CLINICAL AND EXPERIMENTAL OBSTETRICS & GYNECOLOGY
an International Journal

Founding Editor
A. Onnis
Montréal (CND)

Editors-in-Chief
M. Marchetti J.H. Check
Montréal (CND) Camden, NJ (USA)

Assistant Editor
A. Sinopoli
Toronto (CND)

Editorial Board
Andrisani A., Padua (Italy)
Audet-Lapointe P., Montréal (Canada)
Axt-Fliedner R., Lübeck (Germany)
Basta A., Krakow (Poland)
Bender H.J., Dusseldorf (Germany)
Bhattacharya N., Calcutta (India)
Bonilla Musoles F., Valencia (Spain)
Cabero-Roura L., Barcelona (Spain)
Charkviani T., Tbilisi (Georgia)
Chavan N., Mumbai (India)
Dexeus S., Barcelona (Spain)
Eskes T.K.A.B., Nijmegen (The Netherlands)
Farghaly S.A., New York (USA)
Farghaly S.A., New York (USA)
Friedrich M., Homburg (Germany)
Gorins A., Paris (France)

Holub Z., Kladno (Czech Republic)
Kaplan B., Petach Tikva (Israel)
Markowska J., Poznan (Poland)
Marth C., Innsbruck (Austria)
Meden-Vrtovec H., Ljubljana (Slovenia)
Murta E.F.C., Uberaba (Brazil)
Mynbaev O.A., Moscow (Russia)
Papadopoulos N., Alexandroupolis (Greece)
Rakar S., Ljubljana (Slovenia)
Rigó J., Budapest (Hungary)
Sciarrà J.J., Chicago (USA)
Stelmachow J., Warsaw (Poland)
Varras M.N., Athens (Greece)
Winter R., Graz (Austria)

7847050 CANADA, Inc.
Administrative Office (M. Beaucage):
4900 Côte St-Luc, Apt #212 – Montréal, Quèbec, H3W 2H3 (Canada)
Tel. +1-514-4893242 – Fax +1-514-4854513 – e-mail: canlux@mgroup-online.com
Website: www.irog.net

Editorial Office (M. Critelli):
Via Martiri della Libertà, 9, 35137 Padua (Italy)
Tel. +39-049-656521 – Fax +39-049-8752018 – e-mail: irog.canada@gmail.com

CLINICAL AND EXPERIMENTAL OBSTETRICS & GYNECOLOGY – CEOG (ISSN 0390-6663) publishes original work, preferably brief reports, in the fields of Gynecology, Obstetrics, Fetal Medicine, Gynecological Endocrinology and related subjects, (Fertility and Sterility, Menopause, Uro-gynecology, Ultrasound in Obstetrics and Gynecology, Sexually Transmitted Diseases, and Reproductive Biological Section). The Journal is covered by ISI Journal Master List, Index Copernicus International, Science Citation Index Expanded, Current Contents - Clinical Medicine, Web of Science, Index Medicus/MEDLINE, EMBASE Excerpta Medica, PubMed, MedSci, Pubget, Genamics JournalSeek, Sciencescape, Unbound Medicine, and PubFacts.com. CEOG is issued bimonthly in one volume per year by 7847050 CANADA Inc., Montréal (Canada).
Printed in Italy by “Centro Servizi Editoriali S.r.l.” - Grisignano di Zocco - 36040 Vicenza (Italy).
EDITORIAL ARTICLE

Improving the chance of successful implantation – part I – embryo attachment to the endometrium and adequate trophoblast invasion

J.H. Check, J. Aly, E. Chang - Camden, NJ, USA

The first in a series on improving embryo implantation is presented with emphasis on embryo attachment and trophoblast invasion.

ORIGINAL ARTICLES

Flemish obstetricians’ personal preference regarding induction of labor and mode of delivery in term births

H. Sonnemans, A. Schmid, J. Muys, Y. Jacquemyn - Edegem, BELGIUM

A growing number of Flemish gynecologists prefer planned cesarean section for themselves, both in cephalic and breech presentations.

HELLP syndrome is still a serious, life-threatening complication of pregnancy: admission of 34 women to an eastern Turkish intensive care unit

Z. Bedir, A. Ahiskalioglu, Ü. Esenkaya, E.O. Ahiskalioglu, A. Dostbil, M. Aksoy, N. Dogan, H. Kursad - Erzurum, TURKEY

HELLP syndrome and high maternal mortality rate in an intensive care unit are presented.

Rupture risk factors of fallopian tubal pregnancy


Over 3,000 mIU/ml of beta-hCG levels are associated with increased risk of fallopian tubal rupture in ectopic pregnancy.

Urethral instillations of clobetasol propionate and lidocaine: a promising treatment of urethral pain syndrome

B.E. Lindström, D. Hellberg, A.K. Lindström - Uppsala, SWEDEN

Treatment with clobetasol propionate and lidocaine in women with urethral pain syndrome has proven efficacious in a retrospective pilot study including a short- and long-term follow-up.

The influence of sperm parameters on the outcome of intracytoplasmic sperm injection-embryo transfer cycle in poor responder women under 35 years of age

A. Usta, M. Karacan, Z. Cebi, A. Arvas, M. Ulug, C.S. Usta, T. Camlibel - Istanbul, TURKEY

A retrospective analysis in a fertility center evaluates the influence of sperm parameters on the outcome of intracytoplasmic sperm injection cycles in poor responder women under the age of 35 years.

Clinico-biochemical characteristics of 229 Portuguese infertile women with polycystic ovary syndrome: clinical relevance and relationship with fertility treatment results


An original retrospective study evaluates the clinico-biochemical characteristics of 229 infertile polycystic ovary syndrome women and its relationship with fertility treatment outcomes.

Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial?

G.A. Partsinvelos, N. Antonakopoulos, K. Kallianidis, P. Drakakis, E. Anagnostou, R. Bletsas, D. Loutridis - Athens, GREECE

The addition of low-dose hCG to rFSH in a short GnRH-agonist protocol may be associated with better quality embryos and higher pregnancy rates.
Serum carcinogenic antigen (CA)-125 and CA 19-9 combining pain score in the diagnosis of pelvic endometriosis in infertile women

H. Zhu, H. Lei, Q. Wang, J. Fu, Y. Song, L. Shen, W. Huang - Chengdu, CHINA

The utility of serum carcinogenic antigen (CA)-125 and CA 19-9 combining pain score in the prediction of pelvic endometriosis in infertile women is defined.

Impacts of maternal anxiety on non-stress test parameters

S. Nergiz Avcioğlu, S.Ö. Altinkaya, İ. Kurt Ömürlü, M. Küçük, S. Demircan-Sezer, H. Yüksel - Aydın, TURKEY

The association between antenatal maternal anxiety with non-stress test parameters, which is an indicator test of fetal well-being in the third trimester, is assessed.

Management of bladder endometriosis with combined transurethral and laparoscopic approach. Follow-up of pain control, quality of life, and sexual function at 12 months after surgery

A. Pontis, L. Nappi, F. Sedda, F. Multinu, P. Litta, S. Angioni - Cagliari, ITALY

Pain control, quality of life, and sexual function previous to and after bladder endometriosis surgery are discussed.

Is maternal Vitamin D associated with gestational diabetes mellitus in pregnant women in Cyprus?

I. Soytac Inancli, E. Yayci, T. Atacag, M. Uncu - Lefkoşa, CYPRUS

There are conflicting results regarding the relationship between Vitamin D deficiency and gestational diabetes mellitus.

Obstetric outcomes of intramural leiomyomas in pregnancy


The relationships among intramural leiomyoma in pregnancy and miscarriage, preterm birth, preterm premature rupture of membranes, intrauterine growth restriction, fetal malpresentation, cesarean section, and postpartum hemorrhage are assessed.

Immunohistochemical expression of hormonal receptors, collagen, elastin, and proteoglycans in genuine urinary incontinence

E. Pantatosakis, D. Karandrea, E. Liapis, A. Kondi-Pafiti, A. Liapis - Athens, GREECE

Hormonal receptors, collagen, elastin, and proteoglycans in stress incontinence are evaluated.

The assessment of maternal and umbilical cord homocysteine levels in obese pregnant women


The maternal plasma homocysteine levels and umbilical cord homocysteine values are compared between obese and non-obese pregnant women.

Three-dimensional transperineal ultrasound: is there a correlation among age, weight, delivery mode, and a change in the pelvic floor architecture in Korean premenopausal women?

S.J. Kim, M.J. Kim, T.H. Kim - Seoul, REPUBLIC OF KOREA

Delivery mode might be a meaningful factor in the change of contractility of the pelvic floor architecture.

The role of hysteroscopy in unexplained infertility

M. Di Muzio, A.M.L. Gambaro, V. Colagiovanni, L. Valentini, E. Di Simone, M. Monti - Rome, ITALY

The pregnancy rate after diagnostic and operative hysteroscopy in nulliparous patients with infertility of unknown cause is evaluated.

Monitoring and treatment results of 88 HBsAg-positive pregnant women


It is important to screen all pregnant women for hepatitis B (HBsAg) and to assess those found HBsAg-positive, in order to protect both mother and baby using appropriate approaches.

Clinical analysis of 95 cases with ovarian pregnancy

A.W. Le, Z.H. Wang, L. Shan, X.Y. Dai, T.H. Xiao, X.R. Li - Shenzhen, CHINA

Ovarian pregnancy is a rare ectopic pregnancy and surgical exploration should be conducted once cases are suspected.

Effects of estradiol injection on outcome of in-vitro fertilization: a randomized, double-blind, placebo controlled trial

A. Samsami, A. Zarei, S. Shahrivar - Shiraz, IRAN

Estradiol supplementation during the luteal phase in those with unexplained infertility undergoing IVF is associated with decreased serum levels of estradiol after hCG injection.
Using the LigaSure vessel sealing device in the large uterus at laparoscopic hysterectomy
M. Biçer, Z. Güner, C. Karas, A. Güclü, Mert Göl - Izmir, TURKEY
Intraoperative hemorrhage and other operative parameters between patients with large and small weighted uteruses who underwent laparoscopic hysterectomy are compared.

Analysis of the reason of abnormal uterine bleeding induced by copper corrosion of IUD Cu
L. Li, J. Li, N. Li, Y. Zhang, X. Feng - Shijiazhuang, CHINA
The relationship of corrosion of the copper intrauterine device (TCu220 IUD) and abnormal uterine bleeding is analyzed.

**CASE REPORTS**

Bulky fibroid and pregnancy: myomectomy is possible during pregnancy
J. Lepage, B. Merlot, J.P. Lucof, D. Subtil - Lille, FRANCE
The present case confirms that some bulky fibroids can be excised during pregnancy, with a favorable maternal and neonatal prognosis.

A case of a 42-year-old patient with anomalous origin of the left main coronary artery from the pulmonary artery who delivered three times with no complications: presentation, diagnosis, and review
M.S. Arnaout, M. Serhan, C. Saade - Beirut, LEBANON
An unusual case of a multiparity patient who delivered several times without any complications presenting with recurrent dyspnea on exertion in which echocardiographic findings of diastolic flow near the origin of the pulmonary valve were suggestive of anomalous origin of the left coronary artery is presented.

Interstitial ectopic pregnancy after salpingectomy due to previous tubal pregnancy - a case report
A. Marciniak, I. Nawrocka-Rutkowska, I. Szydłowska, B. Wiśniewska, O. Wielgoszewska, A. Starczewski - Police, POLAND
This case demonstrates that even after removal of the fallopian tube, there is still a risk of ectopic pregnancy in the intramural part of this tube.

Asymptomatic isthmico-cervical uterine perforation with IUD – our experience and literature review
Isthmico-cervical IUD uterine perforation, although uncommon, can occur at any time and be asymptomatic for a long period of time.

Successful transvaginal aspiration of interstitial pregnancy after failed methotrexate treatment
I.F. Yang, J.L. Hwang, H.J. Chen, L.W. Huang - Taipei City, TAIWAN
A case of interstitial pregnancy in which the patient failed to respond to multidose methotrexate treatment and successfully treated with transvaginal sonography-guided transvaginal aspiration of the gestational tissue is presented.

P450 oxidoreductase deficiency with maternal virilization during pregnancy
A case of maternal virilization during pregnancy caused by autosomal recessive P450 oxidoreductase deficiency is reported.

Growing teratoma syndrome after ovarian immature teratoma: a case report and review of the literature
O. Lapuente-Ocamica, L. Ugarte, M. Cuadra, A. Lopez-Picado, L. Maestro, I. Lete - Alava, SPAIN
A case of growing teratoma syndrome, a rare complication of malignant germ cell tumours, is reported.

Analysis on two postmenopausal women with clinical symptoms resulting from completely encapsulated intrauterine device by fibrous tissue
D.Y. Wei, L. Yan, H.Q. Wang - Jinan, CHINA
Two cases of postmenopausal women with intrauterine devices severely encapsulated by fibrous tissue are reported.

Large pedunculated angiomyofibroblastoma of the vulva with concomitant anemia: a case report and mini review of the literature
O. Birge, A. Merdin, E.G. Oz bey, D. Arslan - Nyala, SUDAN
Angiomyofibroblastoma is a rare myxoid tumor and is often seen in vulvar and vaginal areas.
Introduction

Successful pregnancy requires adequate sperm concentration, with survival in cervical mucus with sperm, traversing patent non-diseased fallopian tubes, meeting an oocyte that was released from a dominant follicle that was picked up by normal fimbria, and transported to the upper third of the fallopian tube. Furthermore, for successful pregnancy the oocyte and sperm have to be chromosomally normal resulting in fertilization and subsequent embryo cleavage to the blastocyst stage.

The present editorial (and subsequent series to follow) will discuss potential reasons why a chromosomally normal embryo may fail to implant and possible methods to improve implantation failure. The present editorial will deal with embryo attachment and adequate trophoblast invasion.

Normal events enabling implantation

The first step in implantation leading to a successful pregnancy involves the blastocyst. After about six days after fertilization, the blastocyst must invade the maternal decidua. The decidua is a cellular stratum that allows the placenta to co-opt maternal blood flow. Interactions between the stromal and vascular components of the placenta and uterus are mostly regulated by maternal immune cells that populate the decidua.

The outer layer of the blastocyst generates the placenta which is composed of both maternal decidual cells and fetal trophoblast cells. The villous trophoblast cells cover the placental villous tree. The trophoblast cells mediate the transport between the maternal blood in the intervillous space and the fetal circulation. The extravillous trophoblast cells invade deep into the uterine wall. Natural killer (NK) cells destroy and then replace the muscular wall of the uterine arteries. With further development from angiogenic factors secreted by the invading trophoblast, specialized spiral arteries are formed. These spiral arteries ensure adequate blood flow to and from the fetus [1].

White blood cell population at the maternal-fetal interface

The most prominent of the white blood cell population of the maternal-fetal interface are NK cells [1, 2]. These cells are determined by flow cytometry and tissue immunostaining to represent approximately 70% of the cells at the maternal-fetal interface [3, 4]. Macrophages are the next most prominent white blood cell representing about 20% of the population [3, 4]. Most of the remaining cells are T cells with rare dendritic cells, B cells or NK T cells.

