and the rate of cervical ripening at the 12th hour. The length of the latent phase was defined as the lapse of time to cervical dilatation reaching 4 cm. The length of the active phase was defined as the lapse of time from cervical dilatation equal to 4 cm to complete dilatation. Total delivery time was defined as the time from the beginning of the enrolment to delivery.

Statistical analysis was carried out using SPSS 11.0 for Windows (SPSS Inc, Chicago, IL, USA) statistical software. Categorical variables were described using frequency distribution and compared by chi-square and Fisher’s exact test. For continuous variables, descriptive statistics were calculated and reported as mean ± standard deviation. The Student’s t-test was used to compare mean scores of continued variables between the two groups; \( p < 0.05 \) was accepted as the level of significance.

Results

One hundred women were randomly assigned: 50 to dinoprostone pessary and 50 to expectant management. There was no significant difference between the study and control group women in terms of age, gestational age, body mass index, Bishop score, and the rate of multigravida. There was no distinction between study and control groups in terms of uterine hyperstimulation. The other reasons for earlier removal were achievement of effective uterine contractions in seven women and cervical ripening in 19 women. In women where the pessary was removed before the 12th hour, the pessary stayed in the vagina an average of 6.68 hours (range 1-11 hours). There was no difference between the groups in terms of uterine hyperstimulation.

There were no significant differences in mean birth weight, the rate of Apgar score at 5 min and the route of delivery.

Cesarean section was performed in three women of the study group. In two of these, hyperstimulation and fetal distress developed within the first hour after the pessary insertion, and then one women underwent cesarean section. The other women who developed hyperstimulation responded rapidly to removal of the pessary or stopping of the infusion. In one case of the control group, progress of labor arrested at 6-cm cervical dilatation. A healthy 3,900 g neonate was delivered by cesarean section. There were no maternal or neonatal adverse outcomes in either group.

More often cervical ripening was obtained in the study group women who used dinoprostone vaginal inserts than the control group. Latent phase of labor and total delivery time were shorter in study group women than controls.

Discussion

When rupture of fetal membranes occurs before the onset of labor, most cases go into labor spontaneously within 24 hours. Expectant management of term pregnancies with PROM and an unfavorable cervix could increase adverse events related to infection. Early induction of labor could shorten the delivery time and thus reduce the risk of maternal and fetal morbidity related to infection in term pregnancies complicated with PROM and low Bishop scores [2].

Previous studies showed that PGE2 is effective for the total delivery time to shorten labor in term pregnancies complicated with PROM. It improves success rates and reduces morbidity associated with labor induction [3]. Induction of labor with a PGE2 insert was also demonstrated to be successful in women with post-term pregnancies, without any serious complications. However the rate of cesarean section was increased [4].
Some studies have investigated the efficacy and safety of vaginal PGE_{2} inserts in term pregnancies complicated with PROM and low Bishop scores [5, 6]. In a multicenter study, 100 pregnancies with PROM and unfavorable cervices were compared to 180 pregnancies with intact membranes. All patients were treated with dinoprostone vaginal inserts [5]. The rate of labor within 24 hours in the PROM group was higher than in the intact membrane group. The rates of cesarean section, tachysystole or neonatal asphyxia did not differ in either group. In the term pregnancies not only with intact membranes but also with PROM, dinoprostone vaginal inserts were safe and efficient for cervical ripening and induction of labor.

Studies comparing PGE_{2} vaginal inserts with oxytocin for labor induction in pregnancies with PROM reported that oxytocin induction yielded a shorter latent phase of labor and total delivery time, while the rate of cesarean section remained unchanged [6].

In a retrospective cohort study, Park et al. compared use of dinoprostone and oxytocin for labor induction in term pregnancies with PROM and with intact membranes and unfavorable cervices in nulliparous women. They found that labor induction for PROM in term nulliparous women with an unfavorable cervix was associated with longer mean duration of second stage and a higher risk of cesarean delivery for failure to progress than those with intact membranes [7].

Concurrent oxytocin and prostaglandin for labor induction have been used both in pregnancies with PROM and pregnant with intact membranes. When comparing concurrent oxytocin and dinoprostone pessary versus dinoprostone pessary in labor induction of nulliparas with an unfavorable cervix, cesarean rate did not differ [8]. When a similar trial was done in women with PROM, no distinction was found in vaginal delivery and hyperstimulation rate between concurrent oxytocin and dinoprostone pessary versus dinoprostone pessary in labor induction of pregnancies with PROM [9].

In their retrospective non-randomized study, Larranaga-Azcárate et al. compared dinoprostone and expectant management of women with a single pregnancy at term, PROM, and Bishop test < 4. They had started oxytocin at the 12th hour after PROM. The duration from dilatation to labor was shorter and the rate of cesarean section was lower in the dinoprostone group than expectant management group [10]. Our study was planned as randomized and prospective. Oxytocin infusion was started when cervical ripening was provided. Hence, the rate of hyperstimulation was lower than concurrent use of dinoprostone and oxytocin treatment. Not performing power analysis was the limitation of this study.

**Conclusion**

Vaginal dinoprostone inserts following oxytocin infusion to induce labor in term PROM decreased the latent phase of labor and total delivery time without increasing cesarean rate compared with expectant management following oxytocin infusion.

**References**


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Impact of Factor V Leiden, prothrombin and methylenetetrahydrofolate reductase gene mutations on infant birth weight in women with recurrent fetal loss and women with successful pregnancies

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Summary

Purpose: The aim of this study was to verify whether FV Leiden, PT G20210A, MTHFR C677T or MTHFR A1298C mutations influence the risk of recurrent fetal loss in a sample of Turkish women who had experienced recurrent fetal loss and to evaluate whether the aforementioned thrombophilias and recurrent fetal loss may affect the birth weight of subsequent pregnancies. Methods: Fifty-eight women with recurrent pregnancy loss and 30 women with successful pregnancies were evaluated. Results: The average birth weights for infants of all women in the study group and for infants of thrombophilia-positive women in the study group were markedly lower than the birth weight of infants in the control group (p < 0.001 and p < 0.001, respectively). Conclusion: Successful pregnancies in women with a history of recurrent fetal losses may be associated with lower birth weights compared to controls, irrespective of thrombophilia status. This conclusion warrants further research.

Key words: Inherited thrombophilia; Birth weight; Pregnancy; Turkish population.

Introduction

Thrombophilias have been shown to be associated with fetal loss and adverse pregnancy outcomes [1]. Almost 5% of women experience two or more fetal losses during the reproductive period [2]. Inherited thrombophilias can cause thrombosis, vascular damage and fibrinoid necrosis of decidual vessels, all of which can result in adverse pregnancy outcomes such as miscarriage, preeclampsia, intrauterine growth restriction, placental abruption and stillbirth [3-5].

Thrombophilias usually emanate from single nucleotide polymorphisms. Two common polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene are MTHFR C677T and MTHFR A1298C, which can result in elevated homocysteine levels and vascular thrombotic events [6, 7]. Both of these two mutations have been found in women who experienced spontaneous abortions [8]. Factor V Leiden (FV Leiden) and prothrombin (PT) G20210A SNPs are the two most common causes of inherited thrombophilias [9].

Methods and Patients

Data for 90 women with a history of recurrent fetal loss (defined as three or more consecutive fetal losses) [4] were collected from the obstetric unit data bank. The thrombophilia status of all women had been determined previously. No women were detected to have antithrombin deficiency, protein S deficiency or antiphospholipid syndrome. Two women with protein C deficiency were excluded. The remaining 88 women were investigated about their subsequent pregnancy status. Fifty-eight of these women who conceived naturally and who did not develop adverse pregnancy outcomes (e.g., gestational hypertension, gestational diabetes mellitus) served as the study group. All birth weights are corrected for gestational age. No women were given any anticoagulant therapy because as yet there is no consensus report regarding the use of anticoagulants for recurrent fetal loss with inherited thrombophilia [10]. Additionally, more frequent appointments were applied for patients in the study group. Thirty women with a previous uneventful pregnancy, no history of pregnancy loss, no known history of familial thrombophilia who conceived naturally and gave full-term birth without any adverse pregnancy outcomes were selected as the control group. All patients provided informed consent. The local ethics committee approved the study. Genomic DNA was extracted from EDTA-treated whole blood using the spin-column method (MN Nucleospin Blood). FV Leiden (G1691A), PT G20210A, MTHFR C677T and MTHFR A1298C mutations were identified using the 5’ nuclease assay method. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, version 16.0) for Windows; p values < 0.05 were considered statistically significant.

Results

The mean ± SD age was 32.8 ± 4.7 in the study and 29.1 ± 3.6 in the control group (p < 0.05). Maternal weight gain was similar in both groups. Birth weight data was available for 58 patients in the study group. All birth weights after correcting for gestational age were found between the 10th and 90th percentiles. There were nine preterm births in the study group and there was only one low birth weight infant among term births in the study group. There were no preterm births and no low birth weight infants in the control group. All comparisons between groups were performed before and after exclud-
ing preterm births. Birth weight in the study group was significantly lower than that in controls before and after excluding preterm births \((p < 0.001\) and \(p = 0.001\), respectively). When thrombophilia-positive women in the study group were compared before and after excluding preterm births, mean birth weights were 3039 ± 436 g and 3166 ± 342 g, respectively, also significantly lower than that for the controls \((p < 0.001\) and \(p < 0.05\), respectively). Moreover, women with one or two thrombophilias tended to have lower weight infants in the study group than the controls. Maternal weight gain significantly but weakly correlated with birth weight in the study and control groups \((r = 0.206, p < 0.05\) and \(r = 0.135, p < 0.05\), respectively). The distribution of the mean ± SD birth weight in the study and control groups is summarized in detail in Table 1. Additionally, no difference between the number of thrombophilias \((n = 48)\) among women who gave birth in the study group and the control group \((n = 27)\) was found.

**Discussion**

There have been conflicting reports about recurrent fetal loss and inherited thrombophilia in the literature [11-13]. Among 68.9% of women with a history of three or more in-vitro fertilizations and embryo transfer failures at least one thrombophilia was detected, of which MTHFR C677T was the most common [14]. This mutation was also the most prevalent type of thrombophilia observed in our study. Nonetheless, recommendations for thrombophilia screening [15] do not include MTHFR C677T and MTHFR A1298C mutations. In this study, we include these two mutations to determine the frequency in our region.

Previous research by Nath et al. [16] reports that thrombophilia status is not the only cause of low birth weight infants, and that preterm births are the predominant factor. On the contrary, a cohort study reported that MTHFR C677T can be used as a marker to recognize pregnant women who are at risk for having fetuses that are small-for-gestational-age [17]. A recent meta-analysis found a notable association only between FV Leiden mutation and intrauterine growth restriction [18].

In our study, we found that the birth weights of infants born to women in the study group were significantly lower than the birth weight of the infants born to women in the control group. However, we could not link this difference either to preterm births or thrombophilas. Thus, it can be proposed that women with recurrent fetal loss tend to have lower weight infants in their subsequent pregnancy. Additionally, in our study, the mean age of the study patients was higher than that of controls. It is thought that age is an important factor influencing birth weight, but this hypothesis is controversial [19, 20]. It is also possible that the lower infant weight observed in infants born to women in our study group may be associated with paternal thrombophilia status [21], but this is beyond the scope of our study.

One of the limitations of our study is the relatively small population of the study group but our data is solid. Additionally, due to the limited number of patients in the study group it is not possible to make a strict conclusion. However, a substantially larger trial is needed to make a clear decision.

Although, it has been suggested previously that inherited thrombophilias may play an important role in recurrent pregnancy loss, inherited thrombophilias have not been accepted as the sole or adjunct cause of recurrent pregnancy loss or low birth weight infants. In this retrospective case-control study, we demonstrated that irrespective of a woman’s thrombophilia status, past history of recurrent pregnancy loss may be associated with lower birth weight infants in subsequent successful pregnancies. This conclusion warrants further research with prospective randomized trials.

**References**


<table>
<thead>
<tr>
<th>Thrombophilia</th>
<th>Study group</th>
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<th>(p)</th>
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<tbody>
<tr>
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<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>MTHFR A1298C</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>MTHFR C677T + MTHFR A1298C</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>PT G20210A</td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>FV Leiden</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
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<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>Thrombophilia positives</td>
<td>48; 3039 ± 436</td>
<td>27; 3377 ± 256</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Thrombophilia negatives</td>
<td>10; 2803 ± 293</td>
<td>3; 3163 ± 23</td>
<td>ns</td>
</tr>
<tr>
<td>All</td>
<td>58; 2998 ± 422</td>
<td>30; 3353 ± 253</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

BW: birth weight, MTHFR: methylenetetrahydrofolate reductase; PT: prothrombin; FV Leiden: Factor V Leiden; ns: non significant. * The total number of given birth weights for the study group was 58.


Effects of tamoxifen and raloxifene on body and uterine weights of rats in persistent estrus

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Summary

**Purpose:** To evaluate the change in body and uterine weights of rats in persistent estrus, a model developed to mimic polycystic ovary syndrome treated with selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene. **Methods:** Sixty Wistar-Hannover rats induced by a single subcutaneous dose of 1.25 mg testosterone propionate were divided into three groups of 20 animals: Group I (placebo); Group II (tamoxifen, 250 µg/day) and Group III (raloxifene, 750 µg/day). At 90 days of life, the treatment began for 30 consecutive days, in which the animals were weighed weekly. On the 31st day, the animals were sacrificed and the uterus removed. Data were analyzed statistically by analysis of variance and by the Tukey-Kramer multiple comparisons test ($p < 0.05$). **Results:** Means of body and uterine weights (g) after treatment were: 227.3 ± 2.20 and 0.40 ± 0.01; 185.3 ± 2.45 and 0.25 ± 0.01; 186.4 ± 2.20 and 0.27 ± 0.01 in Groups I, II and III, respectively ($p < 0.001$). There was no statistical difference between groups II and III for body and uterine weight ($p = 0.727$ and $p = 0.646$, respectively). **Conclusion:** The present results indicate that, at the doses and during the time of treatment used, both tamoxifen and raloxifene reduce in a similar way the body and uterine weights of rats in persistent estrus showing a possible antiestrogenic effect of SERMs under high levels of estrogens.

**Key words:** Animal models; Estrogen; Tamoxifen; Raloxifene.

Introduction

Selective estrogen receptor modulators (SERMs) are drugs that have attracted the attention of many investigators studying primary chemoprevention of breast cancer in women at high risk for the disease [1, 2]. Tamoxifen was the first drug to be approved in the United States for chemoprevention in women at high risk for breast cancer. However, long-term use of tamoxifen may lead to significant side-effects, especially endometrial carcinoma [3]. For this reason, there has been great interest in identifying other SERMs that may serve as an alternative to tamoxifen [4, 5].

Raloxifene, a second-generation SERM approved in the United States and in other countries for the prevention and treatment of osteoporosis in postmenopausal women, has been shown to exert an antiestrogenic action on the breast without, however, stimulating the endometrium [4]. This fact was confirmed in a recently published study of tamoxifen and raloxifene (STAR) trial, which showed raloxifene to be almost as effective as tamoxifen in reducing invasive breast carcinoma [6].

Little is known about the behavior of SERMs during chronic anovulation in which obesity and constant estrogenic stimulation are a common finding in these patients [7]. Due to ethical issues in humans, studies are required to be conducted in animal models. Female rats receiving steroids, particularly at birth, enter a condition of persistent estrus showing a possible antiestrogenic effect of SERMs under high levels of estrogens.

Therefore, in view of the scarcity in the literature, studies which assessed SERMs behavior on body and uterine weights in states of chronic anovulation and hyperandro-genism are scarce. Thus, to best of our knowledge, we have used a biological model mimicking states of chronic anovulation presenting excess of body and uterine weights to better understand tamoxifen and raloxifene behavior in these conditions.

Material and Methods

The study protocol was carried out in accordance with the ethical principles of the Colegio Brasileiro para Experimentação Animal (COBEA), also by a local Committee on Ethics and Animal Experimentation of the Federal University of Piaui (registers numbers 35/2010 and 54/2010). Sixty female, virgin Wistar-Hannover rats obtained from the animal laboratory of the Federal University of Piaui were used in this study. The animals were kept under controlled temperature (25°C) and lighting conditions (7:00 a.m. to 7:00 p.m.) in individual cages with free access to water and standard laboratory rodent chow (SUPRA-LAB, São Paulo, Brazil). Androgenization or persistent estrus was achieved in the animals by administering a single dose of subcutaneous injection of 1.25 mg of testosterone propionate diluted in 0.1 ml of corn oil on the second day of life. The state of persistent estrus was confirmed in the animals based on the presence of obliteration of the distal third of the vagina and on keratinization of the vaginal epithelium, the principal characteristic of persistent estrus [8, 10], on the presence of polycystic ovaries as observed during histology performed at the end of the study. The rats were randomly distributed into three groups of 20 animals each: Group I (persistent estrus, control) received only vehicle (propylene glycol), whereas Group II (persistent estrus) received tamoxifen and Group III (persistent estrus) received raloxifene.

The animals in Group I received 0.5 ml/animal/day of propylene glycol (placebo), while those in Group II received 250 µg/animal/day of tamoxifen diluted in 0.5 ml of propylene glycol...
Effects of tamoxifen and raloxifene on body and uterine weights of rats in persistent estrus

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and Group III received 750 µg/animal/day of raloxifene also diluted in 0.5 ml of propylene glycol. At 90 days of life, the treatment began for 30 consecutive days by gavage using specific metal tubing.

At the beginning of the treatment the animals were weighed weekly with a digital scale and with the aid of a plastic beaker up to the end of the treatment. On the 1st day after the treatment, the animals were sacrificed and the uterus was removed through a longitudinal abdominal incision and then weighed.

The ANOVA test made comparisons between groups. Post hoc pair wise comparisons between means in groups were conducted using the Tukey method with an overall significance level at 5% [11].

Results

The body and uterine weights of experimental animals treated with placebo (Group I) were significantly higher than the body and uterine weights (g) of animals treated with tamoxifen (Group II) and raloxifene (Group III) (p < 0.001). The mean of body weights of the control and experimental groups are listed in Table 1 and uterine weights are listed in Table 2. There was no statistical difference between Groups II and III for body weight (p = 0.727) (Figure 1) or for uterine weight (p = 0.646) (Figure 2).

Discussion

In the current study, female rats exhibiting persistent estrus were used to evaluate the effects of SERMs directly on body and uterine weights. Tamoxifen, administered at a dosage of 250 µg/animal/day, and raloxifene, administered at a dosage of 750 µg/animal/day, for 30 days, significantly reduced body and uterine weights compared to the group treated with placebo.

The metabolism of rats is faster than in humans, hence the weight-equivalent dose would constitute under-dosage and would not therefore mimic the serum and tissue concentration of SERMs in humans [12]. There are reports of experimental studies in the literature in which rats were given daily oral doses of raloxifene that varied from 1 to 10 mg/kg, without any toxicity for the animals. Therefore, the dosages in this study were chosen because they were sufficient to reproduce tissue levels of the drugs observed in adult women on using this medication [13, 14].

Chronic anovulation syndrome was induced and characterized by keratinization of the vaginal epithelium in this animal model [8, 10]. The adult animals develop characteristics that mimic PCOS in women such as chronic anovulation, sterility and polycystic ovaries [9, 15]. Furthermore, this model of rats in a state of persistent estrus manifested high food intake, elevated body weight and obesity [16, 17] associated with increased of hyperinsulinemia, which in turn is significantly correlated with the risk of cardiovascular diseases [18, 19] and seems to induce hyperandrogenemia [20]. Nevertheless, PCOS is a multifactorial syndrome and probably no animal model has the capacity to reproduce all aspects of this syndrome.
In previous reports, estrogen increased the production of leptin in ovariectomized rats, the ob gene product, which has an important role in regulating body weight [21, 22]. A study performed by Hozumi et al. [23] in ovariectomized rats mimicking postmenopausal women treated with tamoxifen, showed a significantly reduced weight gain, food intake, weight of adipose tissue and leptin concentration. In addition, these animals treated with SERMs become resistant to insulin [23]. Most of the additional effects of tamoxifen on lipid metabolism as changes in body weight and composition, or endometrium are likely to be due to estrogenic effects [24]. However, ovariectomized rats have low levels of circulating estrogen, while the influence of SERMs on adipose tissue under higher levels of estrogen remains unknown.

Our data is the first to evaluate the effect of raloxifene compared to tamoxifen in body mass in this animal model. Nevertheless, these data differ from the study of Patriarca et al. [25], which found no differences in body weight of rats in persistent estrus treated with tamoxifen compared to a control group, probably due to differences in dosages used in trials, with our concentration being more than double that used by the authors.

Similar to this study, Patriarca et al. [25] observed, due to an anti-uterotrophic effect, a reduction in uterine weight in animals receiving tamoxifen. After macroscopic examination of the uterus, lower uterine weights in animals treated with the antiestrogens were likely caused by the inhibitory effect of tamoxifen and raloxifene on fluid retention mediated by estrogen [25], resulting in decrease of mass in this organ.

Conclusion

In conclusion, use of 250 g of tamoxifen and 750 g of raloxifene in rats with persistent estrus significantly reduced body and uterine weights in this experimental model. Even though the results are limited in extrapolating the present study to humans, these findings strengthens the literature and have provided a wider perspective on further studies aimed to elucidate the tamoxifen and raloxifene effect on body and uterine weights in the presence of high circulating levels of estrogen.

References


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Diagnosis and management of cesarean scar pregnancy

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Introduction

Cesarean scar pregnancy (CSP), a very rare form of ectopic pregnancy, is located outside the uterine cavity and completely surrounded by myometrium in the prior lower uterine segment [1]. Because it often is misdiagnosed as cervical or aborting pregnancy, CSP may result in life treating conditions such as uterine rupture, hemorrhage, disseminated intravascular coagulation and even maternal death [1-3]. There is no accepted management protocol, so because of the rarity of this life-threatening disease, each patient should be evaluated individually [1].

The aim of this study was to evaluate the diagnosis and management modalities of CSP at our clinic.

CSP is more common than previously thought. It seems to be on the rise because of increased cesarean delivery rates as well an improvement in the clinical knowledge of clinicians and high clinical index of suspicion [2]. The estimated incidence of CSP ranges from 1:1,800 to 1:2,216 pregnancies [5, 6], but the true incidence has not been determined because so few cases have been reported in the literature [7].

The diagnosis of CSP is mainly accomplished by combining transvaginal sonography (TVS) with Doppler flow imaging [5, 7]. Once the correct diagnosis is made CSP should be terminated to avoid life-threatening complications [7, 8].

Materials and Methods

We searched and analyzed the medical records of patients admitted to our department with a diagnosis of cesarean scar pregnancy from January 2005 to March 2011. There were 12 hospital admissions with the diagnosis of CSP, but six of 12 patients had abortions when re-examined carefully later on.

The diagnosis of CSP was determined based on the following ultrasonographic criteria: 1) empty uterus; 2) empty cervical canal; 3) anteriorly located gestational sac with a diminished myometrium layer between the bladder and the sac; 4) discontinuity in the anterior uterine wall of the uterus on a sagittal view of the uterus after the gestational sac (Figure 1).

Ethical committee approval was given for the study.

Results

The mean maternal age was 32 (range 28-38 years). Five patients had undergone two previous cesarean sections and one patient had undergone four previous cesarean sections. The mean gestational age during the diagnosis was six weeks and two days (range 4 weeks and 4 days - 12 weeks). Fetal cardiac activity was present in only two

Figure 1. — Gestational sac in scar tissue (arrow 1); Empty appearance of uterine cavity (arrow 2).
of the pregnancies. The characteristics of the six patients are given in Table 1. Average gestational age of the patients was six weeks and two days. Fetal pulse of two of those had been detected with ultrasound. In the other four cases, fetal pulse had not been detected.

Clinical presentations were described as follows: mild vaginal bleeding and abdominal bleeding (one patient), mild abdominal pain (two patients) and vaginal bleeding (one patient) and asymptomatic (two patients). Asymptomatic patients were diagnosed during the routine first trimester ultrasonographic (US) screening.

After explaining all the treatment modalities, one patient chose the surgical option due to desire of tubal ligation. The gestational mass was excised and the uterine segment was repaired. During the operation the bladder was damaged due to adhesions and the defect was repaired with success. In the second case methotrexate (1 mg/kg IM, single dosage) was applied initially, but two weeks later suction curettage was applied due to abdominal pain and vaginal bleeding. There were no complications during or at the end of the curettage. Suction curettage was used as an initial treatment for four patients. There were no complications in three of four patients. One patient had heavy vaginal bleeding which started after curettage. On US examination, increasing hemorrhage was seen between the uterus and the bladder and subtotal hysterectomy was performed.

The results of the study were sent as an abstract to ASRM 2011.

**Discussion**

CSP is rare and often misdiagnosed as other diseases like miscarriage and cervico-isthmic pregnancy [3, 8]. What is noteworthy in our research is that before 2010 there had been no admissions to our hospital with this diagnosis. Moreover, after this date, the six patients who were prediagnosed with CSP had been thoroughly examined and checked by a US device with a strong resolution and either miscarriages or missed abortuses had been detected. This situation clearly shows we have information about CSP due to recent case reports and we acted in an over-sensitive manner every time we encountered a pregnant patient with a cesarean history.

Although CSP is rare, without a high index of suspicion and early diagnosis, this abnormal implantation can lead to uterine rupture, hemorrhage, serious maternal morbidity and loss of future fertility, and even maternal death [7, 9].

The invasion of the myometrium through a microscopic tract is the most probable mechanism of development of CSP. This microscopic tract may have occurred from trauma of previous uterine surgeries like cesarean section, myomectomy, dilatation and curettage (D&C), and manual removal of the placenta. However, the exact cause of CSD is unknown [1, 5, 9, 10].

CSP is more common than previously thought and it seems to be on the rise because of increase in cesarean delivery rates as well as improvement in the clinical knowledge of clinicians and high clinical index of suspicion [4]. The estimated incidence of CSP ranges from 1:1,800 to 1:2,216 pregnancies [5, 6], but the true incidence has not been determined because so few cases have been reported in the literature [7]. What attracted our attention is until the year 2010, there had not been any hospital admissions with the diagnosis of CSP. In contrast, after the year 2010, it was noted that six of 12 hospital admissions with the diagnosis of CSP were abortion or miscarriage cases after carefully being re-examined. This indicates that increased knowledge of CSP due to reported case reports led us to over-diagnose and be oversuspicious about pregnancies with prior cesarean deliveries.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Symptoms</th>
<th>Number of previous cesarean sections</th>
<th>GA (week)</th>
<th>Fetal viability</th>
<th>Initial management</th>
<th>Complications</th>
<th>Additional management</th>
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<td>1</td>
<td>33</td>
<td>-</td>
<td>4</td>
<td>CRL = 10.7 mm</td>
<td>+</td>
<td>Laparotomy/hysterotomy</td>
<td>Bladder injury</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 w / 4 d</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>vaginal bleeding</td>
<td></td>
<td>7 w</td>
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<tr>
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<td>28</td>
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<td></td>
<td></td>
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<td>6 w</td>
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<tr>
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<td>pain</td>
<td></td>
<td>4 w / 4 d</td>
<td></td>
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</tbody>
</table>

CRL: crown rump length, GS: gestational sac.
Recently there has been an increase in reported CSP cases. Donald et al. [11] reported 19 CSP cases between 1966 and 2002 in a literature review study whereas Homayoun et al. reported 268 cases between 1995 and 2008 [4]. In our study, we diagnosed and treated six cases of CSP in a 3-month period. The recent increase in the number of cases may reflect high cesarean birth rate worldwide, but it also may be associated with better diagnostic accuracy, improved knowledge, and high index of suspicion [1, 12]. The association between cesarean deliveries for breach presentation and subsequent CSP is reported in the literature. They hypothesize that is most indications for prior cesarean deliveries in CSP cases are elective, and thus there is a poorly developed lower uterine segment that leads to incomplete healing [9, 13, 14]. Some authors have proposed that the increase of these abnormal pregnancies may be the due to change in surgical technique of the hysterotomy. In the past double-layer closure was performed, with sutures inverting the first layer by the second one. As commonly used today, closure of hysterotomy with monolayer noninverting running sutures leads to incomplete postoperative healing and creation of defects in scar tissue [1, 4, 13].

Chuang et al. reported no association between number of cesarean sections and CSP occurrence [15], but Jurkovic et al. reported that the number of cesarean sections affected the occurrence of CSP because the scar surface is increased and the anterior uterine wall may be deficient because of poor vascularity, fibrosis and incomplete healing [6]. In the present study, five of six patients had undergone two previous cesarean sections and one patient had undergone four previous cesarean sections which is compatible with Jurkovic et al.’s study. There are very different presenting manifestations in CSP. It may present with painless vaginal bleeding, lower abdominal pain, vaginal bleeding plus abdominal pain, and it may be an incidental finding on routine ultrasonography in an asymptomatic woman [1, 3, 4, 13]. In the present study, the patients’ clinical presentations also ranged from asymptomatic to mild abdominal pain and vaginal bleeding.

There is no consensus on the best method and criteria to diagnose cases. However the majority of CSPs have been confirmed by TVS. Maymon et al., recommend using combined TVS and transabdominal sonography (TAS) with a full bladder. Thus a ‘panoramic view’ of the uterus is provided with accurate measurement of the distance between the gestational sac and bladder [13]. Occasionally, additional diagnostic modalities such as Doppler flow imaging, three-dimensional ultrasonography, magnetic resonance imaging, and even invasive procedures such as hysteroscopy and cystoscopy may be necessary for the differential diagnosis between cervical pregnancy, cervico-isthmic pregnancy, spontaneous miscarriage in progress and CSP. True diagnosis is crucial because a large number of complications caused by misdiagnosis leads to expectant management and inappropriate interventions [4, 13, 16, 17].

Our all patients were diagnosed by a combination of TVS and TAS. The diagnosis of cesarean scar pregnancy was determined based on the following ultrasonographic criteria: 1) empty uterus; 2) an empty cervical canal; 3) anteriorly located gestational sac with a diminished myometrium layer between the bladder and the sac, and 4) discontinuity in the anterior uterine wall of the uterus on a sagittal view of the uterus following the gestational sac [11, 14, 18].

Because of the infrequency of CSP, there are no universal treatment guidelines for this abnormal pregnancy, although several treatment modalities have been recommended. All reports consist of few cases and there is no agreement on which treatment modality should be preferred [2, 4]. In cases when the patient might request the continuation of the pregnancy, the patient should be thoroughly informed about the possible complications and if the pregnancy is continued it must be closely monitored. Due to the high risk of uterine rupture, invasive placenta and profuse uterine bleeding, the current trend is termination of CSP after the explanation of risks to couples [13, 16].

In a hemodynamically stable patient two management options may be considered: medical or surgical intervention. Surgical intervention has been successfully performed either laparoscopically [19] or by laparotomy [14, 17] in the form of excision of the ectopic pregnancy and repair of the myometrium. However, the surgical operation carries a significant risk of uncontrolled hemorrhage, resulting in hysterectomy and loss of future fertility in some of cases [6, 20].

In one of our four cases, laparotomy was used as an early treatment. The reason for this was the request for tubal ligation and the preference of surgical treatment by the patient. Excision of the scar pregnancy and treatment of the scar was achieved but, during surgery the bladder had been damaged and had to be treated.

Evacuation of CSP by curettage alone has been performed, but secondary salvage treatment has already been proven necessary. Therefore, D&C should not be considered as the first choice of therapy. This is because the majority of the villi are implanted in the myometrium and it seems unlikely that the gestational sac can be expelled by curettage without perforating the uterine wall or damaging the urinary bladder, and may also cause life-threatening hemorrhage [11, 13]. In contrast, some authors have reported successfully treating CSP by D&C under the guidance of ultrasound, without complications [6-7, 21].

Suction curettage was used as the initial treatment on four of our patients. We could not successfully manage in only one of these patients because the patient’s gestational weeks were more than 12 weeks. The other three successfully treated pregnancies were less than seven weeks of gestation. The gestational age is an important factor when curettage is the treatment of choice.

Medical therapy is noninvasive and avoids further damage to the uterus, and therefore may maximize the chance of preservation of the uterus in patients who desire fertility. However, the efficacy and safety of this treatment modality is still unknown [8].

Medical treatment consists of methotrexate (MTX) administered either systemically [22, 23], locally [4] or...
References


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Autopsy findings in fetuses with cystic hygroma: a literature review and our center’s experience


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Summary

Purpose of investigation: To report our experience of autopsied cases of fetal cystic hygroma (CH) and discuss the role of fetal autopsy in genetic counseling. Methods: A review of autopsy reports at our institution revealed 18 cases of fetal CH over a 10-year period (from 2000 to 2010). The clinical data, results of cytogenetic analysis and prenatal ultrasound findings were also retrieved and compared to the autopsy findings. Results: Fetal death was due to intrauterine death in eight cases, therapeutic abortion in eight cases and spontaneous abortion in two cases. The autopsy findings were in agreement with the prenatal ultrasound findings in 13/18 cases (72.2%), while in five cases (27.8%) additional findings were detected during autopsy. The most common placental abnormalities were infarcts and calcifications. Conclusion: In addition to prenatal diagnostic studies, fetal autopsy and pathologic examination of fetal and placental tissues may help to establish the exact cause of death and disclose important information as to the presence of various fetal malformations or placental abnormalities.

Key words: Rat; Autopsy; Cystic hygroma; Fetus; Genetic counseling; Malformations.

Introduction

Lymphangiomas are rare congenital benign lesions of the lymphatic system, which are composed of cystically dilated vascular channels lined by inconspicuous endothelial cells and filled with lymph fluid [1]. Although the debate as to their exact nature has not yet been settled, the belief that most lymphangiomas represent benign malformations of the lymphatic system arising at sites of lymphatic-venous connections, rather than true neoplasms, is the prevalent one [2]. Furthermore, despite the persisting controversy regarding the exact origin and pathogenesis of these lesions it is also generally agreed that their development is closely related to the maturation of the lymphatic system while several different theories attempting to explain the mechanism of their formation have been proposed [1]. Sequestration of lymph tissue, abnormal budding of lymph vessels, lack of fusion with the venous system or obstruction of lymph vessels are all discussed as causes of lymphangiomas, while various factors such as trauma, infection, chronic inflammation or obstruction during embryonic development are considered as potential triggers of this process [1, 3-7].

The most commonly used classification of lymphangiomas separates them into three major forms: capillary, cavernous and cystic [1, 2, 7]. The latter (cystic) form has been traditionally known as cystic hygroma (CH) [2] and accounts for approximately 90% of the lymphangiomas in the head and neck [8], although it may also occur at various other sites including the axilla, mediastinum, abdomen and retroperitoneum [9, 10]. This observation has also led to the concept that CH should be defined merely as a lymphangioma located in the head and neck region, where the formation of a cystic lesion is encouraged by the presence of loose fascia [9].

We present a brief review of the literature related to CH and further discuss the role of fetal autopsy in genetic counseling. The clinical and autopsy findings in 18 cases of fetal CH diagnosed in our center are also presented.

Materials and Methods

A search of autopsy reports at our institution (Pathology Laboratory of Areteion University Hospital) revealed 18 cases of fetal CH over a 10-year period (from 2000 to 2010). Post-mortem examination of the fetuses was done according to a pre-designed protocol including a photograph, skeletal radiography (in cases of suspected skeletal anomalies), and a thorough external and internal examination and microscopic evaluation of fetal tissue as per autopsy findings on gross examination. Histopathologic examination of placental tissue was also carried out when the placenta was available. All fetal autopsies were performed only after obtaining a written consent from the parents. The clinical data, prenatal ultrasound and cytogenetic findings were retrieved from the files of the 2nd Obstetrics and Gynecology Clinic of our hospital and compared to the autopsy findings. Cytogenetic analysis was performed in 12/18 cases (66.7%). The results revealed an abnormal karyotype in 7/12 cases (58.3%), i.e., Turner syndrome in five cases and trisomy 21 in two cases.
Results

The clinical data, the results of cytogenetic analysis and the outcome of our studied cases are presented in Table 1, while the autopsy findings and the placental histopathologic features are summarized in Table 2. The maternal age ranged from 20-40 years (mean 28.6 years), while the gestational week at the time of fetal loss ranged from 13-32 weeks (mean 20.2 weeks). Fetal death was due to intrauterine death in eight cases, therapeutic abortion in eight cases and spontaneous abortion in two cases. The size of CH ranged from 1-12 cm (mean 5.4 cm) (Figure 1). Aside from CH, other structural malformations or findings suggestive of the cause of fetal death were diagnosed in 10/18 cases (55.6%). The most common autopsy findings were hydrops and central nervous system anomalies. The autopsy findings were in agreement with the prenatal ultrasound findings in 13/18 cases (72.2%), while in five cases (27.8%) additional findings were detected during autopsy examination of the fetus and subsequent histological examination of fetal tissue. The additional autopsy and/or histologic findings were: intrauterine pneumonia (case 2), syndactyly of the 3rd and 4th digits of the right hand (case 3), cystic pulmonary malformation (case 5), villous hyperplasia of choroid plexus and polycystic kidneys (case 11). Fibular aplasia was also diagnosed on postmortem X-ray in one case (case 11). The placenta was available in 13/18 cases (72.2%). Histopathological examination of placental tissue was consistent with normal findings in 6/13 cases (46.2%), while in the remaining seven cases (53.8%) various placental abnormalities were diagnosed, i.e., multiple infarcts (cases 5, 8, 10 and 12), extensive calcifications (cases 5, 7, 13), retroplacental hematoma (cases 10 and 11), acute chorioamnionitis (case 7) and chronic placentitis (case 5). In the latter case, suspicion of a specific infection, such as toxoplasmosis, was raised.

Table 1. — Clinical data, cytogenetic analysis and outcome.

<table>
<thead>
<tr>
<th>Case</th>
<th>Maternal age (years)</th>
<th>Gestational age (weeks)</th>
<th>Karyotype</th>
<th>Outcome formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>32</td>
<td>NA</td>
<td>TA</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>20</td>
<td>45XO</td>
<td>ID</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>19</td>
<td>46XX</td>
<td>ID</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>18</td>
<td>47,T21</td>
<td>TA</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>19</td>
<td>NA</td>
<td>ID</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>21</td>
<td>46XX</td>
<td>ID</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>15</td>
<td>NA</td>
<td>ID</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>23</td>
<td>46XY</td>
<td>ID</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>20</td>
<td>45XO</td>
<td>SA</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>19</td>
<td>NA</td>
<td>TA</td>
</tr>
<tr>
<td>11</td>
<td>32</td>
<td>21</td>
<td>NA</td>
<td>TA</td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>30</td>
<td>46XY</td>
<td>ID</td>
</tr>
<tr>
<td>13</td>
<td>35</td>
<td>16</td>
<td>NA</td>
<td>SA</td>
</tr>
<tr>
<td>14</td>
<td>38</td>
<td>13</td>
<td>47,T21</td>
<td>TA</td>
</tr>
<tr>
<td>15</td>
<td>36</td>
<td>19</td>
<td>46XX</td>
<td>ID</td>
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<tr>
<td>16</td>
<td>22</td>
<td>20</td>
<td>45XO</td>
<td>TA</td>
</tr>
<tr>
<td>17</td>
<td>30</td>
<td>20</td>
<td>45XO</td>
<td>TA</td>
</tr>
<tr>
<td>18</td>
<td>20</td>
<td>19</td>
<td>45XO</td>
<td>TA</td>
</tr>
</tbody>
</table>


Table 2. — Autopsy and placental histopathologic findings.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Autopsy Findings (gross and histologic)</th>
<th>Placental histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH (12 cm), hydrops</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>CH (10 cm), hydrops, intrauterine pneumonia</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>CH (3 cm), syndactyly of 3rd and 4th digits (right hand)</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>CH (8 cm)</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>CH (10 cm), cystic pulmonary malformation</td>
<td>Chronic placentitis, multiple infarcts, extensive calcifications</td>
</tr>
<tr>
<td>6</td>
<td>CH (6 cm), nuchal cord</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>CH (1 cm)</td>
<td>Acute chorioamnionitis, extensive calcifications</td>
</tr>
<tr>
<td>8</td>
<td>CH (3 cm)</td>
<td>Multiple infarcts</td>
</tr>
<tr>
<td>9</td>
<td>CH (3 cm), findings consistent with Turner syndrome</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>CH (7 cm), meningo-myeloele, spina bifida</td>
<td>Multiple infarcts, retroplacental hematoma</td>
</tr>
<tr>
<td>11</td>
<td>CH (8 cm), hydrops, cardiac &amp; pulmonary hypoplasia, cerebellar hypoplasia, villous hyperplasia of choroid plexus, polycystic kidneys, fibular aplasia*</td>
<td>Placental hydrops, retroplacental hematoma</td>
</tr>
<tr>
<td>12</td>
<td>CH (3 cm)</td>
<td>Multiple infarcts</td>
</tr>
<tr>
<td>13</td>
<td>CH (2 cm)</td>
<td>Extensive calcifications</td>
</tr>
<tr>
<td>14</td>
<td>CH (1 cm)</td>
<td>NA</td>
</tr>
<tr>
<td>15</td>
<td>CH (2 cm)</td>
<td>NA</td>
</tr>
<tr>
<td>16</td>
<td>CH (7.5 cm), hydrops</td>
<td>Normal</td>
</tr>
<tr>
<td>17</td>
<td>CH (4 cm), hydrops</td>
<td>Normal</td>
</tr>
<tr>
<td>18</td>
<td>CH (7 cm), hydrops</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Postmortem X-ray finding
Discussion

CH typically develops in utero late in the first trimester to early in the second trimester and, due to the widespread use of prenatal sonography, is often detected prenatally by ultrasound [11, 12]. Although complete surgical excision remains the treatment of choice, especially when the anatomic position of the mass causes concern for potential airway compromise, and is usually effective in eliminating the defect, spontaneous regression of this lesion may occasionally occur [13, 14]. Furthermore, nonsurgical therapies such as sclerotherapy or even a non-interventional approach (watchful waiting) have been recently proposed as alternative options to surgery in selected cases to avoid the surgical risk of recurrence or nerve damage [15, 16]. Nonetheless, many of the fetuses with CH also have a variety of additional malformations, commonly associated with an underlying chromosomal anomaly, most frequently Turner syndrome but also trisomy 21 or other genetic disorders [2, 12]. For this reason, the overall prognosis for babies with a prenatal diagnosis of CH is poor, with a fatal outcome for the majority of cases [12, 17, 18]. Cytogenetic analysis is an indispensable diagnostic tool in the overall evaluation of fetuses with CH, and should be always included in the diagnostic work-up of these cases, as it may confirm the presence of a chromosomal abnormality thus allowing for the option of termination of pregnancy, and provide valuable information for genetic counseling [19]. In line with previous reports [11, 13, 17-19], Turner syndrome was the most common chromosomal abnormality among our studied cases with available cytogenetic analysis.