Summary

The first in a series on improving embryo implantation is presented with emphasis on embryo attachment and trophoblast invasion. Purpose: To present knowledge of events needed for embryo attachment to the endometrium and subsequent trophoblast invasion and uterine remodeling leading to successful pregnancy. Materials and Methods: Based on normal events, some practical suggestions are proposed as to possible means of improving pregnancy rates by enhancing possible embryo attachment and trophoblast invasion. Results: Potential benefits of achieving adequate serum estradiol levels at peak follicular maturation, and the benefits of progesterone in the luteal phase are discussed. Also the potential benefits of purposeful endometrial injury is considered. Conclusions: Knowledge of the events leading to embryo attachment and trophoblast invasion could lead to novel research ideas helping to improve pregnancy rates in addition to proper hormone supplementation and endometrial biopsy.

Key words: Spiral arteries; Uterine natural killer cells; Uterine macrophages; Chemokines; Trophoblast invasion.
The decidual NK (dNK) cells first appear in the secretory endometrium prior to implantation. These NK cells are recognized by their CD56 bright CD16-cell surface phenotype. Though having a similar surface phenotype to these same phenotypic cells that are present in 10% of NK cells in the peripheral blood, they have a different transcriptional profile leading to the production of a wide range of chemokines, angiogenic factors, and cytokines [5-8]. Thus the dNK cells differentiate into cells with highly specialized pregnancy specific function [5-8]. Though the majority of peripheral NK cells in the blood have the surface phenotype of CD56dim CD16+, two cytokines, both expressed in the decidua, promote the conversion of CD56dim CD16+ peripheral (P) NK cells to decidua NK like-cells with the CD56 bright CD56- phenotype with the pregnancy specific factors: interleukin (IL-15) and transforming growth factor beta (TGF beta) [9,10].

Maximizing maternal blood flow through the placenta

The dNK cells seem to be the most important of the inflammatory white cells present in the preimplantation time period to promote the change of the high resistance low flow uterine vessels into low resistance (high capacitance) high-flow spiral arterioles that supply the placenta with maternal blood [2]. The NK cells also play a role in replacing the endothelium of the uterine arteries with trophoblasts (termed extravillous trophoblast) that have migrated from the placenta and invade the placenta. Thus, these extravillous trophoblast cells that have migrated into the lining of the uterine arterioles form a pseudoendothelium replacing the previous uterine artery endothelium. The diverted blood flows into the space surrounding the placental villous tree and thus fosters gas and nutrient exchange between mother and conceptus. Thus, if there is insufficient spiral arteriolar transformation, with the consequential failure of trophoblasts to invade into the vessels all the way to the superficial layer of the myometrium, this could result in pathological pregnancies resulting from placental underperfusion, e.g., pre-eclampsia and intrauterine growth restriction [11, 12]. One could easily envision a more serious problem with inadequate trophoblast invasion leading to such defectivespiral arteriole formation leading to demise of the early conceptus before a positive pregnancy test is obtained or a miscarriage from a clinical pregnancy.

The dNK cell is under the influence of the killer cell immunoglobulin-like receptor (KIR) family which encodes NK cells surface receptors. Also there are three HLA1 molecules and it is HLAC which is the dominant KIR ligand and the only one expressed by the extravillous trophoblasts. For further detailed discussion of these interactions, the authors would suggest reading the manuscripts by Parham et al. and by Hiby et al. [13-15].

The trophoblasts have relatively low levels of classical MHC 1 expression which should normally lead to immune rejection [16]. The dNK cells, similar to pNK cells, have granules containing cytotoxic molecules, e.g., perforin and granzymes [17]. There are many theories as to how the trophoblast escapes immune surveillance, but the one favored by the authors based on their research is that it predominantly involves the intracellular expression of a 34-36 kDa molecule known as the progesterone induced blocking factor (PIBF) which stabilizes perforin and granzymes [18]. This topic will be discussed in detail in the next editorial on “Improving the chance of successful implantation – part II – the importance of immune suppression against the fetal semi-allograft.

Macrophages

The bulk of the rest of decidual white blood cells are macrophages. The d-macrophages are well known to produce the interleukin IL10 [19]. Their main function may be to inhibit decidual infection but their possible role in promoting or inhibiting implantation is not known for sure [19]. There have been erudite hypotheses expounded and for further details, the authors recommend the reader to the studies of Renaud et al. and Nagamatsu et al. [20, 21].

Dendritic cells

As mentioned these cells are rare at the maternal fetal interface. Dendritic cells are normally very important cells in the adaptive immune response. When dendritic cells (DC) are exposed to pathogens or inflammation they migrate to draining lymph nodes by lymphatic vessels. At these draining lymph nodes, the dendritic cells present antigens to naïve T cells. This fosters T cell proliferation and polarization. Tagliani and Erlebacher have speculated that part of the process of successful implantation of the conceptus, which is a semi-allograft, is to inhibit DC’s to reduce the tissue’s ability to initiate adaptive T cell responses in the draining lymph notes [22].

T-cells

The presence of T cells in the first trimester human decidua represents about 10% of the white blood cell population. About 30-45% of these T cells are CD3+ TCR2B+ T cells which can be divided into CD4+ T cells (30-45%) and CD8+ T cells (45-75%) [23, 24]. About 5% of the CD4+ T cells are regulatory T Cells (T reg) with the surface phenotype of CD25 bright FOXP3+ [23, 24].

At present the function of decidual T cells is generally unknown. The population remains stable in cases of miscarriage. Their role may become more clear with future research.

Attachment of the blastocyst to the endometrium

In order for the early embryo to produce a live baby, the first step is apposition of the blastocyst to the uterine endometrium. The second step is attachment to the endometrial surface endometrium. In humans, the uterus becomes receptive to attachment five to nine days after fertilization,
which has been referred to as the window of implantation (WOI).

It is during the WOI that one of the two distinct cellular components of the uterine endometrium, the stromal cells, transform into larger and rounded decidual cells (decidualization). The epithelial cells, probably under regulation by corpus luteum progesterone and estradiol secretion, produce cytokines, chemokines, growth factors, and adhesion molecules. Part II of this series will present evidence for the role of progesterone secretion in suppression of immunosurveillance especially by NK cells of the fetal semiallograft. The possibility exists that inadequate early luteal phase secretion of estradiol or progesterone could impair trophoblast attachment to the endometrium. Thus the possibility exists that some cases of infertility may be related to poor embryo attachment which could be corrected by early supplementation of additional progesterone and/or estrogen.

Before a woman can achieve blastocyst invasion and placenta-tion, it must be preceded by attachment of the trophoectoderm to the endometrial epithelium. The uterine surface, however, is covered by various molecules, especially Mucin I (MUC1) carbohydrate that prevents the attachment of the highly adhesive blastocyst to an improper site. Mucin I is upregulated during the implantation period [25]. Thus the possibility exists that the human endometrial surface epithelium prevents blastocyst adhesion, except for the precise spot where the embryo attaches. Dekel et al. have hypothesized that one role of the minority DC’s pres-ent in the uterine stroma is to produce cytokines to induce local degradation of MUC I, and chemokines to attract the blastocyst to that spot [26]. Thus, failure to establish a success-ful pregnancy may be related not only to inadequate decidualization or immune rejection, but to deficient spe-cialized chemokine or cytokine production to allow blas-tocyst recruitment and attachment.

The role of chemokines and cytokines in implantation and placental development

Uterine NK cells are present in low levels pre-ovulation. However, they peak in the late luteal phase [27]. The chemokine CCL4 increases from early secretory phase and may be the main chemokine responsible for the increase in UNK cells and the recruitment of other immune cells, e.g., which are needed to allow implantation, decidualization, and fetal tolerance [28]. The exception may be T reg cells which seem to peak by the rise in estradiol at the peak sexually receptive time [29]. Actually T reg cells decrease with the secretion of progesterone. As mentioned, inadequate progesterone secretion in the luteal phase may lead to in-creased T reg cells during the luteal phase and these cells may be involved in immune rejection. It is not clear what the role of T regs are at peak follicular maturation, but assuming it plays some important role in establishing a suc-cessful pregnancy, it could explain why progesterone supplementation is so successful in correcting infertility and preventing miscarriage when the follicle is making ade-quate estradiol, but where the combination of a follicle maturing drug plus progesterone supplementation in the luteal phase provides the best success when ovulation oc-curs with lower peak serum estradiol levels [30].

Trophoblast invasion is directed by uterine NK cells. The UNK cells express the chemokines IL-8 and IP-10 which bind the receptors CXCR1 and CXCR3 on the extra villous trophoblast cells [31]. The trophoblast invasion and vascular remodeling are influenced by the release of the angio-genic factors VEGF and placental growth factor [31]. Other cytokines play a role. Transforming growth factor beta (TGF-B) may both downregulate inflammatory activity and provide differentiation of UNK cells [31].

The EVT cells express the non-classical class I molecules HLA-E which bind CD94/NKG2A [32]. Another such immu-nomodulatory molecule HLA-G, binds to KIR2DL4 [33]. Furthermore, trophoblast cells express the highly polymorphic classical HLA-C1 and HLA-C2 [34, 35]. HLA-C-KIR interaction is crucial for promoting placental vascularization. Certain combinations of KIR haplotypes and HLA-C groups have a detrimental effect and may in-crease the risk of pre-eclampsia [36].

Can certain treatments improve blastocyst attachment and trophoblast invasion?

Are there other possible treatments that can correct blasto-cyst attachment and subsequent trophoblast invasion be-sides providing adequate estrogen in the follicular phase and by supplementing progesterone and possibly estradiol in the luteal phase?

There is the possibility that local injury to the endo-metrium can result in increased chance of a successful pregnancy. As early as 1907, in the German literature, Loeb reported that scratching the guinea pig uterus during the progestational phase of the estrous cycle provoked a rapid growth of decidual cells [37]. Subsequently, it was found that decidua formation occurred in pseudopregnant rodents by other forms of local injury, e.g., suturing the uterine horn or intrauterine injection of oil [38, 39].

In humans, Barash et al. inadvertently found that per-forming endometrial biopsies in the preceding cycle to measure levels of connexin 43 protein led to a doubling of the pregnancy rates in the subsequent in vitro fertilization-embryo transfer (IVF-ET) cycle [40]. A subsequent study by Raziel et al. found that performing an endometrial biopsy in the preceding luteal phase, and thus performing local injury, could improve subsequent success rates in IVF-ET cycles in women with high-order implantation fail-ure [41]. Zhou et al. showed that this local injury could be performed even during the follicular phase of an IVF-ET cycle and produce improved outcome [42].

A Cochrane meta-analysis reviewed 14 clinical trials (2,128 women) evaluating the effect of endometrial injury...
in women undergoing IVF-ET [43]. Thirteen of the trials had the endometrial biopsy performed in the preceding luteal phase and one study performed the biopsy on the day of oocyte retrieval. Their conclusions were that if 26% of women achieve live birth without endometrial injury, between 28% and 48% will achieve live birth with this intervention when the biopsy is performed in the luteal phase [43]. However, a lower live delivered pregnancy rate occurs if the biopsy occurs on the day of oocyte retrieval [43].

There have been no studies to date on the effect of endometrial local injury on pregnancy rates in non-IVF cycles. A study on the effect of endometrial injury on non-IVF cycles is presently being conducted by our new fellow in reproductive endocrinology and infertility, Dr. Eric Chang, who is also one of the co-authors of this editorial.

References


Address reprint requests to:
J.H. CHECK, M.D., PH.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)
e-mail: laurie@ccivf.com
Flemish obstetricians’ personal preference regarding induction of labor and mode of delivery in term births

H. Sonnemans¹, A. Schmid², J. Muys², Y. Jacquemyn²

¹ Jan Palfijn Hospital, Antwerp; ² Antwerp University Hospital UZA, Edegem (Belgium)

Summary

Introduction: In a 2002 survey, 2% of Flemish gynecologists preferred elective cesarean section for themselves or their partner. This study aims to determine actual preference regarding induction of labor and mode of delivery in term cephalic or breech births for gynecologists or their partners. Materials and Methods: An anonymous postal questionnaire was sent to all gynecologists and trainees in Flanders. Results: Response rate was 28.2% (241/852). In case of an uncomplicated cephalic singleton pregnancy, 39 gynecologists (16.2%) preferred cesarean section. Most (n=134, 66.5%) chose induction at 41 weeks, 26 (13%) at 40 weeks, 37 (18%) at 42 weeks, 26 (13%) at 40 weeks, three (1.5%) preferred induction before 40 weeks and two (1%) would wait until after 42 weeks. Concerning term breech, 30% (n=72) opted for vaginal delivery and 70% (n = 169) for planned cesarean section. Ninety-nine (41%) gynecologists preferred to attempt external version first. Only 115 (47.7%) gynecologists felt professionally capable to assist vaginal breech delivery themselves; about one-third (n=96; 38%) had performed less than ten vaginal breech deliveries in their career. Conclusions: Flemish gynecologists are still in favor of vaginal delivery for themselves in terms of cephalic position, but an increasing number favor planned cesarean section. Most Flemish gynecologists opt for cesarean section for themselves or their partners in case of term breech and state that they do not feel capable in assisting vaginal breech delivery for their patients.

Key words: Cesarean; Induction of labor; Breech; Gynecologist; Obstetrics.

Introduction

In obstetrics the discussion whether we should allow women to choose for cesarean section on demand without any obstetric indication remains a current debate [1]. One of the comments that is regularly mentioned in ethical discussions on this subject is the need for the mother to understand the risks from caesarean section in current and future pregnancies [2]. There is one specific group of pregnant women that is very well informed, namely obstetricians themselves. A very high variation has been reported in obstetricians’ personal preference regarding mode of delivery. From 2% to 60% of obstetricians prefer elective cesarean section for themselves or their partners [3, 4]. In a previous study, performed one decade ago, gynecologists in Flanders demonstrated a very low percentage of personal preference for cesarean section [3] and were very reluctant to perform cesarean section on patient demand. The aim of the current study was to describe Flemish gynecologists personal preference regarding induction of labor for themselves or their pregnant partners and to determine which mode of delivery Flemish obstetricians/gynecologists prefer in case of a term cephalic or breech position for their partners or themselves.

Materials and Methods

A structured anonymous postal questionnaire was used. In January 2014 the questionnaire was sent to all 825 registered gynecologists in the region of Flanders, Belgium. Table 1 presents an overview of the questionnaire content. Results were calculated as absolute numbers and percentages. When appropriate, groups were compared using Chi-squared test with significance accepted at p < 0.05, odds ratios, and 95% confidence interval.