Additional malformations are often disclosed during postmortem examination of fetuses with prenatally diagnosed anomalies, thus leading to the adjustment of the prenatal diagnosis [20, 21]. In addition to establishing the exact cause of death, fetal autopsy is therefore the sole diagnostic method able to confirm, clarify, or even contradict the prenatal diagnostic studies [20-22]. The value of fetal autopsy as an adjunctive tool in genetic counseling has been well established, and a review of the literature reveals several previous studies and literature reviews evaluating the role of autopsy in fetuses with chromosomal abnormalities or structural malformations [20, 23-29]. These studies have emphasized the importance of fetal autopsy in providing an accurate etiologic diagnosis necessary for genetic counseling. Boyd et al. recently reported that in cases of terminated pregnancies because of anomalies which were prenatally diagnosed by ultrasound, by declining an autopsy, parents will remain ignorant of information that might change the recurrence risk in one of four cases and have a one in 13 chance for missing confirmation of a high (one in four) recurrence risk [23]. In another large series of 328 fetuses by Amini et al. [24], comparing the ultrasound and autopsy findings in pregnancies terminated after sonographic detection of fetal anomalies, the authors found that fetal autopsy provided further diagnostic information in 47% of their studied cases, thus concluding that fetal autopsy is essential for a definitive diagnosis. In a similar comparative study by Ramalho et al. [25], additional information after fetal autopsy was obtained in 26.3% of cases, including major structural anomalies in almost half of these cases. In a study of 288 second-trimester abortions because of malformations detected on prenatal ultrasound, discrepancies were noted between sonographic and autopsy findings in about 40% of pregnancies, and the authors concluded that termination of pregnancy should be followed by autopsy [30]. Consistent with these results are also the findings of several other studies [20, 26-29]. Dickinson et al. [29] who also found that fetal autopsy may provide significant information and/or clarify some prenatal findings, further stressed that the currently observed decline in fetal autopsy rates may lead to a loss of significant diagnostic and recurrence risk-counselling information. Needless to say of course that adequate and reliable access to the clinical data, and the prenatal ultrasound and cytogenetic findings is of paramount importance in designating the appropriate autopsy strategy [31, 32]. As reported by Tennstedt et al. [33], the use of an interdisciplinary database, including the prenatal, molecular-cytogenetic, X-ray and autopsy findings in a specialized center of perinatal medicine, may facilitate the identification of very small or rare malformations, thus significantly improving the quality of genetic counseling.

The autopsy findings in cases of fetal CH have been previously reported in a limited number of studies or only as reports of rare cases [17, 34-37]. In the largest series (57 cases) of autopsied CH cases published, to the best of our knowledge, the authors noted that autopsy findings showed various malformations associated with CH, especially hydrops, craniofacial and extremity anomalies and cardiac abnormalities [34]. In another series of 17 cases of fetal CH, hydrops was also the commonest autopsy finding [35]. Carson et al. [37] further reported that the diagnosis of CH was missed on prenatal ultrasound in four of 13 autopsied cases with available ultrasound examinations, while histopathologic examination of the lesion allowed the recognition of lymphatic vascular architecture and the demonstration of immunoreactivity of the endothelium to factor VIII regardless of the degree of autolysis. In our present study postmortem examination revealed additional malformations not apparent on prenatal ultrasound and contributed in the clarification of the exact cause of death in a significant percentage (40%) of cases. In line with the aforementioned previous reports [34, 35], hydrops was the most common abnormality associated with CH in our series as well.

With the notable exception of renal cystic disease, whose adequate diagnosis and classification requires microscopic evaluation of fetal tissue sections [20, 23], the value of histological examination of fetal organs in the presence of structural malformations remains rather controversial. It has been previously supported that histological analysis is unlikely to be helpful in providing significant information as regards the cause of fetal loss, or any predictions for future pregnancies, mainly because abnormal histological findings may be the result of
intrauterine fetal death and not necessarily the cause [20, 38]. On the other hand there is also the view that histopathological examination of all major organs of the fetus is an important diagnostic tool and should always be undertaken [22, 28, 39, 40]. Although histopathological examination of any fetal organ may yield diagnostically valuable information, the most important histopathological findings are frequently retrieved in the central nervous system, the skeletal system and the kidneys [39]. It should also be kept in mind that in dilation and evacuation specimens of aborted fetuses a regular autopsy procedure may be impossible due to the inevitable fragmentation, therefore histological examination in these cases may enable confirmation of the antenatal diagnosis [22, 39]. Furthermore, as rightly pointed out by Millar and Fothergill [41], routine histological examination of fetal tissue may be particularly valuable in units with limited experience in prenatal ultrasound or lack of adequate imaging equipment. Another major benefit of histological examination of fetal tissue is the storage of fetal genetic material, which may be used for the performance of molecular biological studies and the identification of known or suspected genetic diseases either in the present or in the future [42].

In accordance with a previous autopsy study of fetuses with congenital malformations [20], multiple placental infaracts were the most common placental abnormality in our studied cases. Despite the fact that the value of placental examination in clarifying the cause of intrauterine fetal death remains the object of significant controversy, especially in the concomitant presence of fetal anomalies, placental abnormalities are well established causes of fetal demise and should be always evaluated in association with the remaining findings [43]. As previously emphasized the placenta is a maternal-fetal organ involvement in determining fetal disease, and its pathologic examination may provide valuable information in genetic and infectious diseases of the fetus [39]. Histological examination of grossly apparent placental abnormalities may also help to determine the age of the lesion, as in the presence of hematomas attached to the chorion or placenta or within the placental tissue, thus clarifying their relation to a potential iatrogenic injury during the procedures undertaken for sampling [22]. In previous studies placental abnormalities have been associated with conditions causing fetal hydrops [44, 45]. It has also been previously claimed that placental infarcts may represent a major causal factor of fetal death, and an association of placental infarction with prenatal brain damage has been suggested [46–48]. However, other researchers have stated that only massive placental infarction should be considered as an obvious presumptive cause for fetal demise and that placental infarcts have been documented in live and completely normal births as well, and advice for caution as regards any claim that a particular placental lesion has been the direct cause of fetal demise without prior strict evaluation of all the possible factors which could have directly led or contributed to the adverse pregnancy outcome [43]. Previous reports have also revealed that in fetuses with genetic disorders or structural malformations the placenta generally shows nonspecific abnormalities, such as villous immaturity or edema, that are not particularly helpful in establishing a diagnosis [39].

In conclusion, autopsy examination of fetuses with structural malformations such as CH may be of value in revealing additional anomalies not necessarily apparent on prenatal imaging studies and aid in the identification of the exact cause of death. “The family seeks and deserves answers regarding the cause of the loss of a baby” [20] and fetal autopsy may also provide evidence for these answers. On the other hand, as rightly pointed out by Boyd et al. [23], parents should also be fully informed prior to giving their consent for an autopsy examination about the procedures involved and about the benefits in providing information about risks of recurrence.

References


Autopsy findings in fetuses with cystic hygroma: a literature review and our center’s experience


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The role of amino acids in spina bifida

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Summary

Our objective was to measure amniotic fluid amino acid concentrations in pregnant women diagnosed as having fetuses with spina bifida in the second trimester of pregnancy. Fifteen pregnant women who had fetuses with spina bifida detected by ultrasonography (spina bifida group) in the second trimester and 19 women who had abnormal triple screenings indicating an increased risk for Down's syndrome but had healthy fetuses (control group) were enrolled in the study. Amniotic fluid was obtained by amniocentesis. The chromosomal analysis of the study and control groups was normal. Levels of free amino acids were measured in amniotic fluid samples using EZ: fast kits (EZ: fast GC/FID free (physiological) amino acid kit) by gas chromatography (Focus GC AI 3000 Thermo Finnigan analyzer). The mean levels of alanine, cystathionine, cysteine, phenylalanine, tryptophane, and tyrosine amino acids were found to be significantly higher in fetuses of the control group than in the spina bifida group (p < 0.05). The detection of significantly higher amino acid concentrations in the amniotic fluid of healthy fetuses suggests loss of amino acids from the fetus through the spinal cord may contribute to the etiology of spina bifida.

Key words: Spina bifida; Amino acids; Amniocentesis.

Introduction

Spina bifida is a congenital disorder caused by the incomplete closing of the embryonic neural tube. Vertebra overlying the spinal cord are not fully formed and remain unfused and open. There may be a fluid-filled sac surrounding the spinal cord in amniotic fluid of pregnant women. Spina bifida occulta, myelomeningocele, meningocele and lipomeningocele are all types of neural tube defects. The incidence of spina bifida occulta is approximately 10% of the population [1].

The cause of spina bifida is not known in most cases and the etiology remains complex and poorly understood. It is generally agreed that most cases are of multifactor origin. Environmental factors are also important. Maternal age, alcohol consumption, maternal exposure to excess vitamin A, and folic acid deficiency may be associated with the pathogenesis of neural tube defects [2].

The aim of the study was to determine the amino acid concentrations of spina bifida cases in amniotic fluid in the second trimester of pregnancy. We hypothesized that concentrations of amino acids may be a cause or the result of spina bifida cases.

Material and Methods

The study was performed at the Prenatal Diagnosis Unit of our Research Hospital between January 2009 and June 2011 and was approved by the Institutional Review Board and Ethics Committee of the university hospital. Written informed consent was obtained from all participants. All pregnant women who had a fetus with spina bifida (n = 15, study group) in the second trimester were included in the study. Nineteen (n = 19, control group) women who attended our clinic and had abnormal triple screens indicating an increased risk for Down’s syndrome were included in the study as the control group. Detailed ultrasound (US) examination, fetal karyotyping, investigations for fetal cardiac malformations infections and genetic diseases were performed for all cases.

Mean maternal age was 27.3 ± 1.0 years for the spina bifida group and 28.0 ± 0.9 years for the control group. The mean gestational age at sampling was 19.2 ± 1.1 weeks for the spina bifida group and 18.8 ± 1.3 weeks for the control group. Obese patients and those with any systemic or endocrine disorder were excluded from the study. All pregnancies were accurately dated by the last menstrual period and by first-trimester US investigation. Amniotic fluid samples were obtained by routine transabdominal amniocentesis and collected into 10-ml dry tubes. All amniotic fluid samples were free of blood contamination and were immediately centrifuged at 3000 g for 10 min and stored at -20°C until assayed. Levels of free amino acids (histidine, leucine, isoleucine, methionine, phenylalanine, tryptophan, valine, alanine, asparagine, aspartic acid, cystathionine, cysteine, glutamine, glycine, tyrosine) were measured in amniotic fluid samples using EZ: fast kits (EZ: fast GC/FID free (physiological) amino acid kit) by gas chromatography (Focus GC AI 3000 Thermo Finnigan analyzer, Milan, Italy; injection: Split 1:15 at 250°C, 2.5 μ; carrier gas: helium 1.5 ml/min (60 kPa) at 110°C; pressure rise: 6 kPa/min; oven program: 30°C/min from 110° to 320°C, hold at 320° for 1 min; Detector: FID at 320°C; intravariability: 2.4%; intervariability: 3.2%). The results are reported as means ± SD; a t-test was performed for statistical analysis. The statistical relationship between the two variables was checked by Pearson correlation coefficients. A p value of less than 0.05 was considered to be statistically significant.

Results

Fifteen women who had fetuses with spina bifida were included in the study. Spina bifida was diagnosed by US and confirmed after delivery. Detailed US examination, fetal karyotyping, and investigations for fetal cardiac malformation infections were performed for all cases. All investigations were normal. Pregnancy was terminated in the spina bifida group. The 19 control group women were
submitted to amniocentesis performed because of abnormal triple screens indicating an increased risk for Down’s syndrome. None of the control group fetuses showed structural abnormalities in US at the time of amniocentesis and none had chromosome abnormalities. All patients in the control group gave birth to a healthy child. The rates of nulliparity, the mean maternal and gestational ages and body mass index at the time of amniocentesis did not differ significantly between the two groups ($p < 0.05$).

The mean levels of alanine ($37.2 \pm 38.2$ umol/l vs $15.5 \pm 6.5$ umol/l), cystathionine ($14.2 \pm 13.0$ umol/l vs $13.1 \pm 12.3$ umol/l), cysteine ($18.8 \pm 9.5$ umol/l vs $14.6 \pm 5.5$ umol/l), phenylalanine ($56.2 \pm 46.3$ umol/l vs $43.3 \pm 15.8$ umol/l), tryptophane ($80.8 \pm 41.3$ umol/l vs $44.3 \pm 44.2$ umol/l), tyrosine ($44.5 \pm 35.3$ umol/l vs $22.2 \pm 12.5$ umol/l) amino acids were found to be significantly higher in fetuses with spina bifida than in the control group ($p < 0.05$).

**Discussion**

The pathogenesis of spina bifida remains unclear and depends on the underlying disorder. Alcohol consumption, maternal exposure to excess vitamin A, folic acid deficiency, and antiepileptic drugs are the common etiologies of spina bifida [1-4].

Emery *et al.* conducted a study to find out aminoacid concentrations of central nervous system malformations in 33 cases. The study showed that there was a significant increase in the amount of certain neutral amino acids such as methionine, isoleucine, leucine, tyrosine, and phenylalanine [3].

Pettit *et al.* compared amino acid concentrations in amniotic fluid from fetal neural tube defects and normal pregnancies and found that hydroxy amino acids were raised while the branched chain amino acids were lower in concentration [4].

We found that amino acid levels of alanine, cystathionine, cysteine, phenylalanine, tryptophane and tyrosine were significantly higher in fetuses of the control group than in the spina bifida group suggesting that loss of amino acids from the fetus through the spinal cord may contribute to the etiology of spina bifida. Emery *et al.*’s study found a significant increase in the amniotic fluid of tyrosine and phenylalanine amino acids as our study [3].

The loss of alanine, cystathionine, cysteine, phenylalanine, tryptophane and tyrosine amino acids from spinal cord to amniotic fluid might partly explain fetal morbidity and mortality.

This was a preliminary study on amniotic fluid amino acid concentrations conducted on a small patient series. We think that it would be beneficial to conduct further studies with larger groups to determine the amino acid levels of fetuses with spina bifida.

**References**


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Secondary missed abdominal pregnancy due to iatrogenic uterine perforation: a case report

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Summary
Abdominal pregnancy is extremely rare and can result from the reimplantation of an intrauterine pregnancy after spontaneous uterine rupture. In this report, we present the case of a secondary missed abdominal pregnancy resulting from iatrogenic uterine perforation during dilatation and curettage in an early intrauterine pregnancy and subsequently misdiagnosed as intrauterine trophoblastic disease. Transvaginal ultrasound missed the diagnosis, which was finally confirmed by computed tomography. We discuss the particulars of the case along with a review of the relevant literature.

Key words: Abdominal pregnancy; Uterine perforation; Ultrasound; Computerized tomography (CT); Laparotomy.

Introduction
Abdominal pregnancy is a very rare disease, with high maternal morbidity and mortality. Early diagnosis of abdominal pregnancy is essential to prevent the complications of intraabdominal infection and placental hemorrhage. It usually occurs after tubal abortion or rupture. Diagnosis of missed abdominal pregnancy is problematic because it can mimic other pelvic pathology, including pelvic inflammatory disease. We report a very rare case of secondary missed abdominal pregnancy resulting from an iatrogenic uterine perforation, and misdiagnosed as intrauterine trophoblastic disease with pelvic infection.

Case Report
A 36-year-old woman, gravida 1 para 0, was referred due to a 3-week history of abdominal pain. At the time of referral, she had been diagnosed with peritonitis and suspected intrauterine trophoblastic disease. The patient had undergone elective dilatation and curettage (D&C) eight weeks after her last menstrual period. She had experienced mild abdominal pain and vaginal bleeding for two months after the procedure, and returned to the original clinic. The clinic that performed the initial D&C had performed two additional D&Cs two weeks apart for placental remnants in the uterine cavity. However, her abdominal pain worsened, and transvaginal sonography (TVS) showed an intrauterine mass suspicious for trophoblastic tissue, resulting in a diagnosis of pelvic inflammation and intrauterine trophoblastic disease.

She was referred to our center 20 weeks after her last menstrual period with worsening abdominal pain despite a 2-week course of intravenous antibiotics. Her initial blood pressure was 100/70 mmHg, pulse 94/min, and temperature 38.5°C. Her abdomen was soft and flat but there was diffuse tenderness to palpation as well as diffuse rebound tenderness. Initial laboratory tests revealed mild leukocytosis (white blood cell count 11,200/mm³ with 85% neutrophils), an elevated C-reactive protein (CRP) (13.6 mg/dl; normal range: 0.02-0.3 mg/dl). Her urine test was positive for pregnancy.

An ultrasound revealed an echogenic mass measuring 5 × 3 cm, as well as an ill-defined mass of mixed echogenicity, adjacent to the uterine fundus (Figure 1). There was no gestational sac visible in the uterine cavity. Computed tomography (CT) was performed to evaluate the intraabdominal mass and her abdominal pain, which showed a fetal skeleton in the abdominal cavity and an intrauterine mass with air (Figure 2). A missed abdominal pregnancy was diagnosed.

Laparotomy was performed revealing a shapeless necrotic mass, suspected to be a dead fetus due to a recognizable fetal hand, amidst the bowel and omentum (Figure 3). After adhesiolysis, a 2-cm uterine defect, consistent with an iatrogenic perforation made during the D&C, was identified in the posterior body of the uterus (Figure 4). The fetus was connected to the uterine cavity by an umbilical cord passing through the defect. The residual placental mass in the uterine cavity was removed through the uterine defect and a primary repair was performed. The necrotic intraabdominal mass was confirmed to be a fetus of approximately 16 weeks of gestation without an amniotic sac or placental tissue (Figure 5). Pathologic examination confirmed the intrauterine mass as a necrotic placenta. The patient was treated with antibiotics and remained afebrile on postoperative day 2. She recovered uneventfully and was discharged on postoperative day 12 after her CRP level had normalized.

Discussion
Abdominal pregnancy is rare, accounting for approximately 1% of all ectopic pregnancies [1]. While most cases of secondary abdominal pregnancy result from the reimplantation of a ruptured or aborted tubal pregnancy, rare cases result from intrauterine pregnancies in which a uterine scar [2] uterine horn, or other anomaly spontaneously rupture [3, 4]. In our patient, iatrogenic uterine perforation during an induced abortion led to a secondary abdominal pregnancy. Despite several D&Cs, TVS showed an intrauterine echogenic mass leading to the misdiagnosis of placental remnants or gestational trophoblastic disease. Although high-resolution TVS can visualize the pelvic organs in detail, its narrow field of view may miss structures remote from the vagina, such as...
abdominal masses. In our case, we postulated that the intraabdominal fetus was not visible on TVS until it reached 16 weeks of gestation. Furthermore, the intrauterine echogenic mass overshadowed the intraabdominal fetus.

Diagnosis of advanced abdominal pregnancy requires a high index of suspicion. Several cases reported in the literature have been misdiagnosed as normal intrauterine pregnancies despite transabdominal ultrasound [2, 5-7]. These reports suggest magnetic resonance imaging to diagnose a live abdominal pregnancy and to give detailed anatomic information, such as the placental location. In our case, TVS of the necrotic fetus without amniotic fluid was inconclusive, and was ultimately diagnosed by CT. Due to the high mortality and morbidity of post-abortion infections, septic abortions require prompt evacuation [8].

In South Korea, elective abortions are prohibited by law, resulting in illegal abortions that can cause complications such as pelvic infection and uterine perforation.

In this case of secondary missed abdominal pregnancy, the patient returned to the clinic that had provided the abortion with concerning symptoms, but she was misdiagnosed. A high index of suspicion and a careful examination may have yielded the correct diagnosis, but instead the patient underwent unnecessary procedures and delayed treatment, resulting in increased morbidity.

In conclusion, abdominal pregnancy can result from iatrogenic uterine perforation during D&C. Awareness of this possible complication is important to aid in diagnosis and reduce associated morbidity and mortality.

References


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Figure 3. — Intraoperative photograph showing the dead fetus tangled with bowel and omentum in the abdominal cavity outside the uterus.

Figure 4. — Arrow indicates a small defect on the posterior body of the uterus.