Results

In total, 241 out of 852 registered gynecologists replied, resulting in response rate of 28.3% of practicing obstetricians. Of the respondents, 125 were female (52%), 95 (39%) male, 21 (9%) did not answer this question. The male to female ratio is comparable to that of the complete group of registered trainees and gynecologists in Flanders (40% male, 60% females). There were 197 (82%) registered specialist gynecologists and 44 (18%) trainees. Most respondents had children (n= 187, 77.6%). For those that already had a delivery, there were 110 (out of 187, 58.8%) with spontaneous vaginal delivery; three had an instrumental vaginal delivery (1.6%), five (2.7%) underwent planned cesarean section, and eight (4.3%) had secondary cesarean section. Labor was induced in 59 (31.6%). There were 13 (6.9%) inductions before 39 completed weeks of gestational age, 18 (9.6%) inductions were at 39 weeks, 17 (9.2%) at 40...
In case of an uncomplicated term breech, presenting in a cephalic position, 42 weeks uncomplicated pregnancy and a baby 41 weeks

At what gestational age would you for 39 weeks failed external version. This signified that in total, 72 (n=41, failed, and 99 (76% of 130) opted for cesarean section after

Concerning term vaginal delivery, 17% (n=25) had performed more than 50 vaginal breech deliveries in their entire career and 61 (25.3%) had performed more than 50 vaginal breech deliveries.

Comparing these data to an earlier survey reporting on data from 2002 [3], a significant rise can be noted in the percent-age of gynaecologists opting for planned cesarean section for uncomplicated cephalic term singleton for themselves or their partner: from 2% (six out of 295) in 2002 to 16% (39 out of 241) in 2014 (p < 0.0001; OR 5.33, 95% CI 3.91-7.26).

### Discussion

The present authors acknowledge that a weakness of this study was the rather low response rate, but as the ratio of female to male and the ratio of registered specialists to trainees is comparable to the general population, they believe these results are still representative of the Flemish situation. They do not know the proportion of gynecologists active in obstetrics in the complete register of gynecologists. Probably the large majority is practicing obstetrics, as in Flanders, the large majority of uncomplicated pregnancies are under specialist supervision and over 90% of deliveries are in the presence of a gynecologist.

In 2003 in case of an uncomplicated singleton first pregnancy with a cephalic presentation, only 2% of Flemish gynecologists and obstetricians preferred elective cesarean section. In 2014 this has risen to 16%. Clearly there has been an evolution in the last decade with a larger group preferring cesarean section for themselves or their partners: from 2% (six out of 295) in 2002 to 16% (39 out of 241) in 2014 (p < 0.0001; OR 5.33, 95% CI 3.91-7.26).

### Conclusion

While there has been an increase in the preference for cesarean section, it is important to consider the potential risks and benefits associated with this decision. Future research should aim to further investigate the factors influencing these preferences and to evaluate the outcomes associated with different delivery methods. This will help in making informed decisions that are in the best interest of both the mother and the newborn.
chosces cesarean section [6], and two out of three of Turkish obstetricians prefer cesarean section as mode of delivery for themselves or their partners [4]; the same has been noted in China [7].

In a 2004 study in Denmark, only 1.1% of obstetricians would prefer an elective cesarean section in an uncomplicated pregnancy. This is comparable to the 2% in Flanders at that moment [8]. On the other hand, in a 2001 study, 7% of Irish obstetricians would choose elective cesarean section for themselves or their partners [9] and 15% of UK obstetricians in the same period [10].

When looking at the actual mode of delivery for those who have given birth, quite a different image is seen; for the past ten years the cesarean section rate for term singletons in Flanders has been around 15% (and slowly rising), and the total cesarean section rate for all deliveries in Flanders recently reached 20% [11]. In the present group, only 7% had a cesarean section, which is clearly less than the general population. In the general population, 10% of deliveries are instrumental vaginal deliveries, whereas in this group of gynecologists and their partners, only 1.6% had an instrumental vaginal delivery. The rate of induction of labor in Flanders approaches 30%, not different from the present study group.

It is notable that only about half of obstetricians would advise a vaginal delivery in case of a term breech, and it is regrettable that less than half of gynecologists at this moment in Flanders feels personally able to assist vaginal breech delivery, as about 30% has performed less than ten vaginal breech deliveries in their career and it is of no surprise that they feel unsafe. This number can be somewhat lower because this study included trainees. Actually assisting vaginal breech delivery is no longer part of the obligatory obstetrical curriculum for trainees to register for specialization. In reality in Flanders in 2013, 93% breech babies were born by cesarean section [11].

In conclusion the present study demonstrates that the attitude of Flemish gynecologists is still in favor of a vaginal delivery for themselves, their partner, and their patients. However, opinions are clearly changing and significantly more gynecologists now prefer to undergo cesarean section for themselves or their partners, as compared to ten years ago. Most gynecologists do not offer the possibility for vaginal breech delivery and do not consider themselves professionally capable of assisting such a delivery.

References


Address reprint requests to:
Y. JACQUEMYN, M.D.
Department of Obstetrics and Gynecology
Antwerp University Hospital UZA
Witrixstraat, 10
2650 Edgegem (Belgium)
e-mail: Yves.jacquemyn@uz.a.be
HELLP syndrome is still a serious, life-threatening complication of pregnancy: admission of 34 women to an eastern Turkish intensive care unit

Z. Bedir1, A. Ahiskalioglu1, Ü. Esenkaya2, E.O. Ahiskalioglu1, A. Dostbil1, M. Aksoy1, N. Dogan1, H. Kursad1

1 Ataturk University School of Medicine, Department of Anesthesiology and Reanimation, Erzurum
2 Konya Beysehir State Hospital, Department of Obstetric and Gynecology, Konya
1 Erzurum Regional and Training Hospital, Department of Anesthesiology and Reanimation, Erzurum (Turkey)

Summary

Objective: The transfer of the obstetric patient to the intensive care unit is considered as an indicator of maternal morbidity. The most important two indications for admittance of the obstetric patient to the intensive care unit are postpartum hemorrhage and hypertensive disorders. The purpose of this study was to determine maternal morbidity and mortality rates in patients diagnosed with hemolysis, elevated liver enzyme levels, and low platelet count (HELLP) syndrome who required intensive care. Materials and Methods: The charts of 34 patients who were diagnosed with HELLP syndrome and treated in intensive care unit between the years 2005 - 2013 were evaluated retrospectively. Results: During the study period, a total of 151 patients were diagnosed with HELLP syndrome and 34 patients were admitted to the intensive care unit. Mean age of the patients was 28.97 ± 7.26 years and there was no significant difference between survivors and non-survivors (p = 0.442). There were no significant differences between survivors and non-survivors in terms of gestational age, parity, and multiparity rates (p > 0.05). There was 31.2% mortal cases and 77.8% of living cases had received regular antenatal follow-up and the difference was statistically significant (p = 0.006). 30 patients (88.2%) required invasive mechanical ventilation. The average Glasgow Coma Score (GCS) of patients was 6.47 ± 4.34. There were significant differences between patients who lived and who died in terms of Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA) score, and duration of invasive mechanical ventilation (p < 0.05). Twenty-two patients (64.7%) required transfusion of blood and blood products. Maternal mortality occurred in 16 patients (47%). The causes of death were: intracerebral hemorrhage in six cases, acute respiratory distress syndrome (ARDS) in three cases, disseminated intravascular coagulation (DIC) in three cases, sepsis/multiple organ dysfunction syndrome (MODS) in two cases, hepatic rupture in one case, and massive pulmonary embolism in one case. Conclusion: HELLP syndrome is still one of the most serious and life-threatening complications of pregnancy. Mortality rate can be reduced by regular antenatal follow-up and transfer of pregnant women who carry risk to the intensive care unit without delay.

Key words: HELLP syndrome; Maternal mortality; Intensive care unit.

Introduction

Despite advances in diagnosis and treatment, hypertension during pregnancy is still an important cause of morbidity and mortality. HELLP syndrome, characterized by hemolysis, elevated liver enzymes and low platelet count was first described by Weinstein. HELLP syndrome is a multisystemic disorder. Irregular vascular tonus, severe vasoconstriction, and disorders of the coagulation system are remarkable features of the syndrome [1]. HELLP syndrome is one of the most significant causes of maternal and perinatal mortality and morbidity. Its clinical course includes many life-threatening complications such as acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), cerebral hemorrhage, septic shock, acute renal failure, hepatic rupture, and placental abruption [2]. Maternal mortality related to HELLP syndrome was reported to be between 1.1% and 25% [3, 4]. In recent years, while maternal mortality rates have been significantly declining in developed countries, mortality rate is still high in developing countries and in underdeveloped regions where the perinatal follow-up is poor [5, 6]. The intensive care management of the obstetric patient differs from the other patient groups. Admittance of the obstetric patient to the intensive care unit is rare; it constitutes less than 1% of all intensive care patients and transfer of the obstetric patient to the intensive care unit is considered as an indicator of maternal morbidity [7-9] The purpose of this study was to determine maternal morbidity and mortality rates in patients diagnosed with HELLP syndrome who required intensive care.
Table 1. — Clinical characteristics of study patients *.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients</th>
<th>Survivor (n=18)</th>
<th>Non-survivor (n=16)</th>
<th>p value b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>28.97 ± 7.26</td>
<td>29.89 ± 7.68</td>
<td>27.94 ± 6.85</td>
<td>0.442</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>33.97 ± 4.97</td>
<td>34.83 ± 4.96</td>
<td>33.00 ± 4.95</td>
<td>0.290</td>
</tr>
<tr>
<td>Parity</td>
<td>2.71 ± 2.87</td>
<td>3.33 ± 3.51</td>
<td>2.00 ± 1.75</td>
<td>0.421</td>
</tr>
<tr>
<td>Multiparity</td>
<td>15 (44.1%)</td>
<td>8 (44.4%)</td>
<td>7 (43.8%)</td>
<td>NS'</td>
</tr>
<tr>
<td>Primiparity</td>
<td>19 (55.9%)</td>
<td>10 (55.6%)</td>
<td>9 (56.2%)</td>
<td>NS'</td>
</tr>
<tr>
<td>Antenatal follow-up (n, %)</td>
<td>19 (55%)</td>
<td>14 (77.8%)</td>
<td>5 (1.2%)</td>
<td>0.006 *</td>
</tr>
<tr>
<td>Platelets (×10^9/L)</td>
<td>135080 ± 91391</td>
<td>166666 ± 107354</td>
<td>105923 ± 64973</td>
<td>0.097</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>381.36 ± 552.52</td>
<td>183.58 ± 246.44</td>
<td>563.92 ± 692.58</td>
<td>0.85</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>212.56 ± 255.27</td>
<td>144.25 ± 215.58</td>
<td>275.62 ± 280.64</td>
<td>0.205</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>2.13 ± 1.64</td>
<td>2.17 ± 1.75</td>
<td>2.09 ± 1.56</td>
<td>0.825</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>1377.82 ± 873.77</td>
<td>1467.22 ± 965.43</td>
<td>1277.25 ± 776.48</td>
<td>0.535</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.02 ± 0.68</td>
<td>0.9 ± 0.62</td>
<td>1.15 ± 0.75</td>
<td>0.237</td>
</tr>
<tr>
<td>APACHE-II score</td>
<td>26.38 ± 8.84</td>
<td>20.89 ± 6.30</td>
<td>32.56 ± 7.07</td>
<td>&lt; 0.001 f</td>
</tr>
<tr>
<td>GCS score</td>
<td>6.47 ± 4.34</td>
<td>7.89 ± 4.78</td>
<td>4.88 ± 3.24</td>
<td>0.065</td>
</tr>
<tr>
<td>SOFA score</td>
<td>968 ± 4.12</td>
<td>7.11 ± 3.07</td>
<td>12.56 ± 3.16</td>
<td>&lt; 0.001pf</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal (n, %)</td>
<td>9 (26.5%)</td>
<td>6 (33.3%)</td>
<td>3 (18.8%)</td>
<td>NS'</td>
</tr>
<tr>
<td>Cesarean (n, %)</td>
<td>25 (73.5%)</td>
<td>12 (66.7%)</td>
<td>13 (81.2%)</td>
<td>NS'</td>
</tr>
<tr>
<td>Anesthetic management</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regional anesthesia (n, %)</td>
<td>3 (12%)</td>
<td>1 (8.3%)</td>
<td>2 (15.4%)</td>
<td>NS'</td>
</tr>
<tr>
<td>General anesthesia (n, %)</td>
<td>22 (88%)</td>
<td>11 (91.7%)</td>
<td>11 (84.6%)</td>
<td>NS'</td>
</tr>
</tbody>
</table>

APACHE: Acute Physiology and Chronic Health Evaluation; GCS: Glasgow coma score; SOFA: Sequential Organ Failure Assessment; ICU: intensive care unit; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactic dehydrogenase.

* Values are given as number (percentage) or mean ± SD; b For the comparison of survivors and non-survivors.

Materials and Methods

The charts of 34 obstetric patients admitted to the Atatürk University Medical Faculty, Department of Anesthesiology and Reanimation, Intensive Care Unit between 2005 and 2013 were investigated retrospectively, following the approval of Ethics Committee. The diagnosis of HELLP syndrome was made according to Tennessee classification: hemolysis (the presence of fragmented erythrocytes in peripheral blood smear), serum total bilirubin ≥1.2 mg/dl, elevated liver enzymes (alanine aminotransferase ≥ 70 U/L and/or aspartate aminotransferase ≥ 70 U/L), and low platelet count (< 100,000/μl).

The demographic data of diagnosed patients: age, gravidity, parity, gestational week, presence of antenatal follow-up, and delivery mode were identified. Additionally, the laboratory parameters; platelet count, liver function tests (AST, ALT), LDH, total bilirubin, direct bilirubin, renal function tests (BUN, creatinine), and INR values were recorded.

Acute renal failure, DIC, placental abruption, intracranial hemorrhage, subcapsular hematoma, eclampsia, cerebral edema, sepsis, acute respiratory distress syndrome, comorbidities, Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA) score, Glasgow Coma Score (GCS), requirement of mechanical ventilation, duration of mechanical ventilation, duration of stay in intensive care unit, transfusion requirement of blood and blood products (packed red cells, fresh frozen plasma, platelets), vasoactive drug usage, dialysis, hemofiltration, requirement of plasmapheresis, and presence of maternal mortality were investigated in patient charts.

Patients diagnosed as HELLP syndrome who were hemodynamically unstable, or have respiratory failure, require mechanical ventilation, dialysis, plasmapheresis or vasoactive drugs were transferred to the intensive care unit.

Statistical analysis

SPSS 20.0 software package was used for statistical analysis. To compare the variables of living and dead patients categorically, the chi-square test was used. The normal distributions of numerical parameters of the patients were evaluated by Kolmogorov Smirnov test. For comparison of parameters showing normal distribution, t-test was used. Mann-Whitney U test was used for parameters that did not meet normal distribution. A p < 0.05 was considered to be statistically significant.

Results

During the study period, a total of 151 patients were diagnosed with HELLP syndrome and 34 (22.5%) of them were admitted to and treated in intensive care unit. Other patients were treated and followed up in obstetrics clinic.