Figure 5. — Surgical specimen of the dead fetus, approximately 16 weeks gestation. There were no placental parts in the abdominal cavity.
Peritoneal enterobiasis causing endometriosis-like symptoms

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Summary

**Purpose:** Enterobiasis is the most common parasitic disease of the temperate zones and infects the human intestinal tract. In rare cases extraintestinal infections with *Enterobius vermicularis* may occur and can affect the female genital tract and peritoneal cavity. In most cases the infection is asymptomatic, but there are also cases described in which peritoneal enterobiasis can cause abdominal pain. **Methods:** A case report and review of the pertinent literature. **Results:** A 32-year-old patient was admitted with cyclical lower abdominal pain. With suspected endometriosis a diagnostic autofluorescence laparoscopy (DAFE) was performed. At surgery extensive peritoneal deposits were seen. Macroscopically these deposits were not typical for endometriosis. The histological examination showed granuloma caused by *E. vermicularis* eggs. The patient was treated with mebendazole. After completion of treatment the patient was asymptomatic. At the second-look laparoscopy no more peritoneal changes were detected. **Conclusion:** Enterobius vermicularis may cause symptoms similar to endometriosis. In cases with reasonable suspicion it should therefore be considered in the differential diagnosis.

**Key words:** Enterobiasis; Endometriosis; Abdominal pain.

Introduction

Enterobiasis is the most common parasitic disease of the temperate zones infecting the human intestinal tract. Although rarely, extraintestinal infections with *Enterobius vermicularis* occur which can affect the female genital tract and peritoneal cavity. In most cases the infection is asymptomatic, but there have also been cases described in which peritoneal enterobiasis can cause abdominal pain. We present a case of a patient who was admitted with cyclical lower abdominal pain. Endometriosis was suspected, thus diagnostic autofluorescence laparoscopy (DAFE) was performed. At surgery extensive peritoneal deposits were seen. Macroscopically these deposits were not typical for endometriosis. After histological examination, the patient was found to have pinworms.

Case Report

A 32-year-old patient was admitted with worsening lower abdominal pain and dysmenorrhea. The symptoms had not improved under treatment with oral contraceptive pills. The patient had to take analgesics regularly. There were no bowel or urinary symptoms. She worked as a maid in a hotel. The patient had not travelled in tropical regions. Physical examination revealed mild pain of the inner genital tract. During rectal examination there seemed to be little nodules on the peritoneum of the rectovaginal space. With suspected endometriosis a diagnostic autofluorescence laparoscopy (DAFE) was performed. At surgery extensive peritoneal deposits were seen in the Pouch of Douglas. Macroscopically these deposits were not typical for endometriosis (Figures 1 and 2). The suspicious areas were biopsied and the operation was uncomplicated.

Histological examination showed granuloma. In these granulomas a dense infiltration of eosinophilic cells around ovoid structures with eosin red walls were seen. These ovoid structures were the equivalent of *E. vermicularis* (Figure 3).

To rule out other infectious diseases and affection of other organs computed tomography of the chest and abdomen was performed. Neither scan showed any granulomas in the organs.

The patient and her family were treated with 100 mg of mebendazole a daily for three days. This treatment was repeated after four weeks. After completion of the treatment the patient was asymptomatic.

At second-look laparoscopy no more peritoneal changes were detected. There was no sign of any endometriosis-like lesions.

Discussion

Enterobiasis is the most common parasitic disease of the temperate zones and infects the human intestinal tract. In Poland a study revealed 35% of the families examined to be infected with pinworms [1]. *E. vermicularis* normally is found within the human gastrointestinal tract. The female worm migrates out of the host’s anus at night to lay eggs. Sometimes the worm makes it way back into the female genitourinary tract. In these rare cases extraintestinal infections with *E. vermicularis* may occur and can affect the female genital tract and the peritoneal cavity as well as the urinary tract [2]. In most cases the infection is asymptomatic and many patients do not present with the common symptom of pruritus ani, which makes the diagnosis even more difficult. However there have also been cases described in which peritoneal enterobiasis can cause abdominal pain. Due to this, enterobiasis should reasonably be considered in the differential diagnoses of endometriosis [3].

Endometriosis as well as enterobiasis may appear in various entities. There are peritoneal or ovarian lesions, adenomyosis and/or deep infiltrating lesions among endometriosis patients. These different lesions may
present differently and cause various symptoms according to their localization.

Superficial endometriosis lesions often spread to the peritoneum of the Douglas pouch and the uterosacral ligaments. Similar to that *E. vermicularis* may lead to generalized intraperitoneal lesions [4]. Endometriosis of the peritoneum can present with pelvic free fluid and lower abdominal pain. Tandan *et al.* described a case where lower abdominal pain and pelvic free fluid led to a laparoscopy. At the operation no signs of endometriosis but rather of chronic inflammation of the pelvic peritoneum were seen. Histological examination of the inflamed pelvic peritoneum showed pinworm eggs [5]. Cystic lesions of the ovaries can be caused by endometriosis as well as by ascending infections of the genital tract. Even tuboovarian abscesses may be caused by enterobiasis [6]. Besides the acute symptoms endometriosis causes problems among women such as infertility [7]. In these cases infection by *E. vermicularis* should be taken into account. There are cases where a pinworm infection of the genital tract led to tubal obstruction and infertility [8].

Even though endometriosis is a common cause of chronic pelvic pain in young women there are some differential diagnoses to be taken into account such as peritoneal enterobiasis.

**Conclusion**

As the diagnosis of endometriosis continues to be difficult *E. vermicularis* and other infections need to be taken into consideration. This calls for a precise anamnesis including life style and travel. Endometriosis may present with different kinds of symptoms and macroscopic appearances. Recent studies comparing electrocoagulation and sharp excision of superficial endometriosis show only a slightly better outcome in symptoms and relapse rate for electrocoagulation [9]. Nonetheless typical endometriosis should be biopsied to confirm the diagnosis. In cases where thermal destruction of the lesions is performed a wrong diagnosis may lead to false treatment and persistent symptoms of the patient. There might be a number of patients with diagnosed endometriosis not histologically confirmed, that suffer from pinworm infection as it is an ubiquitary infection. There is evidence that excision does reduce endometriosis-related pain [10]. We believe that histological evidence is needed to consistently find the right diagnosis and treatment.

**References**

Peritoneal enterobiasis causing endometriosis-like symptoms


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Uncorrected tetralogy of Fallot and pregnancy: a case report

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Summary

We report a case of pregnancy in a 34-year-old woman with uncorrected tetralogy of Fallot (TOF). There are more risks in patients without surgical correction. In our case, haemoglobin and haematocrit were higher, oxygen saturation was lower, and right ventricular enlargement was observed. Pregnancy was resolved successfully by caesarean section. Improvement of fetomaternal outcome may be related to corrective procedures before conception to achieve better functional heart capacity. Delicate multidisciplinary medical management is essential for these limited cases to achieve optimal prognosis.

Key words: Tetralogy of Fallot and pregnancy; Heart disease in pregnancy; Heart disease and mode of delivery; TOF and mode of delivery.

Introduction

Tetralogy of Fallot (TOF) is the most common cyanotic congenital heart disease (15%), characterized by ventricular large septum defect, pulmonary stenosis, aortic riding of septum communication, and right ventricular hypertrophy. TOF is treated surgically. For cases of TOF surgery is the only possible therapy - sometimes a temporary operation is performed when the patient is a small child. Later in life complete surgical repair must be carried out [1].

Case Report

A 34-year-old woman, gravida 2, para 0, was admitted to hospital at 38 weeks of gestation for elective caesarean section. There was a medical history of tetralogy of Fallot (TOF) with a palliative Blalock-Taussig shunt, oscillation between the subclavian artery and pulmonary arteries, in addition to mild renal failure and mild cyanosis. The patient had explicitly refused for years to submit to complete surgical treatment of her cyanotic heart disease. There was no other significant family history.

The drugs used during pregnancy were allopurinol (100 mg) and lasix tablets (1 x 2 bid). Cardiologic examination revealed a heart rate of 80-100 bpm, blood pressure (BP) of 120/87 mmHg with a normal sinus rhythm, right ventricular hypertrophy, left atria enlargement, a rightward axis and nonspecific T-wave abnormality. There was also a huge interventricular septal defect, sittus solitus, overriding of the ectatic aorta over the interventricular septum, severe constriction of the exitus of the right ventricle and pulmonary artery. The patient's pregnancy had been uneventful and continued normally without complications.

A week before the scheduled caesarean section, a full clinical examination revealed dyspnoea during stress, mild cyanosis, pulse of 98 bpm, BP of 135/90 mmHg, haematocrit of 52%, haemoglobin of 16.5 g/dl, systolic murmur of the pulmonary artery and continuous puffs in the precordium.

On admission to the hospital at 38 weeks of gestation, the presentation of the foetus was cephalic and the cardiotocography (CTG) trace was normal. There was no uterine activity noted. At the vaginal examination there was no effacement of the uterine cervix. Cervical dilation was 4 cm and the membranes were intact. The patient’s pulse was 84 bpm and her blood pressure 95/70 mmHg. Endocarditis prophylaxis was given before the surgical procedure.

On admission to the theatre epidural anaesthesia was performed and right after that a low transverse elective caesarean section was completed. A female neonate was delivered weighing 2,140 g, with an Apgar score of 8-10, at 1 and 5 min, respectively. The baby was in good condition. The patient remained stable during the operation as well as after the delivery without any haemodynamic or ECG changes. The mother was eventually transferred to the Intensive Care Unit (ICU) on antibiotic treatment with ampicillin IV. Due to long term use of cardiological medication she was advised against lactation. During her stay in the ICU she had three episodes of ventricular tachycardia and swelling of the lower extremities. No proteinuria was noted. She was treated with hydrochloride amiodarone IV (150 mg daily).

There was rapid clinical progress over the next 24 hours so the patient was transferred to the ward. Blood tests revealed the following results: Hct 40.6%, Hb 13.5 g/dl, PLT 166,000 k/ul (with no signs of renal failure), urea 49 mg/dl, and creatinine 1.0 mg/dl. LDH 296 U/l, fibrinogen 4.32 g/l and INR 1.02 were all within normal values. Mother and baby were both discharged seven days after the caesarean section with appropriate diuretic therapy through salt restriction and a special low calorie diet. She was also advised bed rest.

At the follow-up appointment almost a month after the delivery, there were no signs of cardiac disturbances. Five months after delivery she was admitted to the hospital in the cardiology clinic with ventricular tachycardia and episodes of unconsciousness. She was discharged 24 hours later on amiodarone (200 mg tablets bid) and lasix tablets (1 x 2 bid). Unfortunately she did not attend the follow-up appointments the first or second year.
TOF is the most common cyanotic congenital heart disease (15%), characterized by ventricular large septum defect, pulmonary stenosis, aortic riding of septum communication, and right ventricular hypertrophy. TOF is treated surgically. In our case a temporary operation was performed a Taussig-Blalock shunt at the age of seven years.

Uncorrected TOF during pregnancy belongs to Group 2 - moderate risk (5-15%) of maternal mortality. A fall in peripheral resistance during pregnancy and hypotension during labour may increase the right to left shunt and aggravate preexisting cyanosis. A rise in blood volume and venous return to the right atrium along with a fall in systemic vascular resistance increase the right to left shunt and cyanosis. Close monitoring of systemic blood pressure and blood gases during labour is essential. Any further systemic (drug induced) vasodilatation should be avoided [1, 2]. Maternal and foetal complications are tied to the degree of maternal cyanosis. The risk is high when oxygen saturation is ≤ 85%. There is a risk of 5% probability of transmission to the offspring and in 15% studies have shown deletion of the short arm of chromosome 22 (genetic cause). Recurrent risk when the father is affected is 1.5%; with an affected mother it is 2-3%, so in one sibling the risk is 2.5 times and in two siblings 8 times [1].

Maternal death rates have been quoted as high as 50% with preterm delivery rate at 55%, IUGR rate at 30% and perinatal mortality rate at 28% [3].

During pregnancy the risk of right ventricular insufficiency and hypoxic attack is increased [4]. There may also be an increased shunt and worse acidosis. Uncorrected TOF in pregnancy leads to deterioration, when the arterial saturation of oxygen is < 85% and the Hct > 60%. In those patients the most significant predictors of foetal hypoxia are the mothers’ persistent cyanosis and congestive heart failure. The prognosis is less favourable if there is already myocardial compromise before pregnancy. In addition, the frequency of abortion, premature birth, foetal distress and congenital malformation of the child is 57% [5]. The indication of assisted delivery in those patients depends on the severity of the disease. In our case caesarean section was indicated and the technique of anaesthetics (epidural anaesthesia) was carefully chosen [6].

On the contrary, the risk of pregnancy in repaired patients depends on their haemodynamic condition. Generally, the risk is low in patients with efficient repairs. In patients with significant residual right ventricular outflow obstruction, severe pulmonary regurgitation with or without tricuspid regurgitation and/or RV dysfunction, the increased volume load of pregnancy may lead to RV failure and arrhythmias [2, 7].

In a study of the American College of Cardiology [8] on 72 women, 43 women with TOF had 112 pregnancies. Eighty-two of these were successful. Eight women had uncorrected TOF at the time of their 20 successful pregnancies. One percent of these women had preterm labour. 8.5% of foetuses were small for their gestational age. Six out of seven were low birth weight infants and 6% of infants had major congenital abnormalities (e.g., congenital heart disease/stomach outlet obstruction, etc.). Finally, 24% of the above pregnancies had adverse obstetrical outcomes [8].

To our knowledge, there is one report as well as four case reports of delivery in uncorrected TOF, but only one without aortic regurgitation [8-10]. One case had a successful delivery by caesarean section - a patient with TOF following a Taussig-Blalock shunt [11, 12]. In that case, there was intrauterine growth restriction and pregnancy-induced hypertension. Our case did not demonstrate such features. All patients with TOF should have genetic counselling preconception with assessment in case of 22q11 deletion syndrome using fluorescent in situ hybridisation (FISH). In its absence the risk of defects in the foetus is low (4%) [13].

It is essential for high-risk patients with congestive heart disease like TOF either uncorrected or with a Taussig-Blalock procedure when becoming pregnant to be supervised by a multidisciplinary team including foetal medicine specialists, cardiologists and anaesthetists.

References


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Mediastinal masses: a case of fetal teratoma and literature review

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Summary

Fetal mediastinal masses are rare congenital formations that could complicate pregnancy. They are usually discovered as space occupying lesions in the fetal chest during routine ultrasound scan. The most important prognostic factors of mediastinal masses are mass location, compressing effect causing pulmonary hypoplasia and/or heart failure, and the presence or absence of hydrops. We report a case of fetal mediastinal teratoma and a review of the literature. A 32-year-old woman carrying a fetus with hydrops due to a mediastinal mass underwent cesarean section at 32 1/7 weeks’ gestation. A well encapsulated tumor was excised by surgery at one day of life. The baby is now eight months old without respiratory difficulty. To our knowledge, this is the fourth case report of a mediastinal teratoma associated with nonimmune hydrops in a fetus that survived the neonatal period. Fetal mediastinal teratoma requires close surveillance and multidisciplinary management by obstetricians, neonatologists, and pediatric surgeons.

Key words: Mediastinal masses; Fetal mediastinal teratoma; Prenatal diagnosis; Fetal management.

Introduction

Fetal mediastinal masses represent a wide diversity of disease states.

The mediastinum is demarcated by pleural cavities laterally, the thoracic inlet superiorly, and the diaphragm inferiorly. It is further compartmentalized into anterior, middle, and posterior divisions based on structural landmarks seen on the lateral radiograph. The anterior mediastinum contains the thymus, fat, and lymph nodes. The middle mediastinum contains the heart, pericardium, ascending and transverse aorta, brachiocephalic veins, trachea, bronchi, and lymph nodes. The posterior mediastinum consists of the descending thoracic aorta, esophagus, azygous vein, autonomic ganglia and nerves, thoracic lymph nodes, and fat [1]. Heart masses are not described. Teratoma and lymphangioma are the most common tumors developing in the anterior mediastinum [2]. Congenital thymic cysts are remnants of the thymopharyngeal duct [1]. Laryngeal atresia is an exceedingly rare anomaly in which the high airways are completely obstructed and the lungs appear severely enlarged and hyperechoic [2]. Fetus in fetu is a particular form of teratoma. This tumor is a parasitic twin that develops within the “main” twin [2]. Neuroblastoma can develop in the posterior mediastinum, appearing as a solid mass [3]. Cystic masses in the posterior mediastinum are most commonly esophageal duplication or neurenteric cyst (presence of both enteric and neural tissue in surgical specimens). Vertebral segmentation anomalies are commonly associated [1]. These tumors may extend both below and above the diaphragm [4]. The term congenital diaphragmatic hernia (CDH) encompasses a range of closure defects of the diaphragm with the abdominal viscera, located near the defect, migrated into the thorax [5]. Congenital intrathoracic stomach may be due to a short esophagus or hiatal hernia.

The likelihood of malignancy of mediastinal masses is influenced primarily by the following factors: mass location, compressing effect causing pulmonary hypoplasia and/or heart failure, and the presence or absence of hydrops.

Ultrasound (US) assessment of the mediastinum can be carried out easily until 25-26 weeks of gestation. After this period, the increased mineralization of the ribs lead to significant acoustic shadowing, which limits the display of the intrathoracic area. However, it has to be underlined that a good number of mediastinal anomalies evolve: they can appear only in the 3rd trimester, or, on the contrary, they can regress before birth [5]. The initial workup of a suspected mediastinal mass involves obtaining the classic 4-chamber view of the fetal heart. If the results from this view are abnormal, and a mediastinal lesion is found, this should be explored further using coronal and sagittal views [5].

In the present study, we report a case of fetal mediastinal teratoma associated with nonimmune hydrops and a review of literature.

Case Report

A 32-year-old white gravida 2, para 1, mother was referred to the Centre of Prenatal Diagnosis at the Hospital Umberto I of Rome at 29 6/7 weeks of gestation for evaluation of a fetal mediastinal mass. Her previous medical and obstetric history had been unremarkable. The mother was group O Rh (+) with a negative indirect Coombs test. On presentation, ultrasound revealed a 47 x 35 x 44 mm left anterior mediastinal mass with displacement of the heart and the left lung, and associated bilateral pleural effusions (Figure 1). The growth of the fetus was consistent with its ges-
Mediastinal masses: a case of fetal teratoma and literature review

Table 1. — Literature review of fetal mediastinal teratoma.