Demographic characteristics and clinical features of 34 patients managed in intensive care unit are shown in Table 1. The mean age of patients was 28.97±7.26 years and no statistically significant difference was found between groups (p = 0.442). There were no statistically significant differences between survivor and non-survivors in terms of gestational week, parity, and multiparity rates (p > 0.05). There was 31.2% of dead patients and 77.8% of living patients had received regular antenatal follow-up and the difference was statistically significant (p = 0.006). When platelet counts, LDH, AST, ALT, total bilirubin, and creatinine values during their first admittance were taken into consideration, there were no significant differences be-
Causes of death for 16 dead patients are shown in Table 3. While 77.8% of the living patients required invasive mechanical ventilation, this ratio was 100% for dead patients (p = 0.045). The duration of mechanical ventilation was 2.61 ± 2.68 days for living patients and 9.06 ± 5.53 days for dead patients, and the difference between them was found to be statistically significant (p < 0.001). Total duration of hospitalization in intensive care unit was 5.67 ± 5.64 days in living patients, whereas it was 9.06 ± 5.53 days in dead patients, and the difference was not statistically significant (p = 0.07). When requirements for inotropic drugs, fresh frozen plasma, and packed red cell transfusion were taken into consideration, they were found to be increased in dead patients and the differences were statistically significant (p values were 0.024, <0.001, and <0.001, respectively). There was no statistically significant difference between groups in terms of platelet transfusion (p = 0.190).

Hemodialysis was required for three patients in each group. Between groups (p > 0.05), APACHE II, GCS, and SOFA scores of the patients at their admittance to the intensive care unit were analyzed. While no statistically significant difference was found in terms of GCS, there were statistically significant differences between groups in terms of APACHE II and SOFA scores (p < 0.001). There were no significant differences in terms of delivery modes and anesthetic management.

Parameters of patients related to intensive care are shown in Table 2. While 77.8% of the living patients required invasive mechanical ventilation, this ratio was 100% for dead patients (p = 0.045). The duration of mechanical ventilation was 2.61 ± 2.68 days for living patients and 9.06 ± 5.53 days for dead patients, and the difference between them was found to be statistically significant (p < 0.001). Total duration of hospitalization in intensive care unit was 5.67 ± 5.64 days in living patients, whereas it was 9.06 ± 5.53 days in dead patients, and the difference was not statistically significant (p = 0.07). When requirements for inotropic drugs, fresh frozen plasma, and packed red cell transfusion were taken into consideration, they were found to be increased in dead patients and the differences were statistically significant (p values were 0.024, <0.001, and <0.001, respectively). There was no statistically significant difference between groups in terms of platelet transfusion (p = 0.190).

Hemodialysis was required for three patients in each group. Causes of death for 16 dead patients are shown in Table 3; they were intracerebral hemorrhage (37.5%), ARDS (18%), DIC (18%), sepsis/MODS (12%), hepatic rupture (6%), and massive pulmonary embolism (6%). Mortality rate was 47% in patients admitted to the intensive care unit. Sixteen out of 151 patients who were diagnosed with HELLP syndrome had died throughout the study period; the mortality rate of this group was 10.5%.

### Discussion

Maternal death is still a significant problem throughout the world. Reducing maternal mortality is an important international developmental goal [6]. Maternal mortality is described as the death of a woman due to the pregnancy or a cause related to its management during pregnancy or within 42 days following delivery, other than accidents or incidental causes [10]. The most common causes are as follows: hemorrhage, thromboembolism, cardiac disorders, sepsis, hypertensive diseases, and amniotic fluid embolism. Despite studies to reduce maternal mortality in recent years, unfortunately, maternal deaths related to HELLP syndrome have not been reduced significantly. The mortality rates of patients with HELLP syndrome admitted to the intensive care unit differs between developed and developing countries. In reports published recently, mortality rate is between 0% and 3.8% in developed countries [11-15]. While mortality rate is significantly low in developed countries, in developing countries with low socio-economic status, this rate is increased to 10-35% [16-20]. In a study conducted in Turkey, maternal mortality rate related to HELLP syndrome was reported to be 30% [21]. The mortality rate being higher in the present study (47%) when compared to other studies might have been related to the present admission criteria to intensive care unit differs between developed and developing countries. In reports published recently, mortality rate is between 0% and 3.8% in developed countries [11-15]. While mortality rate is significantly low in developed countries, in developing countries with low socio-economic status, this rate is increased to 10-35% [16-20]. In a study conducted in Turkey, maternal mortality rate related to HELLP syndrome was reported to be 30% [21]. The mortality rate being higher in the present study (47%) when compared to other studies might have been related to the present admission criteria to intensive care unit.

Sixteen out of 151 patients who were diagnosed with HELLP syndrome had died throughout the study period; the mortality rate of this group was 10.5%.
madan [22] in 1993, on a series of 32 cases with HELLP syndrome is one of the most remarkable studies. In this series, 31% of the cases required dialysis and mortality rate was reported to be 13%. In the present study, the need of patients for dialysis was six out of 34. While half of these patients died, the other half was discharged and renal functions were not affected in the long term. However, requirement of dialysis was not found to be statistically significant in terms of mortality.

Studies have shown that APACHE and GCS scores were useful in predicting the severity of the disease accurately in critical obstetric patients [23]. In the present study, to evaluate the severity of the disease, the authors used APACHE II, SOFA, and GCS scores; the average APACHE II and SOFA scores were found to be higher in patients who died, when compared to living patients. The present authors consider that this high rate might have been related to the existing respiratory failure of the patients requiring mechanical ventilation and lack of their timely transfer to the intensive care unit from external medical facilities. The difference between dead and living patients in terms of their GCS scores during their admissions to the hospital was not statistically significant. This might have been related to the present clinical conditions of the patients being serious.

While no relations were found between maternal age, gestational age, and parity with maternal mortality and morbidity related to HELLP syndrome in many studies in the literature, vice versa, in some other studies, advanced maternal age and high parity were found to be related to poor clinical outcome [14, 24]. In the present study, maternal age, gestational age, and parity did not have any effects on maternal mortality related to HELLP syndrome.

While there are many reports showing that cases with HELLP syndrome require high amounts of blood and blood products, similar results were obtained in the present study. In the present case series, 22 patients (64.7%) required transfusion of blood and blood products. This shows that preparations should be made for these patients in terms of requirements of blood and blood products, and when necessary, these products should be urgently administered to the patients.

Although the most common cause of death related to HELLP syndrome was reported as cerebral hemorrhage, multiple organ failure, DIC, ARDS, and hepatic rupture are the other significant causes of death. Consistent with the literature, in the present study, maternal deaths occurred due to cerebral hemorrhage in six (37.5%) out of 16 patients [25, 26]. Another important cause of death is hepatic rupture. Hepatic rupture-related deaths occur with rates from 18% to 86%. In the present series, hepatic rupture developed in three cases and one of them died related to hepatic rupture.

Limitations of this study were as follows: firstly, this was a retrospective study, not a randomized clinical trial; secondly, the study population was small. The present study revealed significant results regarding mortality since the study population, although small, included only patients with HELLP syndrome and admitted to the intensive care unit.

As a conclusion, in HELLP syndrome, lack of regular antenatal follow-up is a significant cause of death, especially in developing countries. Despite all improvements in the treatment of HELLP syndrome and intensive care, mortality still cannot be significantly reduced throughout the world. In patients with HELLP syndrome, mortality rates can be reduced by determining the risk factors and transferring patients to the intensive care unit without delay, before requirement for intensive care is manifested.

References


HELP syndrome is still a serious, life-threatening complication of pregnancy: admission of 34 women to an eastern Turkish etc.


Address reprint requests to:
A. AHISKALIOGLU, M.D.
Ataturk University School of Medicine
Department of Anesthesiology and Reanimation
Refik Saydam Street, 74
25100 Yakutiye/Erzurum (Turkey)
e-mail: aliahiskalioglu@hotmail.com
Rupture risk factors of fallopian tubal pregnancy


Department of Obstetrics and Gynecology, ASO Iizuka Hospital, Iizuka, Fukuoka (Japan)

Summary

The present authors analyzed patients’ backgrounds and pre-surgical findings to clarify the risk factors of rupture of fallopian tubal pregnancy. The surgical findings 113 cases were clearly diagnosed as fallopian tubal pregnancy with or without rupture. Twenty-six cases of fallopian tubal pregnancy were ruptured and 87 cases were not ruptured at the time of operation. The risk factors of fallopian tubal rupture were assessed by Chi-square for independence test and multiple regression analysis. Obesity (BMI over 26), prior birth history, social welfare entitlement, ultrasonography findings of fetal heart movement, and pre-surgical serum beta-hCG level more than 3,000 mIU/ml patient were significantly higher risk in fallopian tubal rupture. Fertility treatment patient were at significantly lower risk for fallopian tubal rupture. Higher beta-hCG levels, especially >3,000 mIU/ml is associated with increased risk of fallopian tubal rupture in ectopic pregnancy.

Key words: Ectopic pregnancy; Rupture; Serum beta-subunit human chorionic gonadotropin.

Introduction

Ectopic pregnancy remains the leading cause of maternal life crisis [1, 2]. About 1% of pregnancies are in an ectopic location with implantation not occurring inside of the womb [3]. Most of ectopic pregnancies (97%) are implanted within the fallopian tube. Recently the serum beta-subunit human chorionic gonadotropin (beta-hCG) levels and transvaginal ultrasound examination have valuable resources for diagnosing ectopic pregnancy. The knowledge of risk factors associated with the rupture of an ectopic pregnancy may be a valuable tool to identify women at risk for this life-threatening condition. The aim of this study was to discover the fallopian tubal rupture risk factors of ectopic pregnancy in surgical cases. The authors analyzed patient backgrounds and pre-surgical findings to clarify the risk factors of rupture of the fallopian tubal pregnancy.

Materials and Methods

The study group comprised cases of fallopian tubal ectopic pregnancy which were diagnosed in the present hospital from January 2007 to June 2012. On the surgical findings, 113 cases were clearly diagnosed as fallopian tubal pregnancy with or without rupture. The authors classified a ruptured group and an unruptured group. They analyzed patient backgrounds and pre-surgical findings to clarify the risk factors of rupture of fallopian tubal pregnancy. Inclusion criteria were the following: patients diagnosed with fallopian tubal ectopic pregnancy and operated by laparotomy or laparoscopy. Gestational age was calculated according to the last menstrual period at the time of surgery. Exclusion criteria: non-fallopian tubal pregnancy (ovarian, interstitial site). The authors interviewed patients’ and recorded their medical histories. They also analyzed serum beta-hCG concentration, fetal heart beat, and operative findings including intraperitoneal bleeding. The risk factors of fallopian tubal rupture were assessed by Chi-square for independence test and multiple regression analysis. Student's t-test, Mann–Whitney–Wilcoxon test for independent samples, Pearson's chi-square, and Fisher's exact test were applied for comparison of groups where appropriate. Multivariate logistic regression analysis was used to identify predictors of the outcome of ectopic pregnancy (variables with a p value < 0.05 by univariate analysis were entered into the multivariate analysis). Receiver operating characteristic curves (ROC) was used to test the overall predict accuracy of serum beta-hCG, and results were reported as area under the curve (AUC). All statistical analysis was performed with SPSS for Windows, version 20.0. Statistical significance was defined as p < 0.05.

Results

The surgical findings of 113 cases were clearly diagnosed as fallopian tubal pregnancy with or without rupture. Twenty-six cases of fallopian tubal pregnancy were ruptured and 87 cases were not ruptured. The mean of maternal age was 29.0 ± 5.4 years in unruptured group and 32.0 ± 3.9 years in ruptured group. The mean gestational age was 7.72 ± 1.23 weeks in unruptured group compared with 7.23 ± 1.82 weeks in ruptured group. According to t-test, there was no statistical difference between groups. Odds ratios and 95% confidence intervals (CI) for fallopian tube rupture are shown in Table 1. BMI > 26, parity, social welfare entitlement, ultrasonography findings of fetal heart movement, and serum beta-hCG over 3,000 mIU/ml patients were significantly at higher risk for fallopian tubal rupture. Fertility treatment patient were significantly at lower risk for fallopian tubal rupture. Based on the ROC...
curve (Figure 1), the optimal cutoff value of pre-surgical serum beta-hCG levels as an indicator of risk of fallopian tubal rupture was projected to be 3,475 mIU/ml, which yielded a sensitivity of 62.5 % and a specificity of 57.1%, with the AUC at 0.66 ($p = 0.045$). Amount of surgical bleeding is shown in Figure 2. Surgical bleeding was obviously increased in fallopian tubal rupture group.

### Discussion

Diagnosis of fallopian tubal pregnancy requires immediate treatment to avoid rupture remains and is ultimately an arduous task. The high rupture rates were explained by delayed diagnosis due to lack of diagnostic tools such as transvaginal ultrasound and beta-hCG measurements. The rate of fallopian tubal rupture is acceptable (23%) compared with other reports (18-35%) [4, 5]. The present authors discovered five factors: (BMI > 26, parity, social welfare entitlement, ultrasonography findings of fetal heart movement, and serum beta-hCG over 3,000 mIU/ml) that increased the risk of fallopian tubal rupture. Subjects with BMI > 26 were at significantly higher risk for fallopian tubal rupture.

The mechanism behind irregular menstrual cycles is related to disturbances in the normal hormonal balance. Normal menstrual cycles correlate with normal cyclic

---

**Table 1. — Odds ratios and 95% confidence intervals for fallopian tube rupture.**

<table>
<thead>
<tr>
<th></th>
<th>Ruptured (n=26)</th>
<th>Unruptured (n=87)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>10 (38.4)</td>
<td>56 (64.4)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>&gt; 30</td>
<td>16 (61.6)</td>
<td>31 (35.6)</td>
<td>2.9</td>
<td>1.2−7.1</td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 26</td>
<td>17 (65.4)</td>
<td>80 (92.0)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>&gt; 26</td>
<td>9 (34.6)</td>
<td>7 (8.0)</td>
<td>5.1</td>
<td>1.6−15.8</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nullipara</td>
<td>7 (26.9)</td>
<td>47 (54.0)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Delivered</td>
<td>19 (73.1)</td>
<td>40 (46.0)</td>
<td>3.2</td>
<td>1.6−15.8</td>
</tr>
<tr>
<td>History of pelvic surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>19 (73.1)</td>
<td>51 (58.6)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (26.9)</td>
<td>36 (41.4)</td>
<td>0.5</td>
<td>0.2−1.4</td>
</tr>
<tr>
<td>History of STD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>23 (88.4)</td>
<td>66 (75.9)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (11.6)</td>
<td>21 (24.1)</td>
<td>0.4</td>
<td>0.1−1.5</td>
</tr>
<tr>
<td>Habitual smoker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>16 (61.6)</td>
<td>42 (48.3)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (38.4)</td>
<td>45 (51.7)</td>
<td>0.6</td>
<td>0.2−1.4</td>
</tr>
<tr>
<td>Social Welfare</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>22 (84.6)</td>
<td>85 (97.7)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (15.4)</td>
<td>2 (2.3)</td>
<td>7.7</td>
<td>1.3−44.0</td>
</tr>
<tr>
<td>Marriage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6 (23.1)</td>
<td>15 (17.2)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20 (76.9)</td>
<td>72 (82.8)</td>
<td>1.4</td>
<td>0.5−4.2</td>
</tr>
<tr>
<td>Fertility treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>26 (100.0)</td>
<td>79 (90.8)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0.0)</td>
<td>8 (9.2)</td>
<td>0.2</td>
<td>0.1−0.4</td>
</tr>
<tr>
<td>Fetal heart movement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>20 (76.9)</td>
<td>83 (95.4)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 (23.1)</td>
<td>4 (4.6)</td>
<td>6.2</td>
<td>1.6−24.1</td>
</tr>
</tbody>
</table>
production of hormones. An increase in body fat percentage also increases circulating estrogen levels in the body. This excess estrogen causes conflicting signals to the ovaries and to the brain, which results in irregular or ovulation from the ovaries. When ovulation is late or absent, abnormal cycles result. Parity was also a factor for significantly higher risk of fallopian tubal rupture. There is some possibility of relating it to uterine endometrium inflammation and scar.