<table>
<thead>
<tr>
<th>Authors and year</th>
<th>Outcome</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merchant et al. [9] 2005</td>
<td>Case 1: delivered at 25 weeks; alive at 9 months follow-up</td>
<td>Hydrops fetalis; mediastinal mass found on fetal US; in utero resection at 23 weeks</td>
</tr>
<tr>
<td></td>
<td>Case 2: delivered after 34 weeks; alive at 1 year follow-up</td>
<td>Mediastinal mass found on fetal US; surgery in the immediate newborn period</td>
</tr>
<tr>
<td>Kuller et al. [10] 1991</td>
<td>Failed neonatal resuscitation at 27 weeks</td>
<td>Hydrops fetalis; diagnosis at autopsy</td>
</tr>
<tr>
<td>Schild et al. [11] 1998</td>
<td>Failed neonatal resuscitation at 27 weeks</td>
<td>Hydrops fetalis; mediastinal mass found on fetal US; diagnosis at autopsy</td>
</tr>
<tr>
<td>Wang et al. [14] 2000</td>
<td>Delivered at 39 weeks; alive at 3 months follow-up</td>
<td>Surgery at 4 days age; diagnosis at histology</td>
</tr>
<tr>
<td>Liang et al. [15] 1998</td>
<td>Delivered at 39 weeks; alive at 5 months follow-up</td>
<td>Mediastinal mass found on fetal US; surgery at 7 days age; diagnosis at histology</td>
</tr>
<tr>
<td>Weinraub et al. [16] 1989</td>
<td>Fetal demise at 29 weeks</td>
<td>Hydrops fetalis; mediastinal mass found on fetal US</td>
</tr>
<tr>
<td>Froberg et al. [17] 1994</td>
<td>Fetal demise at 27 weeks</td>
<td>Hydrops fetalis; mediastinal mass found on fetal US</td>
</tr>
<tr>
<td>Dumbell et al. [18] 1990</td>
<td>Delivered at 36 weeks; alive at 18 months follow-up</td>
<td>Mediastinal mass or cystic malformation of the lung found on fetal US; surgery in the immediate newborn period and at the age of 3 months</td>
</tr>
<tr>
<td>Takayasu et al. [19] 2010</td>
<td>Delivered at 39 weeks; alive at 6 months follow-up</td>
<td>Hydrops fetalis; mediastinal mass found on fetal US; aspiration of the fetal tumor cyst fluid at 29 weeks; surgery at 30 days of age</td>
</tr>
<tr>
<td>Noreen et al. [20] 2008</td>
<td>Case 1: fetal demise at 19 weeks</td>
<td>Hydrops fetalis; diagnosis at autopsy</td>
</tr>
<tr>
<td></td>
<td>Case 2: stillborn at 27 weeks</td>
<td>Hydrops fetalis; diagnosis at autopsy</td>
</tr>
<tr>
<td></td>
<td>Case 3: fetal demise at 23 weeks</td>
<td>Hydrops fetalis; diagnosis at autopsy</td>
</tr>
<tr>
<td>Wesolowski et al. [21] 2008</td>
<td>Delivered at 33 weeks; died at 50 days of age</td>
<td>Hydrops fetalis; mediastinal mass found on neonatal US surgery at 7 days of age</td>
</tr>
<tr>
<td>Akosy et al. [22] 2002</td>
<td>Delivered at term; died at one day of life</td>
<td>Mediastinal mass found on fetal US; surgery in the immediate newborn period</td>
</tr>
<tr>
<td>Allman et al. [23] 2001</td>
<td>Delivered at 36 weeks; alive at 3 years follow-up</td>
<td>At birth mild hydrops; heart beat detected by 15 minutes of age; surgery at 3 days of age; diagnosis at histology</td>
</tr>
<tr>
<td>Present case</td>
<td>Delivered at 32 weeks; alive at 8 months follow-up</td>
<td>Hydrops fetalis; mediastinal mass found on fetal US; PCO₂ of 45 mmHg, PO₂ of 20 mmHg, bicarbonate of 22.8 mmol/l, and base deficit of 0.8 mmol/l. Surgery was performed on day 1 of life. A well encapsulated tumor was excised, complete and intact. Histological examination confirmed the diagnosis of teratoma. The baby’s postoperative course was complicated by left vocal cord paralysis and left diaphragm paralysis. At 18 days of age a second surgery was performed and the left diaphragm was plicated. The baby has been followed up regularly, and he is now eight months old without respiratory difficulty.</td>
</tr>
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Discussion

Teratomas are tumors composed of multiple tissue elements derived from all three germ layers. These neoplastic lesions could present various degrees of differentiation, ranging from primitive somatic elements to highly organized axial structures [6]. Whereas teratoma is the most common congenital neoplasm, it rarely occurs in the mediastinum in fetal life (4-11% of cases) [7, 8]. While US is usually diagnostic, MRI using both T1-W and T2-W sequences is helpful in determining the extent of the tumor and its content [2]. These congenital malformations could cause compression of vital structures that may result in nonimmune hydrops leading to fetal demise, late gestational polyhydramnios, preterm labor
and respiratory distress at birth [9]. Hydrops fetalis and polyhydramnios are poor prognostic signs. The diseases considered in the differential diagnosis of such lesion are congenital cystic adenomatoid malformation, pulmonary sequestration, intrapericardial teratoma, cardiac rhabdomyosarcoma and diaphragmatic hernia [9-11]. Since each of these pathological conditions may be associated with other congenital and chromosomal abnormalities, a careful anatomic survey and chromosomal analysis are indicated [11]. In particular, karyotyping should be carried out because the prevalence of mediastinal germ cell tumor is 39 to 50 times higher in Klinefelter syndrome than in the general male population [12, 13].

This tumor is extremely rare and there are just few reported cases of fetal mediastinal teratomas in the literature [9-11, 14-23] (Table 1).

To our knowledge, this is the fourth case report of a fetus with mediastinal teratoma associated with nonimmune hydrops that survived the neonatal period [9, 19, 21]. Merchant et al. proposed a management algorithm for large fetal mediastinal teratomas [9]. Management depends on fetal gestation, the presence of hydrops, and the risk of airway compromise. A fetus younger than 30 weeks of gestation with hydrops may require open fetal surgery. A non-hydropic fetus can be managed expectantly with serial US and regular echocardiographic surveillance. Fetuses older than 30 weeks of gestation must be assessed for airway compromise and lung development.

In the case of airway compromise, an ex utero intrapartum therapy (EXIT) may be required [9]. Takayasu et al. recommend aspiration of the tumor cyst fluid as first-line therapy when the tumor is cystic in nature [19]. In the case of Wesolowski et al., the patient succumbed to his illness at 50 days of life [21]. Among the four case reports, Wesolowski’s study is the only one with the diagnosis in the neonatal period rather than in the antenatal period. Detection of teratomas before birth with US or MRI is important because the mortality rate is three times greater with a postnatal diagnosis [24].

Liang et al. point out the importance of Doppler velocimetry: there may be a better postnatal outcome when the hemodynamic changes only involve the central vessels and not the peripheral vessels than in those cases in which flow in both central and peripheral vessels is decreased [15]. Wang et al. state that in the case of immature teratoma the correct diagnosis from the prenatal US can be difficult. Instead, the diagnosis of mediastinal teratoma is comparatively easy to make if a multilobular mass has both cystic and solid components with calcification and acoustic shadows [14].

In 2007, Grethel et al. examined their institutional database and looked at patients with fetal mass lesions to evaluate survival with or without intervention. The development of hydrops conferred a dismal prognosis with greater than 95% mortality. Fetal intervention reduced this mortality to 50% [25]. A 32-week gestational age cut-off point has been identified as the critical time-point before which development of hydrops may warrant prenatal intervention, and after which delivery is indicated [26, 27]. It is highlighted that before consideration of fetal intervention, a full evaluation including karyotyping, fetal echocardiography, and level III US must be carried out [25]. In our case, signs of hydrops were present around 31 and 32 weeks of gestation and an elective cesarean section was performed at 32 1/7 weeks’ gestation after the administration of a course of antenatal corticosteroids for fetal lung maturity.

Obtaining a precise diagnosis has become easier with advances in prenatal imaging as well as karyotype analysis. Understanding the prognosis of the diagnosed anomaly is a more difficult task. At present it is not possible to predict which fetuses will become hydropic, and premature intervention carries unacceptable risk to those that will not progress to hydrops [25]. We still know too little about non-invasive measures to improve outcomes. The diagnosis is often done in the second or third trimester of gestation and a multidisciplinary diagnostic workup where obstetricians, neonatologists, and pediatric surgeons work closely becomes crucial.

Figure 1. — Sonogram showing mediastinal mass and pleural effusion.

Figure 2. — Sonogram showing the large formation occupying all the anterior mediastinum.
References


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Tubo-ovarian abscess presenting as an ovarian tumor in a virginal adolescent: a case report

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Introduction
Pelvic inflammatory disease (PID) is defined as an inflammatory process of the upper genital tract containing one or more of the following: endometritis, salpingitis, tubo-ovarian abscess (TOA) and pelvic peritonitis [1]. TOA occupies 15% of patients with PID, however it is extremely rare in sexually inactive girls [2]. A case of an adolescent virgin with unilateral TOA, severe bowel and omental adhesions is presented.

Case Report
A 13-year-old sexually inactive patient was admitted with abdominal pain and menstrual disorder. Blood pressure was 120/70 mm Hg, pulse was 84/bpm and fever was 37.2°C. The patient had no history of sexual activity, any recent infection or sexual abuse. Physical examination revealed lower quadrant abdominal tenderness, an intact hymen and a mass of approximately 7 x 7 cm in size in the left adnexal area (observed during rectal examination). Pelvic ultrasonography pointed out a semisolid, hyperechogenic mass of 57 x 73 mm in the left adnexal area. A dense cystic semisolid mass (7 x 6.4 cm) with thickened walls and peripheral contrast was detected by computed tomography (CT). Total blood count, biochemical parameters, serologic tests (HIV, Hepatitis B and C) and tumor markers (CA-125, CA 19.9, CA-15.3, AFP, CEA, HCG and LDH) were within normal range. Laparotomy revealed a left unruptured TOA adhering to the bowel and omentum. Abscess drainage and adhesiolysis were performed and postoperative antibiotic therapy was administered. TOA should be considered in the differential diagnosis of females with abdominal pain and adnexal mass whether sexual activity is present or not.

Discussion
TOA, a serious complication of pelvic inflammatory disease, is extremely rare in sexually inactive adolescents and has devastating effects on the genital tract. Neisseria gonorrhoeae is a common cause of PID; however, most cases of acute PID are the result of a polymicrobial infection caused by organisms ascending from the vagina and cervix and infecting the lining of the endometrium and fallopian tube [3]. Approximately 85% of cases are infections in sexually active women at reproductive age. The remaining 15% of infections occur after procedures that break the cervical mucus barrier and allow vaginal flora to infect the upper genital tract [3]. Pelvic abscess and PID are frequent complications of sexually spreading infections and are rarely reported in sexually inactive adolescents. TOA may develop subsequent to a first attack of acute salpingitis however it usually follows recurrent infections of chronically damaged adnexal tissue [4]. Eight cases of sexually inactive patients who had TOAs reported in the English literature are shown in Table 1 [5-11].

As shown in Table 1, the majority of patients were under 20 years of age and the dominant organism was Escherichia coli, however several organisms were considered as the possible etiology. Although our patient had no sign or symptom of lower genital tract or any other systemic infection, we determined that unilateral TOA was a...
result of ascending infection from lower genital tract microorganisms. Cervical secretions act as a barrier against ascending infections. Defending mechanisms and antimicrobial activity of cervical secretions are attributed to lysozyme and lactoferrin. Variation in the composition of cervical secretions may increase the risk of ascending infections and result in serious complications, especially in pregnant women [12]. We consider that the vulnerability of our patient against pelvic infection was related to compositional changes in cervical secretions. Our results support the idea that sexual activity is not an absolute condition for the development of upper genital infection.

Diagnosis of TOA has some clinical and radiological difficulties and confusion due to limited sensitivity and specificity of diagnostic procedures [13]. TOA should be considered in the differential diagnosis of women with abdominal pain and an adnexal mass whether sexual activity is present or not. Earlier and effective treatment plays a crucial role in preventing long-term complications such as infertility, ectopic pregnancy and chronic pelvic pain, especially in younger patients.

Table 1. — Case reports of TOA in female virgins.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Age</th>
<th>Microorganism</th>
<th>Biology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moore et al. 1999 [6]</td>
<td>13</td>
<td>E. coli</td>
<td>Obesity induced vaginal voiding</td>
</tr>
<tr>
<td>Fumino et al. 2002 [8]</td>
<td>13</td>
<td>Colonic flora</td>
<td>Vaginoplasty for cloacal anomaly</td>
</tr>
<tr>
<td>Dogan et al. 2004 [9]</td>
<td>19</td>
<td>E. coli</td>
<td>Unknown a-hemolytic streptococci</td>
</tr>
<tr>
<td>Arda et al. 2004 [10]</td>
<td>15</td>
<td>E. coli</td>
<td>Concomitant urinary tract infection</td>
</tr>
</tbody>
</table>

Case one: 16 Bacteroides uniformis, Coagulase negative staphylococcus, Streptococcus milleri

Case two: 12 E. coli

Recurrent tract infection, obesity, constipation, poor hygiene

References


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Early ovarian pregnancy diagnosed by ultrasound and successfully treated with multidose methotrexate.

A case report

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Summary

A case report of a primary interstitial ovarian pregnancy is presented. A 37-year-old married woman with two children after two Cesarean sections and a spontaneous abortion, with a contraceptive intrauterine device (IUD) inserted three years before, presented at five weeks plus five days amenorrhea with a positive pregnancy test and lower abdominal pain but with no vaginal bleeding. Her previous menstrual cycles had been regular. She was hemodynamically stable. On bimanual examination, the uterus was of normal size, and there was an approximate four-cm tender right adnexal mass. Serum beta-human chorionic gonadotropin (b-hCG) was confirmed positive. Ultrasound revealed a well-positioned IUD in the uterus and a right adnexal mass with normal vascular flow on Doppler, that contained a well-defined gestational sac, well-distinct from the quiescent hemorrhagic corpus luteum. There was no fetal node or cardiac activity or free fluid. The patient received four injections of methotrexate intramuscularly using the multidose regimen that involves the administration of methotrexate calculated according to body weight, alternated with 0.1 mg/kg of leucovorin calcium per os after 30 hours until the values of b-hCG had decreased by 15%. The patient’s post-treatment period was uneventful with a full restoration of ovarian morphology and the complete absorption of the gestational sac. This case is the first where diagnosis was made by endovaginal sonography and treatment was made by multidose methotrexate. Spiegelberg criteria for the diagnosis of ovarian pregnancy are obsolete; new ultrasound and laboratory criteria are needed for a diagnosis as early as possible without the need of surgery.

Key words: Ectopic pregnancy; Ovarian pregnancy; Pregnancy; Multidose methotrexate; MTX; Ultrasound.

Introduction

Ovarian pregnancy is a rare type of extrauterine pregnancy. Its incidence is about one in 7,000 to one in 60,000 pregnancies and accounts for about one to three percent of all extrauterine pregnancies. Recently there appears to have been an increase in ovarian pregnancy due to the improvement in diagnosis ability. Sonography and beta-human chorionic gonadotropin (b-hCG) have made it easier for the early preoperative diagnosis of ectopic pregnancy. In primary ovarian pregnancy the ovum is not guided into the tube but is fertilized in the peritoneal cavity and then implants onto the ovary. It causes the same symptoms as a tubal pregnancy and severe internal bleeding will eventually occur. Primary ovarian pregnancy is a rare entity; the reported incidence being one in 25,000 pregnancies, 0.5 - 3% of extrauterine pregnancies. The diagnosis is difficult and a continuous challenge to the gynecologist [1].

In the secondary type, there is a tubal abortion with secondary implantation of the embryo on the tubal surface. Ovarian pregnancy is probably an accidental event that occurs in fertile women in contrast to tubal pregnancy, which is more frequently associated with impaired fertility.

Early diagnosis of an ovarian pregnancy is perhaps the most difficult compared to all the other types of extrauterine gestations. The signs and symptoms of a ruptured ovarian pregnancy are similar to those of disturbed tubal pregnancy. Although an adnexal mass is palpable in many cases of ovarian pregnancy, the mass is frequently confused for a hemorrhagic corpus luteum cyst or ruptured tubal pregnancy [2]. With a few exceptions, the initial diagnosis is made on the operating table and the final diagnosis only with histopathology on the basis of the four Spiegelberg criteria described in 1878 [3]: the tube must be entirely normal, the gestational sac must be anatomically sited in the ovary, the ovary and the gestational sac must be connected to the uterus by the utero-ovarian ligament, and placental tissue must be mixed with the ovarian cortex.

Until today, histology alone can confirm the diagnosis and distinguish the four forms: intrafollicular, juxtafolicular, juxtacortical, and interstitial pregnancy [4]. New ultrasound and laboratory criteria are needed for a diagnosis as early as possible without the need of surgery.

The present report concerns a primary interstitial ovarian pregnancy with an omolateral corpus luteum.

Case Report

A 37-year-old married woman with two children after two Cesarean sections and a spontaneous abortion, with a contraceptive intrauterine device (IUD) inserted three years before, presented at five weeks plus five days amenorrhea with a positive pregnancy test and lower abdominal pain but with no vaginal bleeding. Her previous menstrual cycles had been regular. She was hemodynamically stable and her hemoglobin was 11.8 g/dl.

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Early ovarian pregnancy diagnosed by ultrasound and successfully treated with multidose methotrexate. A case report

On bimanual examination, the uterus was of normal size, and there was an approximate four cm tender right adnexal mass. Serum b-hCG was 1,866 mIU/ml (reference value is < 5 mIU/ml). Ultrasound revealed a well-positioned IUD in the uterus with an atrophic endometrium and a right adnexal mass with normal vascular flow on Doppler, that contained a well-defined gestational sac of 0.74 cm, well-distinct from the quiescent hemorrhagic corpus luteum, with a more prominent echogenic ring surrounding an echolucent center (Figure 1). There was no fetal node or cardiac activity or free fluid. The gestational sac in the right adnexum was identified and confirmed by three observers.

This patient had nearly all the features that suggest successful medical management; ie, low b-hCG level, endometrial thickness < 12 mm, no cardiac activity, and no yolk sac. Her progesterone levels were not measured. Although her serum folic acid levels were also not estimated, she was not taking peri-conceptional folic acid supplementation.

The patient received an injection of methotrexate intramuscularly the next day (60 mg, ie, 1 mg/kg), using the multidose regimen that involves the administration of methotrexate calculated on body weight, alternated with 0.1 mg/kg of leucovorin calcium per os after 30 hours until the values of b-hCG had decreased by 15% (maximum four cycles of administration). The serum b-hCG measurement was repeated on day three, as per standard protocol, (amenorrhea six weeks plus one day). The result, available the same day, indicated b-hCG was 4,162.8 mIU/ml, but repeated ultrasound scanning showed the same findings without hemoperitoneum. She showed no clinical and laboratory signs of toxicity, so a second dose of methotrexate was administered. The serum b-hCG measurement was repeated on day five (amenorrhea six weeks plus three days), the result was 5,201.8 mIU/ml, the vaginal ultrasound did not show a worsening of the clinical status and low-abdominal symptoms of the patient appeared in remission, despite the rising titers of b-hCG, it was decided to proceed with the third dose of the drug. On day seven serum b-hCG values had decreased to 3,584.6 mIU/ml and the ultrasound showed a smaller gestational sac of 0.65 cm and a corpus luteum partially reabsorbed (Figure 2). Despite the decline in values by over 15%, it was decided to proceed to the fourth administration and complete all four treatment cycles to reinforce the therapeutic response. After two days the patient experienced a massive metrorragia and the IUD was removed ambulatorially. The patient’s post-treatment period was uneventful, she did not exhibit any side-effects to methotrexate, which has also been shown to predict treatment failure. After a week, the b-hCG values were 500 mIU/ml, and after another two weeks they were completely negative (1.28 mIU/ml), with a full restoration of ovarian morphology and the complete absorption of the gestational sac (Figure 3). The patient has been recommended to monitor monthly b-hCG values for at least six months.

Discussion

Environmental conditions favouring tubal ectopic gestation, such as pelvic inflammatory disease, previous surgery, and history of infertility are very rare in ovarian pregnancies [5, 6]. Recurrence is also exceptional and as the fertility of these women is conserved, the next pregnancy is usually intrauterine. However, a few risk factors seem to be present for ovarian pregnancies: endometriosis and IUD usage are reported to contribute in the majority of cases. If the patients have an IUD and a positive pregnancy test, an ectopic ovarian pregnancy must be suspected [7].

The literature shows a strong association between multiparity and IUD usage in cases of ovarian pregnancies [8]. An IUD is effective in preventing intrauterine and tubal pregnancies in 99.5% and 95%, respectively. However it has little effect on the prevention of an ovarian pregnancy [9].

The rate of IUD use in reported ovarian pregnancies is 17% to 25% [10]. Raziel et al. reported that 90% of ovarian pregnancies occured in IUD users [11].
Several theories have been suggested to explain ovarian implantation, such as reflux of the conceptus following a normal fertilization from the Fallopian tube along with blood from the uterus [10] or fertilization occurs within the follicule following defective ovum release at ovulation [12]. Since ovarian pregnancy may result from in vivo fertilization (IVF) of unrecovered oocytes, patients should be informed to avoid intercourse near the time of ovulation [13].

The incidence of ectopic pregnancy per se is on the rise owing to evolution in assisted reproductive techniques (ART). Ovarian pregnancy accounts for 0.5% - 3% of all ectopic pregnancies [11] and the incidence after IVF has been reported to be 0.3% [14].

The diagnosis of an ovarian ectopic pregnancy is seldom made before surgery. The recent advances in h-hCG determination and transvaginal ultrasound have aided the diagnosis. Ultrasound, especially transvaginal scanning has been proven to be an invaluable tool in the diagnosis of this condition. Even then, it can be mistaken for a hemorrhagic corpus luteum or ovarian cyst. The presence of a hemorrhagic lesion on the ovaries should arouse the suspicion of the surgeon of an ovarian ectopic pregnancy. If a concomitant corpus luteum is seen as in this case, then the diagnosis becomes easier.

As for other ectopic locations, the combination of symptoms, such as abdominal pain with or without vaginal bleeding, with a history of antecedent amenorrhoea, raised h-hCG levels, and an ultrasonographically empty uterus, should trigger an investigation for ectopic pregnancy. The diagnosis is usually suggested after ultrasound. A more echogenic wide ring on the ovary, compared with the ovarian tissue, with a yolk sac or fetal parts are key ultrasonographic indicators for ovarian pregnancy [5, 15, 16]; however, an embryo is relatively infrequently seen within the cyst.

Both sonographically and at the time of surgery, the clinical challenge is to distinguish an ovarian ectopic pregnancy from a corpus luteum or hemorrhagic cyst [1, 17, 18], because a cystic adnexal mass with a positive pregnancy test without clear intrauterine gestation could also indicate a corpus luteum in an early or failing intrauterine or tubal pregnancy. Decreased wall echogenicity compared with the endometrium and an anechoic texture suggest a corpus luteum [17]. Color or spectral Doppler sonography do not seem to fulfill additional diagnostic expectations, yet Atriv [19] found that a resistive index of < 0.39 had a specificity of 100% and a positive predictive value of 100% for diagnosing ectopic pregnancy, but was present in only 15% (confidence interval 7% - 23%) of ectopic pregnancies. He concluded that both low- and high-resistive indices discriminate ectopic pregnancy from a corpus luteum cyst.