Social welfare entitlement patients were at a significantly higher risk for fallopian tubal rupture. The cause is uncertain, the possibility are low education level, lack of health knowledge, and unsanitary environments.

Fertility treatment patients were at significantly lower risk for fallopian tubal rupture. Because these patients visited hospital earlier than natural pregnant patient regarding their pregnancy, ectopic pregnancies could be diagnosed before rupture. Serum beta-hCG over 3,000 mIU/ml and ultrasonography findings of fetal heart movement presented were related with activity of ectopic pregnancy. Job-Spira et al. reported that beta-hCG over 10,000 mIU/ml increased the probability of fallopian tubal rupture [4]. Cacciatorre et al. managed 71 cases of suspected ectopic pregnancy based upon quantitative serum beta-hCG and vaginal ultrasonography [6]. They selected expectant management when the beta-hCG was less than 1,000 mIU/ml. Trio et al. successful expectant management about the initial beta-hCG was less than 1,000 mIU/ml in 112 cases ectopic pregnancy [7]. Ylostalo et al. proposed another management of beta-hCG. They calculated percentage by the initial value for beta-hCG and the 48-hour beta-hCG [8]. More than 2% decrease in value had a possibility of unruptured prognosis. The present authors suggest that a serum beta-hCG level of more than 3,000 mIU/ml and ultrasonography findings of fetal heart movement presented in fallopian tubal pregnancy were risk factors for rupture and they needed to decide the surgical procedure. However, it must be kept in mind that fallopian tubal rupture can occur even with lower levels of serum beta-hCG.

Conclusion

There is a dispute regarding the risk factors leading to rupture in fallopian tubal pregnancy. According to the present study, higher beta-hCG levels, especially > 3,000 mIU/ml was associated with increased risk of fallopian tubal rupture in ectopic pregnancy. Patients presenting with these findings can be warned about the risk of rupture and surgical management can be planned according to these risk factors.

Acknowledgements

The authors acknowledge Hidenobu Koga for assistance with statistics analysis.

References


Address reprint requests to:
T. FUKAMI, M.D., PhD
Department of Obstetrics and Gynecology
ASO Iizuka Hospital
3-83 Yoshio-machi, Iizuka
Fukuoka 820-8505 (Japan)
e-mail: fukami1975@msn.com
Urethral instillations of clobetasol propionate and lidocaine: a promising treatment of urethral pain syndrome

B.E. Lindström1,2, D. Hellberg1,2, A.K. Lindström1,2
1 Center for Clinical Research, Dalarna; 2 Department of Women’s and Children’s Health, Uppsala University, Uppsala (Sweden)

Summary
Purpose: To evaluate topical treatment with clobetasol propionate and lidocaine in women with urethral pain syndrome (UPS) in a retrospective pilot study. Materials and Methods: Urethral instillations of two ml clobetasol propionate cream and two ml lidocaine gel in 30 Caucasian women age 15-74 years with UPS between 1999 and 2006 were evaluated retrospectively. Instillations were given approximately once a week until the patient improved. Between one and 15 (median three) instillations were given. In substudy I a review was undertaken of the medical records to register the treatment effect at the end of the treatment (the last instillation) and any relapses six months thereafter. Substudy II was a follow-up at least five years after last instillation based on medical records and a written questionnaire. Results: Substudy I (n=30): By the end of the treatment 18 women had no symptoms and 12 were improved. Five patients had relapsed within six months. Substudy II (n=28): Twenty-eight women responded to the questionnaire. Four women remained with no symptoms, 18 remained improved, and six had the same symptoms as before treatment. Twenty women thought the treatment was very effective, five rather effective, and three women reported poor effect. Twenty-six women would ask for retreatment if a relapse occurred, two patients would not. No side effects, except transient pain, were reported. Conclusions: This retrospective study and long-term follow-up suggests that urethral instillation of clobetasol propionate and lidocaine is effective in treating women with UPS. Randomized control studies are warranted.

Key words: Clobetasol; Lidocaine; Urethral pain syndrome; Urethral syndrome; Urethritis.

Introduction
Gallagher et al. coined “urethral syndrome” in 1965, defined as recurrent urinary irritation without urinary tract infection [1]. In 2002 the International Continence Society changed the terminology to “urethral pain syndrome” (UPS) as a part of the “genito-urinary pain syndromes”. UPS is defined as the occurrence of recurrent episodic urethral pain, usually on voiding, with daytime frequency and nocturia, in the absence of proven infection or other obvious pathology [2]. In this article the authors will consider the urethral syndrome and the UPS as the same condition and they generally use the term UPS for both. UPS is a diagnosis of exclusion and the etiology is unknown. Theories of etiology to UPS include infection of low activity, urethral stenosis, early manifestation of interstitial cystitis (i.e. painful bladder syndrome), stress, trauma, allergy, mechanical obstruction, neuropathic hypersensitivity as a result of urinary infection, traumatic intercourse, an incomplete relaxation or spasm of the external striated sphincter, periurethral fibrosis, estrogen deficiency in the urethral mucosa, dysfunctional epithelium, and inflammation of the paraurethral glands [3, 4].

UPS is a common condition but the true incidence is unknown due to lack of consensus in diagnosis and overlap with other conditions. In a study from England, about half the patients visiting their general practitioner with frequency and/or dysuria did not have significant bacteriuria [5]. UPS is often a recurrent chronic disease. It is not a life-threatening condition but it can have a significant impact on the quality of life. In the most severe cases of UPS the condition can lead to long periods of sick-leave, affect sexual functions, and social relations [4, 6]. A study in 1989 showed higher levels of hostility, irritability, anxiety, dysphoria, and depression in the group of patients with urethral syndrome than in the control group [6].

There is no golden standard in the treatment of UPS. Kaur and Arunkalaivanan stated in 2007 that “treatment at its best is by trial and error” [3]. In 2006 the present authors sent a questionnaire to 21 gynecology clinics and nine urology clinics in Sweden with questions about how they treated UPS in women. Sixteen different modalities of treatment were given singly or in combination. None of the clinics gave treatment with strong or extra strong corticosteroids (Europe class III-IV, US class I-II) to patients with UPS. The response rate from the clinics was 90% (unpublished data).

The present authors’ hypothesis is that an important cause of symptoms of UPS is urethral inflammation. The aim of this study was to retrospectively evaluate treatment with urethral instillations of the more potent corticosteroid clobetasol-propionate (CP) cream (US class I, Europe class IV) with lidocaine (L) gel.
Materials and Methods

This study is a retrospective evaluation of urethral instillations of CP cream 0.05% in 30 women with UPS during the period of September 1999 to November 2006. During this period 40 Caucasian women were diagnosed with UPS at the outpatient gynecology clinic in Bjursås, Sweden. UPS was diagnosed with symptoms urethral pain and or dysuria, with or without urgency, and palpation tenderness of the anterior vaginal wall without signs of infection or other pathology. To exclude other pathology, all patients had, apart from the taking of medical history, dipstick urinalysis, clinical gynecological examination with vaginal ultrasound of the uterus and adnexa to exclude common lower urinary tract infection, gynecological tumors, and atrophic colpitis. If any clinical suspicion of other conditions existed, laboratory tests for sexual transmitted disease, common urine culture or cystoscopy was carried out. Patients without estrogen therapy that were diagnosed with UPS were first offered treatment with local estrogen therapy. If the estrogen therapy did not relieve the patient from the symptoms, she was offered treatment with CP and L. Inclusion criterion was diagnosis of UPS as described above. Exclusion criteria were the finding of any other possible cause of the UPS symptoms as described above, or that the patient achieved any other treatment method for UPS during the study period. Out of the 40 women diagnosed with UPS, ten women were excluded from the study; two patients were lost to follow-up, one patient received one instillation of betamethasone valerate (strong steroid, Europe class III) instead of CP and became free of symptoms, three patients were excluded because UPS occurred only after coitus and were treated effectively with a short course of antibiotics post coitus, one postmenopausal patient received local estrogen, and did not get further relapse of UPS. The remaining 33 patients were offered CP and L instillations; three patients with mild symptoms were satisfied with the diagnosis and explanation and refrained from treatment.

The study included the remaining 30 women. Age at inclusion was 15-74 years (mean 56, median 62). Six women were pre- and 24 were postmenopausal. Two women (7%) were smokers and 20 (67%) were sexually active. Altogether 26 (87%) had estrogen treatment. Thirty (43%) had local estrogens, eight (27%) had local estrogens and hormone replacement therapy (HRT), four (13%) had HRT, and one (3%) combined oral contraceptives. Four patients (13%) did not accept estrogen treatment (two premenopausal and two postmenopausal). All the patients were treated with CP and L the same way and all completed treatment as recommended by the doctor (AKL). At each treatment session, the patient first emptied her bladder to be able to refrain from urination for at least two hours after the urethral instillations. The urine was also checked with dipstick urinalysis to exclude signs of current urinary infection or hematuria. Thereafter two ml CP cream was instilled into the urethra, immediately followed by instillation of two ml of L gel. L was instilled to alleviate the transient burning urethral pain typically caused by the CP cream in the UPS patient. Instillations were given approximately once a week. The patient decided when to stop treatment by stating she was either free from symptoms, improved, unchanged or worsened. Between one and 15 (median three) instillations were given. All treatments were given the same way each time by one of the authors (AKL).

In substudy I, a protocol for retrospective evaluation was constructed by a person not involved in the treatment (DH). A review of the medical records was carried out, to register the treatment effect at the end of the treatment and to document any relapses within six months. A relapse was defined as recurrence of the UPS symptoms, exclusion of other cause of the symptoms was then carried out as described above. Evaluation of the medical records was conducted by a person not involved in the treatment and blinded for patient identification (BEL). Substudy II was a long-term follow-up through a written questionnaire distributed in 2012 to the patients of substudy I at least five years after the end of treatment. Substudy II included 29 women as one woman was deceased. Twenty-eight women responded to the questionnaire (response rate 97%). The patients who stated that they were still free from symptoms had their medical records rechecked and if any of those had attended for UPS in the follow-up time they were not recorded as asymptomatic but improved. The study was approved by the research ethical committee in Uppsala. Written informed consent was obtained.

Results

Effects

In substudy I (n=30), 18 (60.0%) of the patients were totally relieved of their UPS symptoms by the end of the treatment and 12 (40.0%) were improved. By the six-month follow-up, 17 (56.7%) were free from symptoms, eight (26.7%) remained improved, and five (16.7%) patients had relapsed. The number of instillations until the treatment was considered finished were one to 15 (mean 4.9, median three). To 12 (40.0%) patients, one instillation was sufficient, nine (30.0%) patients had two to seven, and nine (30%) patients had eight to 15 instillations (Table 1). All five patients that relapsed within six months in substudy I were further treated with CP and L instillations. In substudy II (n=28) the > five years follow-up showed that four (14.3%) patients were still free from symptoms, 18 (64.3%) patients had symptoms but milder than before treatment, and six (21.4%) had relapsed to the previous degree of symptoms. None had more severe symptoms compared to before treatment.

Side effects

In substudy II two (7.1%) patients reported transient urethral pain in connection with the instillation of CP cream and four (14.3%) patients could not recall whether they had any side effects. Other side effects were not observed in substudy I or reported in substudy II.

The patients' judgment of the treatment

In substudy II (n=28), the patients' reported effectiveness was high (Figure 1) and the large majority would undergo re-treatment in case of relapse (Figure 2). For one of the

<table>
<thead>
<tr>
<th>Substitution I (n=30)</th>
<th>Effect of treatment at completion of treatment and six month follow-up.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>No. of patients</td>
</tr>
<tr>
<td>1 instillation</td>
<td>12</td>
</tr>
<tr>
<td>2-7 instillations</td>
<td>9</td>
</tr>
<tr>
<td>8-15 instillations</td>
<td>9</td>
</tr>
<tr>
<td>Sum</td>
<td>30</td>
</tr>
</tbody>
</table>
two patients who would not undergo the treatment again, the reason was because of the transient urethral pain at the moment of the instillation and the other woman was convinced that in her case the causal factor was estrogen deficiency.

**Discussion**

The search for an answer to the etiology of UPS and related conditions such as trigonitis, interstitial cystitis, and other pelvic pain syndromes is intriguing. Within UPS there might be different conditions, with different etiologies, and therefore different appropriate treatments. The present authors believe that the favorable effects of CP treatment in this study are due to the anti-inflammatory properties of CP. Histopathological examination of biopsies from women with UPS has shown inflammatory changes in trigonum, the urethral mucosa, and the paraurethral glands [7-9]. The search for an infectious cause has so far been unsuccessful [4, 10, 11].

There are studies showing varying effect with antibiotic treatment in treating UPS or trigonitis [12, 13]. In these studies, tetracyklines or macrolides have mainly been used and it is important to remember in this discussion that they also have anti-inflammatory properties [14-16]. There is a theory that UPS is due to dysfunctional urethral epithelia leading to entry of urine and bacteria causing inflammation [4]. One could speculate an analogy to eczema where a dysfunctional epithelial barrier plays a crucial part of the inflammatory process and were microbiological colonization is seen. In dermatological guidelines for treatment of atopic eczema, skin care, emollient treatment, and anti-inflammatory topical corticosteroids without any antibiotics, is the first line of treatment, although it is known that microbial colonization most probably is present [17]. There are strong indications that the paraurethral glands are homologous to the male prostate and that they are not only silent embryological remnants but active glands [18-20]. It has been hypothesized that UPS could be a female equivalent to nonbacterial “prostatitis” [8, 18, 20]. There has been histological findings of inflamed paraurethral ducts in patients with UPS [8, 21]. In case of a female “prostatitis” the present authors believe that the urethral instillation of CP could reach and quench such an inflammation. In male prostatitis, acute bacterial infection of the prostate exists, but in the chronic form of male prostatitis, studies have not been able to prove convincing evidence of an infectious cause but signs of inflammatory activity have been seen [22, 23]. Given the rising global problem of antibiotic resistance, it is desirable to minimize any unnecessary use of antibiotics.