Halla [20], in his study of 25 cases of ovarian pregnancies, reported that the most significant finding was the inability to distinguish an ovarian pregnancy from a hemorrhagic ovary or ruptured corpus luteum. A correct surgical diagnosis was only made in 28% of the cases. In the remaining cases the pathologist made the diagnosis [21].

Ruptured ectopic pregnancy with circulatory collapse [22] or wrong diagnosis of malignant ovarian tumours producing h-hCG [23] may also decrease the accuracy of diagnosis.

Early preoperative diagnosis based on vaginal ultrasonographic findings has resulted in conservative treatment in singleton ovarian cases [24].

There are two possible therapeutic approaches to ovarian pregnancy: surgical (partial or total ovariectomy) and pharmacological (methotrexate, etoposide, and prostaglandins).

Conservative treatment in the ovarian pregnancy, as in tubal pregnancy, is of the utmost importance if the patient is young and desires to bear children. Methotrexate is an effective therapeutic option in the management of unruptured ovarian ectopic pregnancy. It permits to avoid more invasive interventional surgery, with possible complications such as hemorrhage, ovariectomy, or later pelvic adhesions [25].

For selected ovarian pregnancies, an alternative therapy using methotrexate or prostaglandin may possibly minimize adhesion formation and optimize future fertility. The first successful case of treatment of unruptured ovarian pregnancy by progestagen and methotrexate was reported in 1990 by Koike et al. [26]. It is followed by the first successful case of treatment of unruptured ovarian pregnancy by methotrexate by Shamma and Schwartz in 1992 [27]. Mittal et al. [25] reported the third case of successful treatment of an ovarian pregnancy with methotrexate. Similiarly Chelmow et al. treated an ovarian pregnancy diagnosed by laparoscopy with methotrexate [28].

Medical treatment options are reported with etoposide [29] and methotrexate if h-hCG levels are still raised after surgery, indicating persistent trophoblastic tissue [30]. Some authors treated ectopic pregnancies by sonographically-guided injection of methotrexate or potassium chloride into the ectopic gestational sac or fetus and thus see the advantage of the continuation of a concomitant intrauterine pregnancy, and preservation of the uterus for subsequent pregnancies [31-33] by avoiding surgical intervention.

Conclusion

This case report is the first to demonstrate the usefulness of a multidose methotrexate protocol in the treatment of ovarian pregnancy. It is extremely important to select patient candidates according to a pharmacological approach and make a diagnosis as early as possible to improve the therapeutic success and preserve future fertility. Spiegelberg criteria are obsolete; new ultrasound and laboratory criteria are needed for a diagnosis without the need of surgery.

Do not be discouraged if you get an initial increase in the values of the h-hCG during the first cycles of treatment; it must be remembered that the possible number of treatment cycles with methotrexate is four.

Following monthly h-hCG concentrations for six months is important since remaining pregnancy cells may be able to grow post-treatment.
Early ovarian pregnancy diagnosed by ultrasound and successfully treated with multidose methotrexate. A case report

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Silent rupture of an unscarred uterus at third-trimester abortion correlated with an unrecognized perforation

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Summary
Silent uterine rupture in an unscarred uterus during pregnancy is rare. We present a case of silent uterine rupture in an unscarred uterus at third-trimester abortion by use of mifepristone and misoprostol in a patient who had a history of intrauterine procedures. The absence of clinical symptoms suggests that this uterine rupture resulted from an unrecognized perforation in a previous intrauterine manipulation. Routine sonographic follow-up examinations and careful clinical observations are especially recommended for women with previous intrauterine manipulations.

Key words: Uterus rupture; Abortion; Intrauterine manipulation; Uterus perforation.

Introduction
Uterine rupture is a serious pregnant complication, with an increased risk of maternal and perinatal morbidity and even mortality, which usually occurs in women with a known uterine scar, such as cesarean section or myomectomy. An unscarred uterine rupture is relatively rare and has been estimated to occur in approximately one in 8,000-15,000 deliveries [1]. These cases are usually associated with iatrogenic uterine perforation, inappropriate induction or augmentation of labor, multiparity, application of fundal pressure, placenta acreta, and congenital anomalies. Here, we present a case of silent uterine rupture in an unscarred uterus at third-trimester abortion by use of mifepristone and misoprostol in a patient with a history of intrauterine procedures.

Case description
A healthy, 28-year-old woman, gravida 5, para 1, abortion 3, was referred to our hospital at 32+4 weeks of gestation for induction of labor due to ultrasonic (US) findings of oligohydramnios and fetal anomaly. At 27 and 32 weeks of gestation, US examinations both indicated a remarkable decrease of amniotic fluid volume (1.8 and 0.8 cm in the amniotic fluid index, respectively), fetal growth restriction (5.3 and 6.3 cm in BPD, 3.8 and 4.4 cm in FL, respectively) and dysplasia of the fetal kidney. Additionally, Doppler color flow imaging also showed a single-peaked umbilical blood flow in the frequency spectrum.

After an initial clinical assessment, the patient was pretreated with 200 mg of mifepristone orally (50 mg every 12 hr, 4 times) followed by 200 g of misoprostol orally 2 hr later. The signs of labor, including uterus contraction, abdominal pain and vaginal bleeding, were then regularly monitored in the ward. No apparent contraction or bleeding was observed and the patient had no special complaint except slight pain in the lower abdomen. In regular anal examinations, the cervix remained closed, firm and in midposition. The patient’s general condition preceded uneventfully, until 38 hours later when the uterus fundus was found to be lower and the shape of the uterus on the right side was irregular by careful palpation during regular clinical rounds. A bedside US examination was performed to rule out the possibility of uterus rupture or placenta abruption. A retracted uterus with an empty cavity was revealed, but the placenta was fundally sited with no evidence of abruption. No free fluid in the maternal abdominal cavity was demonstrated. Most striking was the finding of an 8 cm defect in the anterior uterus wall, through which the trunk and some limbs of a dead fetus were intruding into the maternal abdominal cavity, which could be visualized between the intestines and bladder. As this situation was considered to carry serious maternal risks, an emergency laparotomy was performed. After opening the abdominal cavity, no hemoperitoneum was observed. An incomplete laceration of approximately 10 cm on the anterior surface extending from the right lower segment to the left uterus body was identified. The dead fetus covered with the uterovesical peritoneum was located between the retracted uterus cavity and the abdominal cavity. Through opening the incomplete laceration, the dead fetus was removed and the clear amniotic fluid and normally placed placenta were identified, with no evidence of abruption. The uterus was preserved by a 2-layer suture. The patient recovered uneventfully, and was discharged seven days later.

Discussion
Based on the dosage of mifepristone and misoprostol and absence of abnormal symptoms, we can almost rule out the possibility of inappropriate use of mifepristone and misoprostol as being the cause of uterine rupture in this case. This patient was multiparous and had no history of cesarean section or myomectomy. Concerning the fact that she had had instrumental abortion three times during first-trimester pregnancy, we hypothesize that this silent uterine rupture may have resulted from an unrecognized perforation in a previous intrauterine manipulation. Although uncommon, uterine perforation is a known...
Silent rupture of an unscarred uterus at third-trimester abortion correlated with an unrecognized perforation

A complication of intrauterine manipulation, such as curettage, instrumental abortion and hysteroscopy. The incidence of uterine perforation associated with abortion by suction curettage has been reported to be 0.08-0.17% [2]. Perforations following the first-trimester intrauterine manipulations may be symptom-free and, in fact, usually be difficult to detect. In a previous report, 14 uterine perforations (2%) in a series of 706 cases with a history of first-trimester curettage were demonstrated by laparoscopic sterilization, of whom 12 (86%) had not been recognized during the procedure [3]. Although the first-trimester intrauterine manipulation is generally considered to be minimally invasive and relative harmless, usually with no short-term consequences, some hazardous maternal and neonatal complications in subsequent pregnancies may be associated with this procedure. Some reports have suggested that a few of unscarred uterine ruptures should have a possible relation with previous uterine manipulations. Notably, these uterine ruptures usually occur in second- or third-trimester pregnancy [4-7]. By stopping bleeding and suturing the laceration in emergency laparotomy, most of these cases have ideal clinical outcomes with preservation of the uterus.

Our patient is not contraindicated for subsequent pregnancies, but we informed her of the substantial risks in subsequent pregnancies. She was advised that the interval to the next delivery should be at least 18 months. Inpatient care after 32 gestational weeks and elective cesarean section after 37 completed weeks seem to be the best care for these women [8].

This case and our comments suggest that clinicians should be aware of the possible occurrence of silent uterine rupture in an unscarred uterus during the late stage of pregnancy. Routine sonographic follow-up examinations and careful clinical observations are especially recommended to women with previous intrauterine manipulations.

References

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Figure 1. — A) incomplete laceration in the anterior uterine surface, and the dead fetus covered with uterovesical peritoneum; B) the incomplete laceration was opened and the dead fetus and placenta removed; C) open laceration and empty uterine cavity; D) the laceration was 2-layered sutured and the uterus preserved.
Treatment of early heterotopic interstitial (cornual) gestation with subsequent delivery of an intrauterine pregnancy - case report

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Summary
We present the case of a 38-year-old woman who was treated for a heterotopic interstitial (cornual) pregnancy diagnosed at the 7th week of gestation. The intervention was performed via transvaginal ultrasound-guided aspiration and instillation of a hypertonic solution of sodium chloride into the cornual sac. The heterotopic cornual pregnancy was successfully aborted, and the intrauterine pregnancy was successfully maintained with delivery of a healthy newborn.

Key words: Interstitial pregnancy; Ultrasound; Hypertonic sodium chloride.

Introduction
The simultaneous existence of intrauterine and ectopic implantation is defined as a heterotopic pregnancy. Ectopic pregnancies located in the cornual (intramural) part of the fallopian tubes outside the cavity of the uterus and a narrow channel connected to the endometrial cavity are usually called interstitial ectopic pregnancies. Both in practice and in the literature, the terms cornual and interstitial pregnancy are often used synonymously [1]. According to the literature data, the frequency of heterotopic pregnancies ranges from one in 30,000 spontaneous pregnancies; however, the incidence of heterotopic pregnancies is significantly greater among patients receiving ovarian stimulation and in those undergoing IVF programs (up to 1-3%). Cornual pregnancies account for 2-4% of the total number of ectopic implantations [2-4]. Early diagnosis allows various therapeutic interventions, including conservative, medical, or surgical treatment [5-7]. The present report describes a case of successful pregnancy and delivery after the treatment of a heterotopic interstitial (cornual) pregnancy and suggests possible approaches for the treatment of this potentially dangerous clinical entity.

Case Report
A 38-year-old patient was admitted to the our clinic due to spotting and light pain in the lower abdominal region after five weeks of amenorrhea. The bleeding and pain began two to three days prior to admission to the clinic. During an ultrasound (US) examination, the patient’s gynecologist diagnosed her with a threatening abortion due to a twin pregnancy, and she was referred to the clinic. The patient had had previous laparotomies six and four years prior to admission on the left and right sides of the uterus, respectively, due to ectopic pregnancies. Moreover, she had participated in three failed IVF programs. In the current pregnancy, three embryos were transferred. At admission, her vital signs were stable, and her blood pressure was 118/65 mm Hg. A transvaginal ultrasound (TVS) examination was immediately performed, and two gestation sacs were detected; one normal appearing intrauterine gestation sac that measured 9.74 mm by 7.47 mm, a crown-rump length (CRL) of 3.1 mm and an embryonic heart rate of 148/min was observed. The other gestational sac was located to the right of the cornual region and measured 7.4 mm by 8.2 mm, with a CRL of 3.3 mm, and a heart rate of 132/min. These US parameters were consistent with a twin pregnancy gestational age of five weeks and six days. The cross scanning revealed that the cornual gestation sac was 7.4 mm away from the endometrial cavity (Figure 1). The patient was informed of the diagnosis and the possible types of intervention. She requested that the proposed intervention not threaten the intrauterine pregnancy and its further development. The intervention was performed under general endotracheal anaesthesia. The objective of the intervention was to terminate the cornual pregnancy and to preserve the intrauterine pregnancy. The US parameters were consistent with a gestational age of seven weeks and four days. The required bacteriological cervical smear was sterile, and an Accuvix V10 Ultrasound machine (Medison) equipped with a 4-9 MHz transvaginal color Doppler transducer (EC4-91S) and a puncture guide was employed. After disinfection of the external genitalia and vagina with a povidone-iodine solution, and antibiotic prophylaxis with 2 g IV sodium ceftriaxone (Longaceph), the uterus was scanned along its longitudinal and cross-sectional axes, and the position of each gestational sac was recorded. A 30-cm long, 1.4-mm outer diameter needle (Labotect GmbH, Gottingen) was inserted through the puncture guide and was advanced through the vaginal fornix toward the right horn of the uterus. The needle was connected to the vacuum pump, which is typically used to aspirate ovarian cysts or follicles. To avoid the major blood vessels of the uterus, the vessels were displayed in color, and the needle was directed toward the cornual gestational sac. Puncture guidelines of the needle toward the interstitial (cornual) gestational sac are presented in Figure 2. The center of the interstitial (cornual) gestational sac was positioned on the software-generated guideline. With a brisk movement through the thick uterine wall, the needle was inserted into the gestational sac, and approximately 1.5-2 cm³ of amniotic fluid was aspirated by vacuum (-0.8 mmHg). The aspirate was sent for histopathological analysis. According to the color scan, aspi-
Treatment of early heterotopic interstitial (cornual) gestation with subsequent delivery of an intrauterine pregnancy - case report

...caused the immediate cessation of fetal cardiac activity. Subsequently, under direct US visualization, 2 cm³ of a 20% sterile solution of sodium chloride was instilled into the gestation sac, and the needle was removed. The intrauterine pregnancy was monitored for 10 min to determine the fetal viability of the pregnancy. Bed rest and observation in our clinic was advised for several days. One month later a check up was done. The US parameters were consistent with an intrauterine pregnancy age of 11 weeks. Moreover, the required histopathological analysis confirmed the presence of chorionic villi. The development of the intrauterine pregnancy was monitored monthly, and a normal course was observed. At 38.5 weeks of pregnancy, due to exhibiting symptoms of premature membrane rupture and a prolapsed umbilical cord, an emergency caesarean section was performed. A 5 x 8-mm lump was palpated on the surface of the uterine horn at the puncture site, and a healthy male weighing 3,050 g was born. The postoperative course was uneventful.

Discussion

One heterotopic cornual implantation occurs in every 3,600 pregnancies obtained via IVF programs [8]. Increased probability of cornual gestation in women with a history of repeat tubal ectopic pregnancies, especially after IVF, in our opinion, the consequences are probably not sufficient excision of the interstitial part of the oviduct during the previous salpingectomy and as results of multiple embryo transfer in IVF. The mortality rate of patients with cornual pregnancies ranges from 2.0-2.5% [9, 10]. High maternal mortality rates are attributed to the rupture of the overly stretched cornual myometrial wall [11]. The thick myometrial wall, which surrounds the interstitial gestation, causes late complications and permits a timely diagnosis. Late diagnosis and delayed treatment of ruptures may be associated with massive hemorrhage and even patient death. Thus, most cornual pregnancies end in hysterectomies. Currently, diagnosis and therapy for heterotopic cornual pregnancies remain a significant challenge. In the presence of risk factors, such as previous salpingectomy, tubal pregnancy, induction of ovulation, and the transfer of multiple embryos, gynecologists should consider this rare but dangerous condition. In recent years, due to the timely diagnosis of cornual pregnancies, a variety of therapeutic approaches have been developed [12, 13]. The most common conservative method for the treatment of cornual pregnancies is the TVS-guided administration of prostaglandin (15-methyl-PGF2α), potassium chloride solution (KCL), or methotrexate [14]. These approaches have a limited role in heterotopic (cornual) pregnancy and can have negative effects on survival and development of intrauterine pregnancy [15]. In the present study, the heterotopic pregnancy was successfully terminated by the TVS-guided instillation of 20% NaCl solution. When a vital intrauterine pregnancy is present in patients with a concurrent heterotopic gestation, determination of the β hCG concentration is not required to assess the success of the procedure; only the size and disappearance of the heterotopic gestational sac should be monitored [16]. After the TVS-guided administration of any drug, the size of the gestational sac is typically monitored until it decreases by 75% [17]. According to the literature, the remains of cornual pregnancy can be monitored by TVS in the range of 47 to 64 weeks [18]. In our patient, the rapid disappearance of the US-detected remnants of the interstitial (cornual) pregnancy four weeks after the intervention was attributed to the aspiration of the amniotic fluid prior to the instillation of the hypertonic sodium chloride solution into the gestational sac. To avoid injury to the uterine blood vessels, the needle should be advanced using the color display produced by the vaginal probe. The gestational age and size of the cornual gestation sac at which this procedure can be applied have not yet been established [19]. We consider the lower limit of the feasibility of intervention is the size of the sac in which it can be safely punctured. To determine the upper limit of the pregnancy age for intervention the individual patient should be carefully considered because of the possible risks of spontaneous cornual rupture and consequences of surgical cornual resection.

Figure 1. — Heterotopic interstitial (cornual) pregnancy at 5 weeks/6 days of gestation.
Figure 2. — Puncture guidelines of the needle toward the interstitial (cornual) gestational sac.
Conclusion

This case report demonstrated that our approach is a successful method especially for the treatment of heterotopic cornual pregnancies and allows patients to avoid surgery and preserve intrauterine pregnancies. Prior to selecting a treatment strategy for heterotopic pregnancies, the survival and normal development of the intrauterine pregnancy must be considered. Adequate excision of the interstitial part of the fallopian tube during salpingectomy and avoidance of multiple embryo transfers, could be a good prevention for interstitial (cornual) pregnancy.

References


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Obstetric rupture of the rectovaginal septum and sphincter complex despite an intact perineum: report of three cases

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Summary
Obstetric injury comprising tearing of the rectovaginal septum, rectal mucosa, and anal sphincter complex with limited or no involvement of the perineal body is highly uncommon and reports have rarely been published. There are no guidelines as to how to respond to this obstetric emergency and there is no time for consultation. In order to reach clinical recommendations on repair and management of this unexpected obstetric injury for the midwife or obstetrician, we report three such cases. The three described cases with their corresponding sequence of events and interventions illustrate that successful repair of these types of injury can often be achieved. To minimize factors leading to long-term complications, repair requires the involvement of an experienced gynaecologist and sometimes even a colorectal surgeon.

Key words: Labour; Rectovaginal septum; Perineal body; Anal sphincter.

Introduction
Tearing of the rectovaginal septum, rectal mucosa, and sphincter complex during the second stage of labour with limited or no involvement of the perineal body is highly uncommon and reports have rarely been published. There are no guidelines as to how to respond to this obstetric emergency and there is no time for consultation. What should we advise the midwife or obstetrician who as a 'once in a lifetime experience' is unexpectedly confronted with such obstetric injury? In order to reach clinical recommendations on repair and management, we report three such cases and review the appropriate literature.

Case Report

Case 1
A 32-year-old nulliparous woman presented at term with signs of fetal distress during labour. After hospital arrival, the fetal heart-rate tracing did not demonstrate any abnormalities so that spontaneous labour was allowed to progress to full dilatation. During active pushing, an elbow appeared in the right upper quadrant of the anal orifice. After performing a mediolateral episiotomy, the elbow was pushed back and redirected into the vagina. During the next contraction, a healthy infant was delivered with a birth weight of 4,080 g and an Apgar score of 9/10/10 at 1, 5, and 10 min, respectively. Inspection under spinal anaesthesia revealed a normal rectal tone and positive anal wink. The patient demonstrated no signs of anal sphincter dysfunction.

Case 2
A second case concerned an elderly primigravid woman, pregnant after a period of unwanted infertility. After 39 weeks of pregnancy, spontaneous labour commenced. Simultaneous to vaginal crowning of the fetal head, hairs appeared in the anal orifice. During the next contraction the baby, weighing only 2,700 g, was born in the occiput anterior position without requiring an episiotomy. The surgeon on call was consulted, after which the still-intact perineal body of the perineum was cut for better exposure and anatomically restored as a fourth degree rupture. During inspection the anal sphincter appeared to have ruptured prior to the perineal incision. Repair followed under general anaesthesia according to the standard method for fourth degree lacerations: closure of the vaginal mucosal apex using a continuous inverting 3-0 Vicryl suture on anatraumatic needle up to the mucocutaneous junction, closure of the rectum mucosa with interrupted 3-0 Vicryl sutures, followed by reinforcement using a series of interrupted sutures in the surrounding perirectal fascia. The retracted ends of the sphincter were sutured in an end-to-end fashion using 3-0 Vicryl. The levator ani muscles were sutured in the midline, after which the repair was completed in a similar manner as in a second degree laceration. At follow-up six weeks later the patient reported good continence for stools and flatus.

Case 3
A third case occurred during delayed progress in the second stage of labour of a primigravid woman with the child in complete breech presentation. In between contractions, a sudden copious outflow of amniotic fluid was emitted from the anal orifice, shortly after which a foot prolapsed out of the anus.

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Intuitively, the foot was pushed back. Subsequently, a median episiotomy was performed and extended through the anal sphincter into the rectovaginal perforation. Following this intervention the child was born rapidly and in good condition. The fourth degree lesion was sutured under general anaesthesia. The technique applied was the same as described in case 2, both cases dating back to the time before the introduction of the overlapping method of sphincter repair. At follow-up there was complete anatomical and functional recovery.

Discussion

Perineal injury remains the commonest form of maternal obstetric injury. The perineal body is a fibromuscular elastic structure found in the midline between the rectum and the vagina and is a point of convergence of a number of structures - the external anal sphincter, the perineal membrane, the superficial and deep transverse perineus muscles, the bulbocavernous muscle, some fibers of the levator ani (puborectalis and pubococcygeus muscles), and the posterior vaginal muscles. The apex of the perineal body is continuous with the rectovaginal septum (the fascia of Denonvilliers), as illustrated in Figure 2.

Third-degree (injury to the perineum involving the anal sphincter complex) or fourth-degree perineal tears (including the anorectal epithelium), as classified by Sultan [1, 2], are not uncommon during vaginal delivery. Reported incidence figures vary between 0.5% and 2.5%, with 75% of the cases involving nulliparous women. Alongside nulliparity, the relative risk of suffering obstetric anal sphincter injuries increases with fetal weight, induction of labour, epidural analgesia, shoulder dystocia, a narrow suprapubic arch, persistent occipitoposterior position, prolonged second stage of labour, and forceps or vacuum delivery [1, 3-5]. Satisfactory healing is reached in 90-95% of cases, when repaired promptly at the time of labour [6]. Anal sphincter tears are, however, an important risk factor for long-term anal sphincter dysfunction: up to 60% of women who experience a sphincter tear are reported to experience symptoms of dyspareunia, perineal pain, or anal incontinence [7].
Obstetric rupture of the rectovaginal septum and sphincter complex despite an intact perineum: report of three cases

Obstetric injury comparable to our three cases is highly uncommon and reports have rarely been published. A medline search from 1970 through January 2010 using the keywords labour, delivery, rectovaginal septum, perineal injury, and anal sphincter, revealed numerous reports of fourth degree anal sphincter injuries and rectovaginal tears, all involving the perineal body [1, 3, 4, 6] (Figure 2). Only two case reports describe obstetric injuries involving (averted) transperineal deliveries with rectovaginal septum injury. Stern et al. described a transperineal delivery with tearing of the anal sphincter [8] whereas Kovoor et al. presented a case of an averted delivery of the fetal head through the perineal body [4]. In both cases the rectal mucosa remained intact. To our knowledge, tearing of the rectovaginal septum and sphincter complex including the rectal mucosa during the second stage of labour with limited or no involvement of the perineal body, as reported in our three cases, has not been described to date. In case 1, tearing commenced in front of the anal sphincter and soon extended through the sphincter into the rectal mucosa. The latter two cases, however, demonstrated a different sequence of events with an initial transmural rupture of the rectovaginal septum, extending into the sphincter complex.

In the three described cases, the obstetricians’ response varied from no episiotomy to a mediolateral episiotomy or median episiotomy into the rectovaginal rupture. It is important to realise that even with a rectovaginal rupture with an intact perineum, the anal sphincter will usually have suffered a complete rupture. As our first case demonstrated, leaving the larger portion of the perineum intact can allow successful repair, but hampers recognition of involved structures so that the presence of the sphincter injury may be overlooked. Hence, the obstetrician may perform a median episiotomy extending into the rectovaginal perforation, thus producing a clear operation field similar to the ‘familiar’ fourth degree perineal rupture. Assistance of a colorectal surgeon in these cases is strongly recommended.

The complication risk after tearing of the rectovaginal septum and sphincter complex is substantial. Persistent faecal incontinence is a known complication of anal sphincter injury of obstetric origin [3]. An incidence of 3% of persistent incontinence to solid stool after repair of a third degree perineal rupture is mentioned [9]. However, the true incidence is unknown as detection bias exists in many studies. A rectovaginal rupture accompanying anal sphincter injury will further increase the expected complication risk. Despite the knowledge that rectovaginal septum defects of obstetric origin are generally surrounded by highly vascularised tissue and may therefore heal well after repair [10], approximately 1/10% of third to fourth degree perineal injuries will result in a rectovaginal fistula [11]. Failure of a repair may be due to poor surgical technique including a lack of tension free repair. Haematoma formation, wound infection, faecal impaction, as well as an unreconised second sphincter injury have also been suggested to be potential causes [12]. In case of incontinence or pain at follow-up, referral to a specialist gynaecologist or colorectal surgeon for endoanal ultrasonography and anorectal manometry should be considered. A small number of women may require referral to a colorectal surgeon for consideration of secondary sphincter repair.

Obstetric injury comprising anal sphincter and rectovaginal septum defects are complex types of injury which may lead to a disappointing outcome with a negative impact on the patient’s physical and emotional health [3, 13]. To minimise factors leading to long-term complications, repair requires the involvement of an experienced gynaecologist and sometimes even a colorectal surgeon. As our three cases with their corresponding interventions have demonstrated, successful repair can often be achieved.

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Quadruplet pregnancy complicated by ovarian hyperstimulation syndrome with spontaneous ovulation

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Summary
Ovarian hyperstimulation syndrome (OHSS) commonly occurs as a complication of ovarian stimulation with gonadotrophins. Spontaneous OHSS is an extremely rare event, but can occur as a result of stimulation with pregnancy-derived hCG. We herein report a case of quadruplet pregnancy complicated by OHSS with spontaneous ovulation. The patient had previously undergone ovarian stimulation with clomiphene citrate plus FSH. After that, she conceived spontaneously and developed OHSS after three weeks of amenorrhea. The OHSS was managed by conservative treatment and improved at six weeks of gestation. However, a quadruplet pregnancy became apparent on ultrasound examination. The patient therefore elected to have an induced abortion. Besides the conception in the cycle without administration of exogenous gonadotrophins, the symptoms in this case had the same kinetics as iatrogenic OHSS caused by ovarian stimulation.

Key words: Ovarian hyperstimulation syndrome; Quadruplet pregnancy; Ovulation induction; Spontaneous ovulation.

Introduction
Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening complication. OHSS occurs as a result of excessive ovarian stimulation, typically as a result of ovarian stimulation with gonadotrophins. However, OHSS also has been reported to occur in spontaneous pregnancies [1-3]. In this paper, a case of quadruplet pregnancy complicated by ovarian hyperstimulation syndrome is reported. The patient conceived after spontaneous ovulation. However, she had previously undergone ovarian stimulation. This case is uncommon and interesting with regard to the kinetics of the symptoms.

Case Report
A 32-year-old female, gravida 1 para 1, was referred to another clinic because of secondary infertility on December 2, 2010. Her past obstetrical and familial histories were unremarkable. She had an ovulatory disorder, but her hormonal testing was normal. She had been receiving ovarian stimulation using clomiphene citrate (CC) and FSH. The last administration of CC started on February 12, 2011, on day 5 of her menstrual cycle, and a 150 IU FSH injection was administered on days 8 and 10 of the cycle. The development of two follicles was detected on ultrasound (US) examination. She was followed up without hCG injection. Her basal body temperature (BBT) continued to be low. After that, menstruation began on March 14. She visited that clinic again due to a complaint of abdominal discomfort on May 2. Her urinary pregnancy test was positive. Her last menstrual period was from April 1 to April 6. Ovulation was estimated to have occurred on April 20 based on the BBT (Figure 1). US examination showed enlarged ovaries and ascites. Moreover, hemoconcentration (hct 45.0%, hgb 13.0 g/dl) was noted on laboratory tests. Her thyroid-stimulating hormone and free T₄ levels were normal (3.6 uIU/ml and 0.82 ng/dl, respectively). CA125 was elevated (363.6 U/ml). CA19-9 and CEA were normal (11.6 U/ml and 0.4 ng/ml, respectively). The serum LH and FSH levels were both < 0.1 mIU/ml. The serum estradiol level was 2759.8 pg/ml. The urinary hCG level was 114.2 mIU/ml on the day of admission and 693.0 mIU/ml two days later. Intravenous fluids were administered to maintain a fluid balance and prevent hemoconcentration. On May 9, her complaints of abdominal pain and discomfort were decreased. On May 18, at six weeks of gestation, she was discharged from our hospital. At this time, four intrauterine gestational sacs were found on US examination (Figure 4). On May 24, at six weeks and six days of gestation, a fetus with a heart beat was detected in each of the four gestational sacs on US examination. The patient requested an induced abortion. After obtaining informed consent, the operation (dilatation and curettage) was performed on June 2. Her postoperative course was uneventful. Bilateral ovaries were normalized on June 24.

Figure 1. Menstrual cycle and ovarian stimulation.
Discussion

OHSS is a disease characterized by massive ovarian enlargement together with a fluid shift into extravascular compartments. The development of ascites, hypovolemia, oliguria, and thromboembolism occur as a result of the hemoconcentration and coagulation disturbance. This condition is typically seen as a complication of pharmacological ovarian stimulation.

On the other hand, spontaneous OHSS has been reported to be associated with a mutation in the FSH receptor gene [4], hypothyroidism [5], and hydatidiform moles with abnormally high hCG values [6]. Delbaere et al. [7] have described the differences of chronology between iatrogenic and spontaneous OHSS. The development of OHSS is thought to be related to hCG, with exogenous hCG inducing ovulation in iatrogenic OHSS or endogeneous pregnancy-derived hCG in spontaneous OHSS. The follicular recruitment and enlargement occur during ovarian stimulation with exogenous FSH in iatrogenic OHSS. Iatrogenic OHSS has been reported to usually develop at between three and five weeks of amenorrhea and then it starts to improve after six weeks of pregnancy [7]. On the other hand, the follicular recruitment and enlargement by pregnancy-derived hCG has been reported to start at between six and ten weeks of amenorrhea in patients with spontaneous OHSS [7]. The hCG level usually peaks between eight and ten weeks of pregnancy. The development of spontaneous OHSS is therefore expected to occur at between eight and 12 weeks of amenorrhea [7].

In this case, ovarian stimulation has been previously performed using CC plus FSH. However, the patient conceived after spontaneous ovulation. Her symptoms of OHSS appeared after three weeks of amenorrhea and improved at six weeks of gestation. Besides the concep-
tion in the cycle without the administration of exogenous gonadotrophins, the symptoms had the same kinetics as that of iatrogenic OHSS. This case is uncommon and interesting with regard to the kinetics of the symptoms. The correlation between previous administration of CC plus FSH and the development of symptoms are uncertain.

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Figure 4. — Ultrasound image showing four gestational sacs in the uterus.
Missing ductus venosus: a case report

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Summary

Background: The ductus venosus is a short vessel, present in the newborn infant on the dorsal surface of the liver, connecting the portal and umbilical circulation with the inferior vena cava. Agenesis of the duct is a rare anomaly. Case: A 28-year-old woman was referred to our department for the first trimester ultrasound evaluation. Detailed scanning revealed agenesis of the duct. Fetal echocardiography showed cardiac disproportion at the level of the ventricles. Conclusion: Agenesis of the duct can be related to either cardiac or congenital abnormalities.

Key words: Ductus venosus and aneuploidy or heart problems; Agenesis of ductus venosus; Ventricular disproportion; Congenital liver abnormalities.

Introduction

The embryological development of ductus venosus affects, to a great extent, the prenatal course and pregnancy outcome. Agenesis of the tiny duct is related to cardiac structural anomalies and various congenital anomalies. An abnormal course of the umbilical vein seems to be the first indication that the duct is probably not there. Some cases have been reported in the past [1].

Case Report

A 28-year-old woman was referred to our fetal medicine clinic in the first trimester (13+1 weeks) for a routine scan evaluation. She was a non-smoker, with a pre-pregnancy weight of 75 kg and a BMI of 26; no allergies or comorbidities were reported. Nuchal translucency (NT) was 1.8 mm, the nasal bone was present and blood flow across the tricuspid valve was normal. Maternal serum biochemistry (free beta hCG: 1.223MoM, PAPP-A: 1.2988MoM) gave her a low risk for Down’s syndrome. Detailed fetal ultrasound (US) examination showed a single viable fetus with agenesis of the ductus venosus and an umbilical left iliac vein-inferior vena cava aberrant connection (Figure 1). Fetal echocardiography revealed ventricular disproportion. No hydrops had developed and the portal vein was absent. Fetal karyotyping detected no chromosomal abnormalities. After birth, the newborn’s heart on US confirmed ventricular disproportion (the right side larger than the left). During the infant’s neonatal period the intracardiac pressure reached the normal values and the heart size became normal.

Discussion

The most common congenital malformations are cardiac defects. The ductus venosus is a small fetal vessel that transfers oxygenated blood originating from the placenta to the fetal heart through the umbilical vein. Ductus venosus shunts a significant majority of the blood flow of the umbilical vein directly to the inferior vena cava. In this way, it allows oxygenated blood from the placenta to bypass the liver, thus playing a critical role in preferentially shunting oxygenated blood to the fetal heart and brain. The amount of shunting is determined by the diameter of the vessel, the pressure gradient and blood viscosity. The blood from the ductus venosus is then directed towards the left atrium with high velocity due to the small diameter of the vessel. This flow bypasses the right atrium, reaches the left atrium through the foramen ovale and enters the ascending aorta towards the coronary and brain vessels [1]. It is one of the three physiological shunts determining blood distribution during intrauterine life and is greatly involved in the regulation of fetal circulation, acting as a sphincter to protect the fetus from placental overcirculation. The volume of its flow is altered according to the pressure gradient between the umbilical vein and the heart. Absence of the ductus venosus leads to direct umbilical venous return into the heart. The prenatal diagnosis of an abnormal waveform on the ductus during first trimester scanning can be portrayed as a sign of an abnormal pregnancy course [2].

The absence of the ductus venosus is usually related to adverse pregnancy outcome [2]. Similarly, the abnormal flow pattern of the ductus is associated to structural, cardiac and chromosomal defects of the embryo in both singletons and multiple pregnancies [3]. The ductus venosus normal waveform is altered during the second trimester, mainly due to severe intrauterine growth restriction, twin-to-twin syndrome, heart defects and cardiac vessel abnormalities [4]. Today, the assessment of ductus venosus flow is proposed as an integral part of the 11-13-week scan. Ductus venosus flow assessment improves the performance of screening by NT thickness and serum PAPP-A and free -hCG [3, 4].

A negative a-wave on the ductus venosus waveform in combination with a backflow along the tricuspid valve can be a reliable indicator of serious cardiac abnormalities, i.e., ventricular disproportion and unfavorable fetal outcome [5]. Fetuses with no ductus venosus should be carefully scanned for any additional anatomical abnormalities [6]. Moreover, techniques such as two-phase helical computed tomography, magnetic resonance (MR) imaging and MR angiography can shed light on the vascular anomaly of ductus venosus [7].
In the early second trimester of pregnancy, fetal cardiac malformations can be detected by the ductus venosus Doppler waveform and the four-chamber view [8]. Going back in the past, sonographical detection of the nasal bone was part of first trimester prenatal testing, while in the second trimester the nasal bone length was put into consideration [9].

During the first trimester, the ductus venosus can be easily imaged with color Doppler and its flow waveform can be visualized by pulsed Doppler. It is identified as the part of the vessel bearing the highest blood velocity, following the umbilical vein. Ductus venosus has a typical waveform with three phases: a) the highest velocity peak that corresponds to the ventricular systole while the pressure gradient between the umbilical vein and the atrium is the highest; b) the peak of forward flow that corresponds to early diastole, throughout the opening of the atrioventricular valves and early passive filling of the ventricles; and c) the lowest velocity that corresponds to the atrial contraction, during late diastole. In this last phase the atrial pressure is high and there is a low pressure gradient [10, 11].

Fetuses with abnormal vascular connections in the liver parenchyma require detailed scanning and evaluation [12]. The number of cases of ductus venosus agenesis with a normal or abnormal course of the umbilical vein have been found postmortem, postnatally or prenatally [1].

Absence of the ductus venosus vessel is a very rare pathological finding, which may be compatible with normal fetal development. Non visualization of the vessel’s anatomical position combined with increased amniotic fluid, an abnormal heart structure and a deviated umbilical vein during scanning suggests non formation of the ductus. Careful US assessment of the ductus venosus and the umbilical vein should be performed on every fetus with unexplained cardiomegaly, polyhydramnios, ascites or hydrops [13-15].

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Figure 1. — Umbilical vein-left iliac vein-inferior vena cava aberrant connection.
Spontaneous rupture of splenic hemangioma in puerperium

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Summary

Atraumatic splenic rupture is a rare clinical entity and in the absence of trauma, the diagnosis and treatment are often delayed. In this article the authors discuss a case of a 45-year-old woman, gravida 5, para 4, with spontaneous splenic rupture on her second postpartum day. The rupture was related to a splenic hemangioma that is a vascular malformation and the most common neoplasm of the spleen. Despite the fact that hemangiomas are the most common primary neoplasms of the spleen, only few cases of splenic rupture have been described in pregnancy or puerperium. However, spontaneous splenic rupture is a rare event and the rupture should be suspected in woman with unexplained abdominal pain or with clear signs of haemorrhage.

Key words: Spontaneous splenic rupture; Splenic hemangiomas; Puerperal disorder; Postpartum splenic rupture; Splenectomy in postpartum.

Introduction

The first reported atraumatic spleen ruptures were described in 1891, by Rokitansky, in a patient with leukemia [1] and in 1874 by Atkinson who reported the rupture of an apparently normal spleen [2]. Since then numerous atraumatic splenic ruptures have been described in literature and often a distinction has been made between the rupture of a normal spleen defined as a “true spontaneous” rupture and the rupture of a diseased spleen described as a “pathologic” or “occult” rupture.

True spontaneous splenic rupture has been reported in literature, but its validity has often been challenged. Wright and Prigot stated “There is no such clinical entity as spontaneous rupture of the normal spleen” implying that thorough questioning and investigation will reveal a history of trauma or splenic pathology [3]. After reviewing reports of spontaneous splenic ruptures through 1958, Orloff and Peskin found that most cases had an identifiable pathologic or traumatic source [4].

Splenic rupture in pregnancy has been attributed to the patient’s hypervolemic state, splenic enlargement, and diminished peritoneal cavity volume due to the enlarged uterus and muscular contractions during pregnancy [5]. Several authors have suggested that contributing factors to splenic rupture may include congenital malpositioning of the spleen or anatomical characteristics such as a short splenic pedicle or deeply recessed location [6]. This would predispose the spleen to trauma from a compressing diaphragm during coughing, sneezing or contractions [7].

Case Report

A 45-year-old woman was admitted to the San Salvatore Hospital of Aquila at 39 weeks of gestation, gravida 5, para 4. She had previously been successfully treated for a superficial phlebitis in her left leg and during late pregnancy had developed a mild hypertension that was treated with M ethyldopa.

Two days later she gave birth to a healthy child weighing 3,800 grams. The morning after, the patient was anxious but well-oriented as to time, place, and person. She was febrile, pale, and had a mild circulatory decompensation (blood pressure of 90/60 mmHg, pulse rate 110 hr/min). There was no previous history of direct trauma and the labor was uneventful.

Physical examination revealed a distended abdomen. Tenderness was elicited in the left and right hypochondrium and in the left subcostal region on deep palpitation with no guarding or rebound tenderness. No mass could be palpated. The liver and the spleen were not palpable. Kehr’s sign was positive. Bowel sounds were present with no signs of peritonitis. Blood counts were as follows: Hb 9.6 g/dl, Hct 32.8%, Plt 296 mmc, WBC 25,000 mmc, Pmn 84%, lymphocytes 7.6%, monocytes 6.4%.

X-rays showed a marked distension of the central abdominal jejuno-ileum loops and a relaxed cecum with plenty of fecal material.

An abdominal ultrasound scan of the spleen showed large, round low-density multiple areas similar to cysts, the largest of which was located in the lower pole of the spleen.

Blood tests were repeated two hours later and a fall in the hematocrit was noted: Hb 8.5 g/dl, Htc 28.8, Plt 299 mmc, WBC 17,000 mmc, Pmn 69%.

Urgent computed tomography (CT) with contrast was performed showing significant haematomatous effusion, probably in the parieto-colic space. A midline laparotomy was performed urgently for hemoperitoneum.

Marked bleeding in the left hypochondrium and the presence of a lesion was noted on the hilar surface of the spleen. Approximately 2,000 cc of free blood were found in the peritoneal cavity.

Splenectomy was performed. The patient was transfused with five units of blood and 600 ml of fresh frozen plasma during the operation. She had an uneventful recovery and was discharged from the hospital on the tenth post-operative day. At one month follow-up, the patient was healthy.

The histopathological examination of the spleen revealed cystic cavities of 2, 1.5, and 0.4 cm. The biggest cavity measured 17 x 10 x 4 mm. A laceration of 3 cm in length was noted on the outer surface of the spleen. Histopathologic examination confirmed capillary hemangioma as the source of hemorrhage.

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After careful questioning, trauma and delivery complications were ruled out as possible causes of the rupture, leading the authors to conclude that the rupture was spontaneous.