The present findings suggest that the strong anti-inflammatory properties of CP [24] could alleviate or remove the symptoms of UPS, although an effect of L could not be ruled out. Perhaps CP cream or the L gel could also anoint and provide protection to a possible dysfunctional urethral epithelia and in this way relieve symptoms. The only observed side effect in this study was transient urethral burning pain in association with the instillation. The local anesthetic L was added to the treatment to remove this side effect. Instead of CP 0.05% cream with the addition of L, the authors now use CP 0.05% ointment without addition of L as the clinical experience is that the ointment more seldom gives this side effect. The present authors have not found any studies on the pharmacokinetics of CP on the urethral or bladder epithelia and its possible local or systemic side effects. Topical treatment with CP is used on other types of mucosa such in treating oral lichen planus (OLP), mucous membrane pemphigoid (MMP), lichen sclerosus (LS) in vulva, as well as children with severe phimosis. In treating OLP, MMP, phimosis, vulvar LS the frequency of topical application of CP (ranging from three times per day to twice weekly) is higher and the length of treatment (from four up to 48 weeks) is often longer than
the treatment with CP and L given in this study, suggesting that this treatment of UPS is not aggressive [25-27]. The present authors have not found any studies of urethral administration of CP, though a few studies can be found in treating interstitial cystitis with triamcinolone or hydrocortisone as ingredients in bladder instillations. To their knowledge, two earlier studies have reported corticosteroid treatment of UPS, but these were on weaker corticosteroids. In a Swedish randomized study from 1972, urethral dilatation combined with instillation of chloromycetin-hydrocortisone (very low potency corticosteroid US class VII, Europe class I) was shown to relieve or remove symptoms in 44% of women with symptoms of cysto-urethritis without concurrent findings of urinary tract infection [28]. In 1976 an American study presented a very good response in 54 women treated with sub-mucosal injections of the corticosteroid triamcinolone acetonide (moderate potency corticosteroid US class III, Europe class II) around the paraurethral glands [29]. In these studies no side effects were observed or mentioned.

Although not noticed in this study, there could theoretically be a risk of local side effects such as infection, atrophy, eczematous reactions or systemic such as adrenal suppression, hirsutism or moon face from urethral instillations of CP. Systemic side effects due to installation of CP in this dosage seem unlikely.

Local deficiency of estrogens is a differential diagnosis to UPS, or an aggravating factor. Postmenopausal women presenting with UPS should initially be given local estrogen therapy [30], even premenopausal women may sometimes benefit. The present authors believe that local (vaginal) estrogen therapy in doses that give effect on the urethral epithelium is important, at least in postmenopausal patients, in order to exclude it as a sole cause or for general improvement of the UPS. In this study 86% of the studied women had some kind of estrogen therapy which could be a confounding factor. Nevertheless in all cases the estrogen therapy was started in a sufficient amount of time before the CP and L treatment so that any estrogen effect on the UPS already ought to have been shown. Most of the patients had their estrogen therapies since years before starting CP and L treatment. As mentioned earlier patients whose UPS were efficiently treated by estrogens were excluded from the study.

When evaluating treatments to UPS, one has to keep in mind the common intermittent course of the UPS [4], spontaneous remission independent of the treatment is also a risk of bias. This intermittent course also gives a risk for recall bias in the follow-up questionnaire. Other risk of bias is the risk of placebo. Also, in this study the physician treating the patients had set the model of treatment and had a positive attitude to the effect of the treatment. There is a risk that the patients declared better results so as to not affect their doctor-patient relation negatively. The high number of satisfied patients and their positive responses to the treatment could also be because they, in the present authors’ experience, often describe what they experience as lack of interest or knowledge by other medical practitioners, while they in this study are met with interest.

In conclusion, this study indicates a good effect in treating the UPS with CL and L instillations. No significant side effects have been noticed. Some previous findings support the rationale for local treatment with potent corticosteroids. It will always be important to rule out infection or other pathological conditions before the treatment is administered and to be vigilant for possible side effects. All UPS patients may not benefit from this treatment since there could be different and so far unknown causes to the UPS. Multicenter double blinded randomized placebo controlled clinical trials with urethro-cystoscopy and preferably urethral histology prior to and after treatment are warranted.

Acknowledgements

The authors would like to thank PhD Karin Åhrling for writing and editing assistance.

References

Urethral instillations of clobetasol propionate and lidocaine: a promising treatment of urethral pain syndrome


Address reprint requests to:
A.K. LINDSTROM, M.D., Ph.D.
Center for Clinical Research
Nissers väg 3, Falun
SE- 791 82 (Sweden)
e-mail: annika.lindstrom@ltdalarna.se
The influence of sperm parameters on the outcome of intracytoplasmic sperm injection-embryo transfer cycle in poor responder women under 35 years of age

A. Usta¹, M. Karacan², Z. Cebi², A. Arvas², M. Ulug², C.S. Usta¹, T. Camlibel²

¹ Obstetrics and Gynecology Clinic, Ministry of Health Balikesir State Hospital, Balikesir
² Assisted Reproduction Unit of Ota-Jinemed Hospital, Istanbul (Turkey)

Summary
To evaluate the influence of sperm parameters on the outcome of intracytoplasmic sperm injection (ICSI) cycles in poor responder women under the age of 35 years in a retrospective analysis in a fertility center. Materials and Methods: A total of 432 poor responder women who underwent ICSI cycles were evaluated. The interventions included ICSI and microdissection testicular sperm extraction (m-TESE). Main outcome measures included fertilization, cleavage, clinical pregnancy, and delivery rates. Results: Patients were divided into four groups according to the sperm parameters and the source of sperm; testicular spermatozoa obtained from men with azoospermia (group 1; n=26), severe oligoasthenoteratozoospermia (OAT) (group 2; n=35), OAT (group 3; n=104), and normal semen analysis (group 4; n=267). Average age of the women, antral follicle count, FSH level, male age, number of previous ICSI cycles, duration of infertility, and the maximal endometrial thickness were similar among the groups. In group 1, the fertilization rate was lower than those in all other groups. Cleavage, clinical pregnancy, and delivery rates were similar among the groups. Conclusions: Neither sperm parameters nor the source of spermatozoa affects delivery rate through ICSI in poor responder women < 35-years-old.

Key words: ICSI, microdissection TESE, male factor, OAT.

Introduction
Infertility affects approximately 15% of sexually active couples and male factors account for about half of the cases [1]. Idiopathic oligoasthenoteratozoospermia (OAT) is the most common medical diagnosis of abnormal semen quality [2]. Several studies reported increased rate of centrosome dysfunction, sperm DNA fragmentation [3, 4], deficiency of oocyte-activating factors [5], higher sperm chromosomal abnormalities, and sperm related aneuploidies in the deriving embryos [6-11] in patients with OAT, severe OAT, obstructive azoospermia (OA), and non-obstructive azoospermia (NOA). Thus, ICSI with spermatozoa of these men may affect outcome.

Intracytoplasmic sperm injection (ICSI) has been commonly used for couples with male infertility since its first successful introduction in 1992 [12]. Reliable pregnancy rates were achieved by using spermatozoa from OAT patients as well as testicular spermatozoa [13].

ICSI provides a mechanical assistance of injection of a morphologically normal and motile spermatozoon into the oocyte. Although aneuploid spermatozoa may retain the ability to fertilize an oocyte through ICSI, resultant embryo has an increased risk of chromosomal abnormalities which may negatively affect ICSI outcome.

A poor ovarian response, although the definition varies widely, to ovulation induction is a common problem which severely diminishes live birth rate [14]. Even though the number of oocytes affects the outcome, female age seems a better predictor on pregnancy rate.

In the current study, the authors aimed to evaluate the influence of semen quality on the outcome of ICSI in relatively young women (< 35-years-old) with poor ovarian response.

Materials and Methods
Patients and design
A retrospective analysis of 432 women with the diagnosis of poor ovarian response (POR) that underwent ICSI-ET cycles between May 2005 and June 2012 at the Assisted Reproduction Unit of Ota-Jinemed Hospital was carried out. The study was approved by the Institutional Review Board of Ota-Jinemed Hospital. Women who had < five oocytes as a response to controlled ovarian stimulation were classified as poor responders. All women had normal uterine cavity confirmed with hysterosalphingography and/or saline infusion sonography.

Semen evaluation and preparation
Semen analysis of male partners of all couples attending ICSI cycles were recruited from hospital database. All semen samples were analyzed according to the World Health Organization 2010
criteria. Couples were divided into four groups according to male partners' semen analysis: 1) testicular spermatozoa obtained from men with azoospermia (group 1; n=26), severe OAT (group 2; n=35), 3) OAT (group 3; n=104), and 4) normal semen analysis (group 4; n=267). Baseline characteristics of the patients are given in Table 1.

Semen samples were collected by masturbation at the laboratory after two to four days of abstinence. All semen analyses were carried out manually within one hour after the semen collection. The semen samples were left to liquefy at 37°C for 20 minutes. Following liquefaction, a drop of the well-mixed specimen was placed on a clean glass slide, covered with a coverslip, and left for a few minutes. The preparation was examined at ×400 magnification. Sperm parameters were divided into four groups as severe OAT (sperm concentration ≤1×10^6/ml, motility < 25% and morphology < 1%), OAT (sperm concentration = 1–15×10^6/ml, motility < 25% and morphology = 1–4%), normal (sperm concentration > 15×10^6/ml, motility > 25%, morphology ≥ 4%), and testicular sperm obtained with m-TESE. The semen samples were prepared by centrifugation on a density gradient and washed with HEPES buffered medium containing human serum albumin.

Azoospermia was confirmed on at least two semen samples and microdissection testicular sperm extraction was performed as described previously [15]. Procedures in which fresh motile testicular spermatozoa used for ICSI were included in the study. Twisting was accepted as a minimum criterion for motility. Four patients had the diagnosis of OA and 22 had NOA.

**Ovarian stimulation and oocyte retrieval**

Women were treated with either down-regulation protocol starting GnRH-agonist in the previous luteal phase or GnRH-antagonist protocol. Recombinant FSH was started when down-regulation was achieved or on cycle day 3 until at least one follicle reached to 17 mm in diameter. GnRH antagonist was administered routinely on cycle day 6 regardless of follicular size. Oocyte retrieval was performed by transvaginal route 35 hours after hCG injection. After removing the cumulus cells attached to the oocytes with hyaluronidase (type VIII) after two hours of incubation, ICSI was performed as described elsewhere [16]. Fertilization was assessed 16 to 18 hours and cleavage was checked 48-72 hours after ICSI. The embryos were transferred three days after oocyte retrieval. The luteal phase was supported by 50 mg of P in oil injections IM and continued until the detection of fetal heart beat. Clinical pregnancy was verified by the presence of fetal cardiac activity with transvaginal ultrasonography performed at six to seven weeks of gestation. The implantation rate was shown by the ratio of the number of implanted embryos to the number of embryos transferred into the uterus. Miscarriage was defined as disappearance of gestational sac.

**Statistical analysis**

MedCalc Statistical Software Program version 13.1.0 was carried out. Female age, antral follicle count, FSH level, male age, number of previous ICSI cycles, duration of infertility, and maximal endometrial thickness were evaluated by one-way analysis of variance (ANOVA) and the log-transform Schefé’s method. Fertilization, cleavage, implantation, clinical pregnancy, miscarriage, twin pregnancy, and delivery rates were evaluated by one-way analysis of variance (ANOVA). A p-value < 0.05 was accepted as significant.

**Results**

A total of 432 poor responder women were included in this study. Patients’ characteristics are summarized in Table 1. Female age, antral follicle count, FSH level, male age, number of previous ICSI cycles, duration of infertility, and maximal endometrial thickness were similar among the four groups classified according to sperm parameters. Ovarian response to controlled ovarian stimulation in terms of number of oocytes retrieved did also not differ significantly among the groups (Table 2). Sperm count was significantly higher in group 4 than those in group 3 and 2 (33.7 ± 1.0 × 10^6 ml) and 2.1 ± 1.3 × 10^6 ml, p < 0.01, respectively). It was also higher in group 3 than that in group 2 (p < 0.05). Scrotal hematoma was noted in one case following m-TESE and disappeared within a few days.

Fertilization rate was significantly lower with testicular spermatozoa as compared to the ejaculated spermatozoa (57.4% vs. 74.2% for sOAT, 76.4% for OAT, and 76.2% for normal sperm parameters) (p = 0.007). However, there was no significant difference in the cleavage, number of embryos transferred, implantation, clinical pregnancy, miscarriage, twin pregnancy, and delivery rates among the four groups (Table 2). Major malformation was not noted in the 142 children who were born.

**Discussion**

Despite being the only effective therapy for couples with male subfertility, ICSI is one of the most unphysiological methods of assisted reproductive technologies (ART), since spermatozoa is somewhat selected arbitrarily by an embryologist. Theoretically, testicular spermatozoa as well as spermatozoa of men with sOAT and OAT may carry higher
Table 2. — Outcome of ICSI-ET cycles of poor responder women in four different groups as classified according to semen parameters.

<table>
<thead>
<tr>
<th>Poor responder women</th>
<th>Group 1: azoospermia (n=26)</th>
<th>Group 2: severe OAT (n=35)</th>
<th>Group 3: OAT (n=104)</th>
<th>Group 4: normal (n=267)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of oocytes retrieved</td>
<td>2.6 ± 1.3</td>
<td>2.8 ± 1.2</td>
<td>2.9 ± 1.1</td>
<td>2.8 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>No. of MII oocytes</td>
<td>2.0 ± 1.2</td>
<td>1.9 ± 1.4</td>
<td>2.2 ± 1.5</td>
<td>2.1 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate per oocyte(%)</td>
<td>57.4</td>
<td>74.2</td>
<td>76.4</td>
<td>76.2</td>
<td>0.007</td>
</tr>
<tr>
<td>Cleavage rate (%)</td>
<td>87.2</td>
<td>81.8</td>
<td>82.8</td>
<td>78.5</td>
<td>NS</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>1.8 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td>1.7 ± 0.6</td>
<td>1.7 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>10/47 (21.3)</td>
<td>12/61 (19.7)</td>
<td>38/170 (22.4)</td>
<td>97/459 (21.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy rate, n(%)</td>
<td>9 (34.6)</td>
<td>10 (28.5)</td>
<td>33 (31.7)</td>
<td>80 (29.9)</td>
<td>NS</td>
</tr>
<tr>
<td>No.of miscarriages, (%)</td>
<td>1 (11.1)</td>
<td>1 (10)</td>
<td>4 (12.1)</td>
<td>9 (11.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Delivery rate, n(%)</td>
<td>8 (30.7)</td>
<td>9 (25.7)</td>
<td>29 (27.8)</td>
<td>71 (26.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Twin pregnancy rate(%)</td>
<td>1/9 (11.1)</td>
<td>2/10 (20)</td>
<td>5/35(15.2)</td>
<td>17/80 (21.3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: values are expressed as the mean±SD. NS= not significant.

aneuploidy and DNA fragmentation rates, thereby may be associated with poor outcome in an ICSI cycle [3-11, 17-20]. In the current study, the authors compared ICSI outcomes of men with different sperm parameters in poor responder women. Since female age is an independent predictor of success with ART [21], the authors included women aged less than 35 years. Moreover, poor responder women were selected to assess the effect of semen parameters on the limited number of oocytes. Also this inclusion criterion has helped to overcome heterogeneity in terms of infertility factors.

A reduced fertilization rate was observed with testicular spermatozoa compared to those achieved with other semen parameters in the present study. This finding is in contrast to some of the prior studies which reported the same fertilization rate with testicular sperm as ejaculated sperm [22], but in accord-ance with Loutradi et al. who found a decreased fertilization potential of testicular spermatozoa with ICSI [23]. Possible explanations of the reduced fertilization rate are high rates of DNA fragmentation, mitochondrial dysfunction, and chromosomal aneuploidy found in the sperm of men with azoospermia [8]. The present authors observed decreased fertilization rate even though they only used motile testicular spermatozoa. Aside from fertilization, other variables such as implantation rate, miscarriage rate, and delivery rate did not differ.

In the ICSI procedure, only a single spermatozoon is required for injection and, individual sperm features such as motility [24] and morphology [25] seem to be the important factors for the successful outcome. However, Burrelo et al. found that even normally shaped spermatozoa from OAT patients had an increased aneuploidy rate [18]. Ushijimal et al. determined a significantly higher frequency of disomy for chromosomes 13, 21, sex chromosomes, and diploidy in the OAT group than the control group [26]. Vegetti et al. detected that patients with abnormal semen parameters showed a significantly higher aneuploidy rate for chromosomes 13, 18, 21, X, and Y in their spermatozoa compared to controls [27]. The risk of chromosomal aneuploidy in spermatozoa seems to be inversely correlated to sperm concentration and total progressive motility. These studies indicate that spermatozoa of men with OAT could influence the fertilization process and the potential viability of ICSI embryos. However, in the present study, the outcome of ICSI cycles in men with diminished sperm parameters did not differ from that with normal semen analysis.

Nagy et al. demonstrated that only microinjection of an immotile (presumably dead) spermatozoon into the oocyte had a strongly negative influence on the result of ICSI procedure [24]. Thus, the only ultimate criterion for successful ICSI seemed the presence of at least one living spermatozoon per oocyte in the pellet of the treated semen sample.

The association between sperm morphology and ICSI outcome has also been analyzed. Previous studies found an inverse relationship between the percentage of atypical forms and the percentage of aneuploidies [28]. Furthermore, globozoospermia, flagellar abnormalities, large-headed and multiple-tailed spermatozoa, and elongated-head spermatozoa were linked to increased aneuploidies [29,30]. De Vos et al. evaluated the influence of morphology of spermatozoa on the fertilization and pregnancy outcome and found lower fertilization, pregnancy, and implantation rates with the injection of morphologically abnormal spermatozoa (irrespective of origin) compared to the injection of morphologically normal spermatozoa [25]. Since only cases with morphologically normal motile spermatozoa were included, the present authors are not able to comment on the effect of morphologically abnormal spermatozoa on pregnancy rate via ICSI.

Minor sperm nuclei abnormalities (such as vacuoles) which may be related to poor outcome cannot normally be identified during the ICSI procedure [31]. The present authors selected sperm under ×400 magnification, at the periphery of the PVP microdroplet. The rate of sperm nucleus normality was significantly higher when intracytoplasmic morphologically selected sperm injection (IMSI) was performed with sperm selected under a magnification level above ×6,000 [32]. The role of more detailed sperm selection needs to be studied to improve the ICSI outcome.

The present authors do recognize some weaknesses such as its retrospective nature and the limited number of poor re-
sponder patients having ICSI with testicular spermatozoa. Moreover, they included only cases having motile/morphologically normal spermatozoa in each group. They assume that inclusion of men with no motile spermatozoa available could have changed the results.

In conclusion, neither sperm parameters nor the source of spermatozoa affects delivery rate through ICSI in poor responder women < 35 years of age, when motile/morphologically normal spermatozoa is present.

References


Address reprint requests to: A. USTA, M.D.
Obstetrics and Gynecology Clinic
Ministry of Health Balikesir State Hospital
Yildiz mah. Soma cad. no. 82
Balikesir (Turkey)

E-mail: drakinusta@gmail.com
Clinico-biochemical characteristics of 229 Portuguese infertile women with polycystic ovary syndrome: clinical relevance and relationship with fertility treatment results

P. Marques¹, F. Ferreira², A.P. Soares³, J. Nunes³, S. Sousa³, A. Aguiar³, C. Calhaz-Jorge³

¹ Department of Endocrinology, Portuguese Institute of Oncology, Lisbon
² Department of Endocrinology, Hospital Santa Maria, CHLN, Lisbon
³ Department of Obstetrics and Gynecology, Reproductive Medicine Unit, Hospital Santa Maria, Lisbon (Portugal)

Summary

Purpose: Polycystic ovary syndrome (PCOS) affects 6-20% of reproductive-age women. The authors aimed to evaluate the characteristics of PCOS women and its relationship with fertility treatment outcomes. Materials and Methods: The authors reviewed records of PCOS women assisted at Hospital Santa Maria. Fertility treatment results were assessed as pregnancy rate, number of cycles, and miscarriage rate. Results: They identified 229 PCOS women, 179 (78.2%) had waist circumference > 80 cm, 72 (31.4%) had type 2 diabetes mellitus (T2DM) familial history and glucose abnormalities, hypertriglyceridemia and low cholesterol-HDL were detected in 23 (10.1%), 15 (6.6%) and 103 (45.0%), respectively. Pregnancy was achieved in 164 women. The mean number of cycles to achieve pregnancy was 2.7 (±2.2). Statistical analysis identified factors associated with longer/higher number of treatments: primary infertility, T2DM familial history, hypertriglyceridemia, and low cholesterol-HDL. Waist circumference > 80 cm, older age, and increased LH level were associated with miscarriage. Conclusions: Primary infertility, T2DM familial history, hypertriglyceridemia, low cholesterol-HDL, older age, waist circumference > 80 cm, and high LH may confer poorer fertility treatment results.

Key words: Polycystic ovary syndrome; Infertility; Oligomenorrhea; Clinical pregnancy; Spontaneous abortion.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies affecting 6-20% of the reproductive age women [1-3]. The definition of PCOS, according to 2003 ESHRE/ASRM Rotterdam consensus, requires the presence of two out of three diagnostic features: oligomenorrhea or anovulation, clinical or biochemical evidence of hyperandrogenism, and presence of polycystic ovarian morphology [4].

PCOS is a heterogeneous syndrome with ethnicity, geographic region, genetic, and environmental factors contributing to different phenotypes of PCOS and associated comorbidities [5, 6]. Although not necessary for diagnosis of PCOS, obesity, type 2 diabetes mellitus (T2DM), hypertension, cardiovascular disease, dyslipidemia, and infertility are common in this condition. Thus, PCOS adversely affects endocrine, metabolic, cardiovascular, and reproductive health [7].

Women with PCOS have a normal number of primordial follicles and primary/secondary follicles are increased, but due to derangements in factors involved in follicular development, the recruitment and progression of the dominant follicle and ovulation does not occur normally [5, 8]. Menstrual disturbances in PCOS include oligoamenorrhea, anovulation, and prolonged menstrual bleeding. PCOS is the most frequent cause of anovulation, accounting for 75-90% of ovulatory disorders [5, 9]. Nevertheless, up to 30% of women with PCOS may have normal menses [10]. Prolonged periods of anovulation is the primary mechanism of infertility, but there are other subfertility factors in PCOS such as diminished oocyte competence, delay in early embryo kinetics, and endometrial changes interfering with implantation [11]. Moreover, spontaneous abortion occurs more frequently in women with PCOS [12]. Therefore, infertility is highly prevalent in this setting, affecting approximately 40% of PCOS women [5].

The first-line treatment in these women is ovulation induction with clomiphene citrate (CC), which restores ovulation and result in pregnancy in about 50% of PCOS women. Second-line intervention includes either exogenous gonadotropins (Gnd) or laparoscopic ovarian drilling (LOD). Recommended third-line strategy is in-vitro fertilization (IVF) [13]. Some studies have studied the impact of multiple factors that may influence the fertility treatment outcomes, but conclusions are controversial; factors such as overweight/obesity, insulin-resistance, hyperinsulinemia, higher fasting blood glucose, higher systolic blood pressure, and smoking habits may adversely affect cumulative live
Materials and Methods

The authors conducted a retrospective study involving infertile women with PCOS referring to the Reproductive Medicine Unit of Hospital de Santa Maria and surveilled within the period of January 2004 to June 2013. Women eligible for inclusion were those with PCOS diagnosis based in the Rotterdam 2003 criteria [4], who either underwent at least one fertility treatment in the present unit and/or reached pregnancy. Patients with other infertility factors, namely endometriosis, male factor (mild to moderate abnormality on spouse semen analysis) or partial tube dysfunction (at least one patent tube) were included. Exclusion criteria were primary ovarian failure (FSH ≥ 40.0 U/L), uncontrolled hypothyroidism (TSH ≥ 4.76 mU/mL), late-onset congenital adrenal hyperplasia (17-hydroxyprogesterone ≥ 3.0 ng/mL), hypogonadotrophic hypogonadism (low or inappropriate low-normal levels of FSH and LH, plus undetectable or low concentrations of serum estradiol [18]), history of chemotherapy, radiotherapy or pelvic surgery.

Two-hundred twenty-nine infertile PCOS women were identified and their clinical records were reviewed. All records had medical data, physical examination, smoking habits, menstrual irregularities, signs of clinical hyperandrogenism (hirsutism considered for modified Ferriman-Gallwey scores ≥ 8 [19]), family history, blood pressure, body mass index (BMI), waist circumference (measured on bare skin at the narrowest indentation), menstrual and LH, plus undetectable or low concentrations of serum estradiol [18]), history of chemotherapy, radiotherapy or pelvic surgery. Baseline characteristics in the whole group of infertile women with PCOS.

| Table 1. — Baseline characteristics in the whole group of infertile women with PCOS. |
|---------------------------------|------------------|
| **Baseline characteristics**    | **PCOS (n=229)** |
| **Age (years)**                 | 29.7 (±3.9)      |
| **Age of menarche (years)**     | 12.8 (±1.9)      |
| **Duration of infertility (months)** | 41 (±29)   |
| **Primary infertility**         | 185 (80.8%)      |
| **Existence of additional infertility factor** | 38 (16.6%) |
| **BMI (kg/m²)**                 | 27.8 (±6.4)      |
| **Weight excess (BMI ≥ 25 kg/m²)** | 134 (58.5%)   |
| **Weight (kg)**                 | 73.1 (±17.6)     |
| **Waist circumference (cm)**    | 93.6 (±14.5)     |
| **Waist circumference > 80 cm** | 179 (78.2%)      |
| **Hypertension**               | 12 (5.2%)        |
| **Familial history of T2DM**    | 72 (31.4%)       |
| **Smoking habits**              | 61 (26.6%)       |
| **Polycystic ovarian morphology** | 235 (60%) |
| **Clinical and/or biochemical androgen excess** | 110 (48.0%) |
| **Oligoamenorrhea**             | 229 (100%)       |

Biochemical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (2.5-10.2 U/L)</td>
<td>5.0 (±2.1)</td>
</tr>
<tr>
<td>LH (1.9-12.5 U/L)</td>
<td>9.0 (±5.7)</td>
</tr>
<tr>
<td>Estradiol (19.5-144.0 pg/mL)</td>
<td>67.9 (±65.0)</td>
</tr>
<tr>
<td>Total testosterone (&lt; 73 ng/dL)</td>
<td>63.6 (±33.0)</td>
</tr>
<tr>
<td>Prolactin (2.8-29.0 ng/mL)</td>
<td>12.5 (±6.3)</td>
</tr>
<tr>
<td>TSH (0.55-4.78 mU/mL)</td>
<td>2.6 (±1.9)</td>
</tr>
<tr>
<td>17-hydroxyprogesterone (0.1-3.0 mg/mL)</td>
<td>1.5 (±0.7)</td>
</tr>
<tr>
<td>SHBG (18-144 nmol/L)</td>
<td>43.3 (±38.1)</td>
</tr>
<tr>
<td>Insulin (3.25 mU/L)</td>
<td>12.1 (±9.6)</td>
</tr>
<tr>
<td>Total cholesterol (&lt;190 mg/dL)</td>
<td>179.7 (±33.3)</td>
</tr>
<tr>
<td>Cholesterol-LDL (&lt;110 mg/dL)</td>
<td>111.5 (±33.7)</td>
</tr>
<tr>
<td>Cholesterol-HDL (&gt;50 mg/dL)</td>
<td>53.4 (±15.1)</td>
</tr>
<tr>
<td>Triglyceride (&lt;150 mg/dL)</td>
<td>86.8 (±42.9)</td>
</tr>
<tr>
<td>Fasting glyceria (&lt;100 mg/dL)</td>
<td>96.1 (±27.7)</td>
</tr>
<tr>
<td>2-h glycemia on 75g-OGTT (&lt;140 mg/dL)</td>
<td>96.1 (±27.7)</td>
</tr>
<tr>
<td>Hypertriglyceridemia (&gt;150 mg/dL)</td>
<td>15 (6.6%)</td>
</tr>
<tr>
<td>Low cholesterol-HDL (&lt;50 mg/dL)</td>
<td>103 (45.0%)</td>
</tr>
<tr>
<td>Positive 75g-OGTT</td>
<td>23 (10.1%)</td>
</tr>
<tr>
<td>[IFG=9; IGT=14]</td>
<td></td>
</tr>
<tr>
<td>Biochemical hyperandrogenism</td>
<td>63 (27.5%)</td>
</tr>
<tr>
<td>LH/FSH ≥ 2</td>
<td>90 (39.3%)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.6 (±2.1)</td>
</tr>
<tr>
<td>HOMA-IR ≥ 2.5</td>
<td>78 (34.1%)</td>
</tr>
</tbody>
</table>

Data is shown as mean (± standard deviation) or n (%).

PCOS: polycystic ovary syndrome; BMI: body mass index; T2DM: type 2 diabetes mellitus; OGTT: oral-glucose tolerance test; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; HOMA-IR: homeostasis model assessment for insulin-resistance index.
Number of treatment cycles to achieve 2.7 (± 2.2) spontaneous abortion outcomes in the infertile PCOS women. Pregnancy was achieved in 164 (71.6%) women. Spontaneous abortion was verified in 34 women (14.9%); five women had more than one abortion and the mean number of treatment cycles needed to achieve pregnancy was 2.7 (±2.2). The mean duration between the first fertility treatment and clinical pregnancy was 9.9 (± 10.8) months.

The data analysis and statistical tests were carried out using SPSS (version 20.0). Descriptive statistics were used to summarize the data. Chi-square analysis and Fisher’s exact test were used to compare categorical variables. Student t-test, ANOVA, and Wilcoxon tests were used for continuous variables. The Pearson correlation coefficient was used for correlations between continuous variables. A p value < 0.05 was considered statistically significant.