**Discussion**

Hemangiomas are vascular neoformations composed of rapidly proliferating endothelial vascular channels filled with blood cells which are usually found incidentally. Indeed most cases are of a sporadic nature although they can be inherited in a autosomal dominant pattern [8]. Splenic hemangiomas are thought to be congenital in origin [9]. They can be solitary or multiple and are frequently detected by chance during CT or ultrasounds in patients in their fourth or fifth decade of life or at autopsy. Serious complications of hemangiomas include rupture or malignant transformation [10]. Risk of rupture is thought to be increased in late pregnancy or in puerperium, especially in multiparous women, secondary to the effects of estrogens on hemangiomas. In fact, hemangiomas show immunopositivity to estrogen receptors [11]. Other factors that increase the risk of splenic rupture in late pregnancy or in puerperium are spleen enlargement, diminished peritoneal cavity, a hypervolemic state, uterus enlargement, and contractions [12]. All these factors increase the overall risk of rupture of an hemangioma which in this case occurred after labor.

**Conclusion**

Spontaneous spleen rupture is an uncommon event and delay in its diagnosis can lead to an increase in morbidity and mortality. The diagnosis of splenic rupture should be taken into consideration when a patient presents with sudden onset of upper abdominal pain. The clinical diagnosis can be backed up by radiology and ultrasound with power Doppler. However magnetic resonance imaging is the most sensitive and specific means of diagnosing of splenic rupture. Without immediate splenectomy the prognosis is always fatal.

**References**


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Adenomyosis completely encapsulated by muscle-like cavity in the mesorectum: a case report

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Summary
A case of adenomyosis, completely encapsulated and located in the mesentery between the sigmoid colon and rectum, was admitted to our hospital. We have reported the symptoms, diagnosis, and treatment of the present case.

Key words: Adenomyosis; Muscle-like cavity; Mesorectum.

Introduction
Adenomyosis is a benign disease of the uterus caused by ectopic translocation of endometrium to the myometrium. A typical symptom of adenomyosis is aggravating dysmenorrhea, and is frequently accompanied by menstrual disorders, anemia, infertility, and sexual pain [1, 2]. Adenomyosis is not considered as a tumor per se because it does not have a capsule and cannot be completely removed by regular surgery [3].

Case Report
The patient (female, 39 years old) was admitted to the hospital due to five years of dysmenorrhea and three months of aggravating symptoms. The patient had regular and average amount of emmenia without abdominal pain. Over the past five years, she also experienced dysmenorrhea and the symptoms had gradually worsened, but the patient could still work and live normally without treatment. During the recent three months, dysmenorrhea had particularly become aggravated and pain was not even relieved during the non-menstrual period. Analgesic drugs were needed and the patient had difficulty in working normally, which severely affected the quality of life. One month ago, she was found to have an encapsulated mass with a diameter of 5 cm in the left annexal area during the ultrasonic and gynecological examinations in another hospital. Surgical treatment was recommended. During the course of disease, there were no changes of menstrual amount, stool quality and quantity. The patient did not have other discomfort except for pain caused by dysmenorrhea. The patient had been pregnant four times and gave birth twice. Currently, she has two healthy daughters. The patient had had two abortions with the last abortion in 2006. Since 2006, a contraceptive intrauterine device was placed. Gynecological examination revealed that the uterus was slightly enlarged without tenderness. There was a mass with a diameter of 5 cm in the left back side of the uterus. The mass was solid and mobile with obvious tenderness. No abnormalities were observed in the right accessories. Laboratory examination showed that both electrocardiogram and chest X-ray were normal. Blood CA125 concentration was 13.4 U/ml (normal value < 35 U/ml). B-ultrasonic examination indicated that the right accessories had a mixed mass. The mass (size: 5 × 5 × 4 cm³) with a thick capsule had spotted strong echoes in the low intensity areas. There was no adhesion seen in abdominal laparotomy examinations. The uterus was slightly full and purple in color, which is similar to adenomyosis. Accessories on both sides were normal. A capsulated solid mass with a diameter of 5 cm could be detected in the mesentery of retro-rectum between the sigmoid colon and rectum. There was no anatomical adhesion between the mass and uterus. Mass cystectomy was performed and the uterus tissue on the bottom side was collected for biopsy examination. During mass cystectomy, the capsule of the mass was completely removed. The mass itself was completely removed. The surface of the mass was smooth. A 1 cm-thick muscle-like cavity structure of the mass was cut open and approximately 4 ml of chocolate-like viscous liquid came out from the cavity (Figure 1). Pathological examination on both cryo-sample intraoperative and postoperative paraffin-embedded sample confirmed that the patient had adenomyosis. A biopsy of the uterus also reconfirmed adenomyosis. Pathological examination of the mass and biopsy showed that endometrial glands and stroma had an island-like distribution in the uterine myometrium (Figure 2). Endometrial glandular epithelium showed proliferation including expansion of the glands and flat epithelium surrounded by endometrial stroma. Immunohistochemical analysis showed that estrogen and progesterone receptors were positive. It was recommended that the patient use ovarian function suppressive drugs after surgery. However, the patient refused to apply any medication. The patient was observed for two months. Menstruation normalized and dysmenorrhea disappeared.

Discussion
Adenomyosis is a benign gynecological disease caused by invasion of endometrium to the myometrium, and is accompanied by diffuse hyperplasia of myometrium [4, 5]. The cause of the disease is the direct expansion of the basal layer of endometrium to the muscle layer, but without cultivation or metaplasia [6]. Ectopic endometrium is diffused in the mural muscle of the uterus (most commonly in the posterior wall). The muscle fiber has diffusive and reactive proliferation. The uterus is evenly enlarged with hard quality, and its size varies before and after men-
Adenomyosis is often associated with endometriosis, fibroids, and pelvic adhesions [7]. One of the characteristics of adenomyosis is an undefined border from the surrounding tissues [8]. The main clinical manifestation of adenomyosis is secondary progressive aggravation of dysmenorrhea and extended time of menstrual period with increased amount. Biopsy during surgery is the gold standard for the diagnosis of adenomyosis [9]. Currently, extremely valuable biochemical markers for diagnosis and monitoring the progression of adenomyosis are unavailable. For example, CA125, anti-endometrial, and anticardiolipin antibodies may be increased, but not specifically in adenomyosis [10]. The clinical symptoms of the patient were secondary progressive aggravation of dysmenorrhea, which was consistent with the manifestation of adenomyosis. In addition, dysmenorrhea disappeared after surgery. We speculated that dysmenorrhea caused the encapsulated mass of adenomyosis. The mass with a smooth surface was located between the mesentery and had a clear border with the surrounding tissues. The features of the mass can hardly be explained by the presence of adenomyosis. Some reports showed that leiomyomatosis peritonealis disseminata (LPD) was a very rare disease and could be manifested by the symptoms of endometriosis, e.g., progressive aggravation of dysmenorrhea, infertility, and sexual pain [11, 12]. White or gray red nodules can be diffusively distributed on the surface of the peritoneum in the abdominal and pelvic cavities. The color of the nodules is associated with the course of the disease. The surface of the nodules is smooth and the quality of the nodules is hard. The nodules have a clear border with a size of several micrometers to millimeters. There could be several hundred nodules commonly located in the omentum, mesentery, serosa surfaces of the small intestine and colon, and on the surface of the uterus. Histological examination manifests benign smooth muscle changes. Adenomyosis with similar characteristics to the patients in this study has not yet been reported. Currently, there are a few number of adenomyosis cases reported, and epidemiological studies for adenomyosis are unavailable. Therefore, factors that cause this disease are generally unknown. As histological analysis showed that estrogen and progesterone receptors were positive, we believed that the disease occurring in this patient was related to estrogen or progesterone. The occurrence of LPD is believed to be associated with the increased level of female hormones. We consider that adenomyosis can occur in both diffuse uterus fibroids and myometrium.

Figure 1. — The signs of mass displayed in each figure. A shows the smooth surface of the mass. B and C show the 1 cm-thick muscle-like cavity structure of the mass that was cut open and approximately 4 ml of chocolate-like viscous liquid that came out from the cavity.

Figure 2. — Histopathologic signs in mesentery adenomyosis in each figure. A and B show detected smooth muscle, endometrial gland, and stroma. Original magnification × 200.
The patient reported in this study refused any drug medication and was only followed by outpatient service. We continue to follow-up the clinical symptoms and monitor mass recurrence.

References


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The challenging trisomy 16: a case report

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Summary

Background: Trisomy 16 is a very frequent autosomal anomaly accounting for about 2% of first trimester abortions. In most pregnancies the chromosomal genome found in the fetus is also present in the placenta. Confined placental mosaicism is frequently detected in the placent al region along with a structurally normal fetus. Case: We present the case of a 39-year-old primigravida with confined placental mosaicism diagnosed with chorionic villus sampling. Amniocentesis showed a normal karyotype (46, XX). Detailed scanning revealed no structural fetal anomalies, but severe oligohydramnios. Conclusion: Diagnosis of trisomy 16 does not necessarily mean that the newborn has anatomical abnormalities.

Key words: Confined placental mosaicism and fetal anatomy; Triploidy; Trisomy 16; Mosaicism; Oligohydramnios; Chromosome 16.

Introduction

The identification of two or more cell lines with different genomes is called chromosomal mosaicism. All cell lines are derived from a single zygote. Mosaicism may involve both fetal tissues and extraembryonic membranes. This case report demonstrates our concerns about possible anatomical abnormalities and pregnancy outcome due to the restricted ability of the fetus to move freely in the uterus and the positioning of the skeleton and extremities during intrauterine life due to severe oligohydramnios.

Case Report

A 39-year-old primigravida woman visited the clinic at 12 weeks of gestation. She was rhesus positive, a non-smoker with a pre-pregnancy weight of 56 kg, and no allergies or co-morbidities reported. Maternal serum biochemistry (free beta hCG: 2.42 MoM, PAPP-A: 0.13MoM) gave her an increased risk for Down’s syndrome (1 in 19). After an informed discussion with the woman and her husband, they requested invasive testing; an uncomplicated transabdominal chorionic villus sampling was performed at 13 weeks of gestation. The results of karyotyping reported trisomy 16 within the chorionic villi. The couple agreed to have an amniocentesis in order to establish whether it was confined to placental mosaicism. Amniocentesis was performed at 16 weeks and showed a normal karyotype (46, XX). Detailed sonography at 20 weeks showed no obvious structural abnormalities (Figure 1), but severe oligohydramnios (AFI~6 cm, amniotic fluid index) with a large placental mass (width 45 mm) with multiple “cysts”, covering a large posterior surface of the uterus (Figure 2). Doppler assessment of the uterine, umbilical, and middle cerebral arteries was normal. Fetal growth velocity slowed down in the abdominal circumference. In view of raised human chorionic gonadotropin (ß-hCG) and a diagnosis of confined placental mosaicism (CPM) the fetus was monitored for growth due to risk of early onset of intrauterine growth restriction (IUGR). Caesarean section was performed due to fetal distress at 33 weeks and a female neonate of 2000 g was born with an Apgar score of 1 at 1 min and 2 at 5 min. The newborn breathed normally on and growth improved in the first four months.

Trisomy 16 diagnosed prenatally is common and associated with variable pregnancy outcomes making counselling challenging.

Diagnosis of CPM does not necessarily mean the presence of birth defects; children identified prenatally with CPM function in a similar way compared to normal children [1]. CPM diagnosed in the non-mosaic state can be lethal and not compatible with normal fetal development [2]. The detection of trisomy 16 cells in the amniotic fluid increases the risk of fetal abnormalities [3].

Discussion

Trisomy 16 is a chromosomal abnormality in which there are three copies of chromosome 16 rather than two. It is the most common cause of miscarriage during the first trimester of pregnancy.

Mosaicism occurs when two or more different genomes are expressed in one individual developing from a single fertilized egg. Mosaicism can occur in different fetal tissues and affect various stages of embryonic development. Mosaicism may result from a mutation during development which is propagated to only a subset of adult cells. Mosaicism may be present in the fetal and extra embryonic tissues (cytotrophoblast, villous stroma), or it may be present in the placental unit only.

In CPM there is a discrepancy between the fetus karyotype and the placenta; the mosaic cells are strictly limited to within the placental area.

In CPM, the cell can be trisomic; the chromosome can be of either paternal or maternal uniparental disomy (UPD), or of unknown origin [4]. It seems that maternal UPD 16 exerts a stronger influence on the prevalence of IUGR and fetal anomalies [5].

CPM may be found in the placental tissue along with a normal fetus [6]. It has been observed in spontaneous abortions, with trisomy of chromosomes 7, 16, 18, being the most common [7].

Trisomy 16 mosaicism is associated with IUGR, preterm delivery, fetal death and fetal anomalies, i.e., two-vessel cord, clinodactyly, congenital diaphragmatic hernias [8].

Confined placental mosaicism occurs in about 1% of pregnancies, where there is a mixture of cells with different karyotypes located in the placental regions [9].
90% of these, the fetus has a normal karyotype, while in the remainder the fetus is also a mosaic with variable phenotypic results [10]. Some studies showed that there is no clear correlation between IUGR and CPM [11]. The phenotypic effects of mosaicism may be dramatic, however not all cases are serious. In about 20% of cases, pregnancy outcome is absolutely normal [12].

It is not possible for a child to be born with an extra copy of chromosome 16 present in all cells (full trisomy 16). It is however possible to be born with the mosaic form [12]. Examination of multiple tissues is highly recommended before determining the actual identity of CPM. The first step is the performance of a chorionic villus sample (CVS). A follow-up amniocentesis shows the euploid cell line on the fetus in contrast to the placenta mosaic cells. Chorionic villi illustrate a much higher proportion of mosaic cells compared to fetal tissue. Mosaicism should be confirmed by amniocentesis before any clinical decisions are made. CPM detected through invasive testing is not a benign finding (the findings always depend on the chromosome involved), it is associated with abnormal outcomes, most commonly IUGR, cardiac defects, congenital diaphragmatic hernias, hypospadias, and global developmental delay [6, 12]. A very rare finding in trisomy 16 mosaicism is fetal artery stenosis [13].

Elevated titres of maternal hCG and alpha fetoprotein characterize the syndrome, indicate possible placental dysfunction, and should be markers for fetal echocardiography and serial scanning evaluation [14].

Prenatally diagnosed CPM can cause IUGR and preeclampsia. Pregnant women suspected of trisomy 16 mosaicism should be offered testing for preeclampsia since they are at increased risk for severe preeclampsia [15].

References


Acute generalized exanthematous pustulosis during the puerperal period: a case report

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Summary

Background: Acute generalized exanthematous pustulosis (AGEP) is an uncommon adverse cutaneous reaction, most commonly associated with drugs. Case: A 38-year-old primigravida whose labor had been induced developed erythema over her chest and abdomen. She was transferred to our department after a failed vacuum extraction, and delivered a mature infant by forceps. On day three postpartum she developed a 40.4°C fever. Although ceftriaxone was administered, her fever persisted (> 38°C). On day six of the puerperium, diffuse non-follicular pustules appeared over her neck and trunk, and AGEP was suspected. Two days after ceftriaxone was withdrawn, the eruptions started to resolve without any medical intervention. Conclusion: Once the diagnosis of AGEP has been made, the antibiotics being administered must be discontinued. If continued treatment is required, pharmacologically distinct antibiotics must be used instead to aid the rapid self-limitation of the disease.

Key words: Acute generalized exanthematous pustulosis; Antibiotics; Puerperium.

Introduction

Although recent advances in the use of antibiotics during pregnancy have improved maternal and perinatal outcomes, inappropriate antibacterial treatment and overuse of antibiotics can sometimes cause harmful side effects to pregnant women and their fetuses. We report a case of acute generalized exanthematous pustulosis (AGEP) that manifested during the puerperal period.

Case Report

Labor was induced in a 38-year-old primigravida post-term woman at 41+3 weeks gestation by her practitioner using an intratracheal extra-amniotic Foley catheter. She started to take an empirical cephalosporin (cefditoren pivoxil, CDTR-PI) after the procedure. Her previous medical history did not include drug eruption or psoriasis, and her current pregnancy was otherwise uncomplicated. Two days later she developed erythema over her chest and abdomen. At 41+5 weeks gestation she was referred to us following a failed vacuum extraction. On admission, the patient appeared strained and was wet with perspiration. Her body temperature was 37.2°C, and her blood pressure was 139/74 mmHg. She had the following clinicopathological characteristics: white blood cell count, 20,400/mm³ with 95.5% neutrophils; hemoglobin, 12.4 g/dl; platelet count, 32.6 × 10⁴/mm³; and C-reactive protein (CRP), 2.20 mg/dl. Her uterine cervix was fully dilated, and one hour after admission a mature infant was delivered by forceps following an episiotomy. The infant weighed 3,690 g and had Apgar scores of 7 and 8 at 1 and 5 min, respectively. After labor, the patient was treated prophylactically with two 1 gram doses of intravenous oxacephem (flo- moxef sodium, FMOX) followed by an oral cephalosporin (ceftixime pivoxil, CFTM-PI) for two days. On day two postpar- tum she developed a high fever (> 39°C). Her body temperature was 40.4°C the following day, her erythema was exacerbated and her serum CRP was elevated to 13.7 mg/dl. Although the antibiotic she was taking was changed to ceftriaxone (CTRX) and administered at a dosage of 2 g/day for three days, her body temperature remained elevated above 38°C. On day six postparturition, diffuse non-follicular pustules appeared over her neck and trunk on an erythematous base (Figures 1a, 1b). CTRX administration was discontinued, and intravenous fosfomycin (FOM) and oral clarithromycin (CAM) were administered instead. Two days later the eruptions began to resolve and desquamation occurred without any medical intervention (Figure 1c). These findings indicated that she had suffered from acute generalized exanthematous pustulosis (AGEP). Histopathological analysis of the skin biopsy revealed a spongi- form subcorneal pustule with marked infiltration of neutrophils (Figure 1d), which is consistent with the typical features of AGEP. However, lymphocyte transformation tests (LTT) performed on day 10 and 26 of the puerperium failed to find possible associations between AGEP and CTRX, FMOX, CFTM-PI or CDTR-PI.

Discussion

AGEP is an uncommon (one to five cases per million/year) adverse cutaneous reaction that is characterized by: (1) numerous, small non-follicular, intraepidermal or subcorneal pustules (< 5 mm) on an erythema- tous background; (2) typical histopathological changes; (3) fever (> 38°C); (4) blood neutrophil count > 7,000/mm³; and (5) acute evolution with spontaneous res- olution of pustules in less than 15 days [1, 2]. The EuroSCAR study group developed a validation score system for diagnosis of AGEP based on three aspects: (1) morphology (pustules; erythema; distribution/pattern; postpustular desquamation); (2) course (mucosal involvement; acute onset (≤ 10 d); resolution ≤ 15 d; fever ≥ 38°C; polynuclear neutrophils ≥ 7,000/mm³); and histol- ogy [3]. Our patient scored 10 points using this system, which was interpreted as “definite” AGEP.

AGEP is drug induced in more than 90% of cases, with β-lactam and macrolide antibiotics being the most common causative agents, followed by acute infections.
Acute generalized exanthematous pustulosis during the puerperal period: a case report

Once the diagnosis of AGEP is made, the causative drug must be discontinued and other antibiotics must not be administered unless there is a clear and well documented associated infection [3]. In the present case we hesitated to cease administration of antibiotics even though repeated blood cultures failed to detect microorganisms, because the patient had devastating vaginal injuries caused by the forceps delivery. Instead, we substituted CTRX, a β-lactam, with two pharmacologically distinct antibiotics, FOM and CAM, which resulted in a rapid self-limitation of the disease. We performed lymphocyte transformation tests (LTT) on day 10 and 26 of the puerperium to investigate possible associations between AGEP and CTRX, FMOX, CFTM-PI and CDTR-PI. However, we were unable to definitively determine the causative agent of AGEP in this patient. LTT measures the proliferation of T-cells in response to a drug in vitro, indicating sensitization, but has a limited sensitivity (60-70% for β-lactams) [4].

Pregnancy associated with AGEP is rare, and only five cases have been reported to date [5-9]. Although the pathogenesis of AGEP remains unknown, possible involvement of drug-specific T-cells has been raised. Drug-specific T-cell clones from lesional skin and the circulation have been found that are positive for the neutrophil-attracting cytokine interleukin (IL)-8 [2]. It is of note that these pregnancy-related AGEP cases developed following procedures that exposed patients to possible intrauterine infection, such as amniocentesis [5], cesarean section [8] and labor induction [9]. The present case of AGEP was also preceded by induction of labor using an intrauterine extra-amniotic Foley catheter. Reich et al. [6] reported a case of AGEP following premature labor that was likely caused by chorioamnionitis. The human placenta constitutively produces IL-8 during pregnancy and enhances its production in chorioamnionitis [10]. Further investigations are required to determine if there is an association between the specific immunological environment during pregnancy and the pathogenesis of AGEP.

In summary, once the diagnosis of AGEP has been
made, the antibiotics being administered must be discontinued. If continued treatment is required, pharmacologically distinct antibiotics must be used instead to aid the rapid self-limitation of the disease.

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