Results

The baseline features of the 229 infertile PCOS women included are summarized in Table 1. The mean age was 30 years. The duration of infertility was 41 (± 29) months and 80.8% were primary infertilities. Out of the 229 women, 134 (58.5%) were overweight or obese, 179 (78.2%) had waist circumference > 80 cm, 12 (5.2%) were hypertensive, 126 (76.8%) were smokers, and 72 (31.4%) had familial history of T2DM. Clinical and/or biochemical androgen excess was detected in 150 (91.5%), 55 (33.5%), 53 (32.3%), and 132 (80.5%) women, respectively.

Table 2 provides an overview of the fertility treatment outcomes in the infertile PCOS women. Pregnancy was achieved in 164 (71.6%) women. Spontaneous abortion was verified in 34 women (14.9%); five women had more than one abortion and the mean number of treatment cycles needed to achieve pregnancy was 2.7 (±2.2). The mean duration between the first fertility treatment and clinical pregnancy was 9.9 (± 10.8) months. Sixty-one percent of the pregnancies achieved clinical pregnancy (months) 9.9 (± 10.8) months. Sixty-one percent of the pregnancies achieved clinical pregnancy (months) 9.9 (± 10.8) months.
them reached spontaneous pregnancy postoperatively without additional treatments.

Table 3 summarizes treatment fertility results in the infertile PCOS women who became pregnant, regarding the number of cycles and spontaneous abortion. Coefficients of correlation regarding the relationship between clinico-biochemical parameters and treatment cycles or spontaneous abortion are represented in Table 4. For the considered clinico-biochemical parameters, no statistical significant differences were detected between women who became pregnant and women who did not reach pregnancy (Table 5).

The history of primary infertility, hypertriglyceridemia, clinical and/or biochemical androgen excess, familial history of T2DM, and low cholesterol-HDL were associated with higher number of cycles to obtain pregnancy. Waist circumference > 80 cm was the only feature significantly associated with higher rates of spontaneous abortion. Significant positive correlations were seen only between both mean age/LH and abortion rate.

### Discussion

The results of the present study suggest that infertile PCOS women with primary type infertility, hypertriglyceridemia, low cholesterol-HDL, and T2DM familial history may have poorer fertility treatment results, possibly requiring more treatment cycles. These features may justify more intensive and effective reproductive approaches in the management of those infertile PCOS women. On the other hand,
the present data suggest that increased ages, waist circumference above 80 cm, and/or higher LH levels may be associated with spontaneous abortion. In the present series, no prognostic significance was found for the other clinico-biochemical features, such age, obesity, insulin-resistance, glucose abnormalities, hypertension or smoking habits. Dyslipidemia is very common in women with PCOS, with a prevalence of up to 70% [25]. In the present series, the frequency of lipid abnormalities was considerably lower. The most common abnormal lipid profile of these patients includes decreased levels of cholesterol-HDL and increased levels of triglycerides and/or cholesterol-LDL, and it is strongly associated with insulin-resistance and obesity [26].

A recent meta-analysis of lipid levels reported triglycerides levels 26 mg/dL higher in PCOS than in controls; on the other hand, cholesterol-HDL concentrations were six mg/dL lower in PCOS than in controls [27]. Moreover, cholesterol-HDL encompasses different classes of lipoproteins, and it has been reported that PCOS women have not only lower concentrations, but also alterations in their quality [26].

Testosterone has been implicated in lowering the cholesterol-HDL [28]. The association between dyslipidemia and cardiovascular morbidity, which result from the increased atherogenesis, insulin-resistance, oxidative stress, pro-inflammation, and platelet hyperactivity, has been described [25-30]. However, the implication of lipid abnormalities in reproductive life and fertility treatment results are not conclusively established. In the present study, the presence of hypertriglyceridemia and/or low levels of cholesterol-HDL was significantly associated with poorer fertility treatment results. No relationship with spontaneous abortions was detected for both hypertriglyceridemia and low cholesterol-HDL. Thus, it seems reasonable to control the lipid profile prior fertility treatment as it may interfere negatively with the results. These findings highlight the importance of lipid profile evaluation in infertile women with PCOS seeking pregnancy. Lifestyle modification should be implemented as first-line approach for those who have dyslipidemia. The use of statins in pregnancy is contraindicated; therefore, contraception is obligatory for severe dyslipidemias that implies the use of those drugs [31,32].

The assessment of family history of T2DM should be routinely done in PCOS women, as it allows metabolic risk stratification. T2DM familial history is associated with adverse metabolic profile, namely increased risk for obesity, central fat accumulation, metabolic syndrome, glucose abnormalities, and low cholesterol-HDL [33]. The present study indicates that assessment of T2DM family history may be a valuable prognostic indicator for fertility treatment, with its existence associated to worse results.

In PCOS women, primary infertility is more frequent than secondary infertility [33]. In teh present study, the prevalence of primary infertility was remarkably higher than secondary infertility and the women with primary infertility required more treatment cycles. In this population, other infertility factors were present in 22.9% of the primary infertile women, whereas this was true in only 6.2% of secondary infertilities (p < 0.05). By including anovulatory PCOS women with concomitant endometriotic, tubal or spouse sperm abnormalities, the authors believe that these results are more applicable to real-life clinical setting of infertile PCOS women in fertility centers. Thus, fertility workup is required in an important proportion of anovulatory PCOS women, especially those with primary infertility refractory to fertility treatments [13].

Multiple studies have been conducted to address the impact of different factors in the outcomes of fertility treatments. The present study found no impact of overweight/obesity, insulin-resistance and hyperandrogenism on fertility treatment results, although these parameters are frequently reported as relevant adverse factors for fertility treatment [15, 21, 34, 35]. Reduced ovarian responses, suppressed oocyte developmental competence, and lower implantation rates may directly explain the poorer outcomes in this subset of PCOS women [16, 36]. Some reports refer also to negative impact of hypertension or smoking in fertility treatment results in PCOS women [15, 37, 38], which was not confirmed in this series. Obesity, hyperinsulinemia, and insulin-resistance have being implicated in early pregnancy loss [14, 16, 39]. Moreover, high LH levels and hyperandrogenemia increase the risk of first-trimester abortion [11, 14]. These features are highly prevalent in infertile PCOS women, conferring a high risk for miscarriage. First-trimester abortions may reach 50% in PCOS women, which is threefold higher than the rate of normal women [14, 40, 41]. The present study failed to demonstrate the association between BMI, insulin-resistance or hyperandrogenemia with higher rates of spontaneous abortion. However, the authors found significant associations between older age, waist circumference > 80 cm (a marker of metabolic disturbance and insulin-resistance [42]) and higher LH levels with miscarriage occurrence.

In conclusion, this study demonstrated that infertile PCOS women with primary infertility, hypertriglyceridemia, low cholesterol-HDL, and T2DM familial history may have poorer fertility treatment results, possibly requiring more treatment cycles and longer treatment periods to achieve clinical pregnancy. Older ages, waist circumference above 80 cm, and high levels of LH may be associated with spontaneous abortion. Thus, these features may confer worse prognosis for fertility treatments and should orientate the clinicians for a careful evaluation and management. Preconceptional control of these factors, namely the lipid profile, may positively affect the prognosis of infertility treatments and should be largely stimulated.

References


Address reprint requests to: P. MARQUES, M.D.
Endocrinology Department
Instituto Português de Oncologia de Lisboa
Rua Professor Lima Basto
1099-023 Lisboa (Portugal)
e-mail: pedro.miguel.sousa.marques@gmail.com
Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial?

G.A. Partsinevelos¹, N. Antonakopoulos¹, K. Kallianidis¹,², P. Drakakis¹, E. Anagnostou¹, R. Bletsa¹, D. Loutradis¹,²
¹Division of Human Reproduction, 1st Department of Obstetrics and Gynecology, Alexandra Hospital, Athens University Medical School, Athens
²Fertility Institute, Diagnostic and Therapeutic Centre S.A., Athens (Greece)

Summary

Purpose: The aim of the study was to assess the effect of the addition of low-dose human chorionic gonadotropin (hCG) to ovarian stimulation with recombinant follicle stimulating hormone (rFSH) on in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) outcome.

Materials and Methods: This retrospective clinical study was conducted on 141 women undergoing ICSI through a short GnRH-agonist protocol with rFSH and the addition of low-dose (100 IU/day) hCG. The control group consisted of 124 women undergoing ovarian stimulation with a similar protocol devoid of hCG. Statistical analysis in the study population along with a subgroup analysis for age ≥ 35 years and ≥ 36 years was performed.

Results: Women in hCG group were statistically significant older and with higher basal FSH compared to control group. Despite this fact and the fact that several ovarian stimulation parameters, such as peak estradiol levels, number of oocytes retrieved, number of mature oocytes, and fertilization rates were in favor of the control group, the quality of transferred embryos and pregnancy rates were in favor of hCG group. Similar results were obtained in the subgroup analyses apart from peak estradiol levels, which did not differ among the study groups.

Conclusions: The addition of hCG to rFSH may be associated with better quality embryos and higher pregnancy rates, even in women of advanced reproductive age with higher basal FSH levels, which are often considered to have poorer ovarian reserve.

Key words: hCG; ICSI; IVF; LH activity.

Introduction

The diversity of ovarian response among women undergoing controlled ovarian stimulation (COS) for in vitro fertilization (IVF), especially in cases with a history of previous failed attempts, has led researchers to investigate the factors that determine and potentially improve this response [1].

In physiology, it is uniformly recognized that luteinizing hormone (LH) is drastically involved in follicle maturation from the antral stage onwards. Basically, primordial and primary preantral follicle development is considered gonadotropin independent, given that both cumulus cells and theca cells are devoid of follicle stimulating hormone (FSH) and LH receptors. However, in cumulus cells, the presence of FSH receptors and LH receptors has been confirmed from the secondary preantral and from the antral stage onwards, respectively. With regards to the theca cells, although FSH receptors are lacking, LH receptors are present from the secondary preantral stage onwards. Gonadotropin receptor allocation in follicular cells is in line with the two-cell two-gonadotropin theory [2], according to which, LH induces androgen production by the theca cells and FSH promotes aromatase enzyme activity and thus the utility of androgens as a substrate for estrogen biosynthesis. In fact, FSH and LH act synergistically and complementally in the process of follicular growth, given that FSH drives recruitment, selection, and dominance, whereas LH contributes to dominance, maturation, and ovulation [3-4].

On the basis of this theory, preantral stage can be reached in the absence of LH. However, this hormone is considered essential for antral formation and further follicle development and differentiation from the antral stage onwards. In this context, hypogonadotropic hypogonadal women respond to FSH alone with follicle development with blunted estradiol production and poor luteinization following human chorionic gonadotropin (hCG) administration for triggering final follicle maturation [5, 6]. However, in gonadotropin-releasing hormone (GnRH)-agonist downregulation cycles, despite gonadotropin suppression, residual endogenous LH secretion is considered adequate for effective ovarian stimulation [7]. Basically, in assisted reproductive technology (ART), follicle development can be accomplished in the absence of LH in the stimulation protocol, suggesting that the addition of this hormone in COS protocols for IVF may be optional, determined by clini-
ian’s preferences.
Currently, LH activity can be provided by human menopausal gonadotropin (hMG), recombinant LH (rLH), human derived chorionic gonadotropin (hCG) or recombinant human chorionic gonadotropin (rhCG). hMG is a urinary product, which has been estimated to provide around 75 IU of FSH and 75 IU of LH activity per ampoule. Studies have shown that the hCG content of the hMG preparation is around five IU per ampoule. Given that hCG is around six-fold more potent than LH [8], it is concluded that of the 75 IU of LH activity provided in the hMG preparation, actually, about 30 IU are provided by hCG. Thus, hCG content contributes considerably to hMG-mediated LH activity [9].

Theoretically, LH activity mediated by hCG sounds quite attractive in the clinical setting due to its unique characteristics. In particular, hCG shares structural similarities with LH and function through the same receptor, LH/CGR. However, hCG has a longer half-life of 36 hours [10] compared with recombinant LH whose elimination half-life is estimated to be around ten to 12 hours [11], has stronger LH/CGR receptor binding affinity probably due to differences in the carbohydrate moiety, which may make the molecule more sensitive to the binding receptor [12], and is much more potent than LH [8].

Taking into consideration the accumulating evidence of a potential beneficial effect of hCG-mediated LH activity in ART outcome, the authors retrospectively collected data of ICSI cycles, in which as low as 100 IU of hCG were added in COS with rFSH and compared them with cycles, in which COS was conducted with rFSH only.

Study endpoints
The aim of this study was to assess the effect of the addition of low-dose hCG to rFSH throughout the follicular phase in COS conducted with a short GnRH-agonist protocol on ART outcome. In particular, the primary endpoint was to assess the effect on pregnancy rates, whereas secondary endpoints were the effect on various COS parameters, such as total rFSH dose used, duration of stimulation, peak serum estradiol levels, number of oocytes retrieved, number of mature oocytes, fertilization rates, and embryo quality.

Materials and Methods
Patients
This retrospective clinical study was conducted in 141 women undergoing intracytoplasmic sperm injection (ICSI) through a short GnRH-agonist protocol with rFSH and the addition of low dose (100 IU/day) hCG (hCG group). The control group consisted of 124 women undergoing COS with a similar protocol devoid of hCG. In fact, from July 2012 to June 2014, the medical records of a total of 645 women, who underwent in vitro fertilization (IVF)/ICSI in Fertility Institute, Diagnostic and Therapeutic Centre S.A., a private Fertility Centre in Athens, were assessed for eligibility. Among them, 283 were found eligible and finally 265 agreed to participate in the study, 141 in the hCG group and 124 in the control group.

Figure 1. — Flow chart. Medical records of a total of 645 women, who underwent IVF/ICSI were assessed for eligibility. Among them, 283 were found eligible and finally 265 agreed to participate in the study, 141 in the hCG group and 124 in the control group.
Similarly, oocyte grading, fertilization, and embryo grading were conducted by either of the two senior embryologists of the Centre.

Ovarian stimulation, IVF/ICSI and embryo transfer

Short GnRH-agonist protocol was conducted according to the strict routine practice of Fertility Institute. On day 2 of cycle, a baseline ultrasound scan was performed. Serum E2 and progesterone levels were determined and provided that they were reassuring, daily subcutaneous injections of buserelin acetate were started on cycle day 2 at a dose of 0.5 mg and continued until triggering of final oocyte maturation with hCG. Recombinant FSH administration started on day 3 at a dose of 200 IU and the dose was adjusted according to ovarian response on a daily basis, six days after the onset of rFSH administration.

In hCG group, hCG was administered intramuscularly at a dose of 100 IU per day along with rFSH, starting on day 3 of cycle throughout the follicular phase, until the day of triggering of final oocyte maturation.

Serum E2 levels were measured daily from day 5 of ovarian stimulation with gonadotropins (day 7 of cycle) until the day of triggering final oocyte maturation. Follicular tracking began on day 6 of stimulation (day 8 of cycle) and subsequent ultrasound scans were performed daily until oocyte retrieval. Follicular aspiration and oocyte retrieval took place 35-36 h ours after the intramuscular administration of 10,000 IU hCG, by transvaginal ultrasound-guided puncture. Oocytes were assessed for their maturation under the microscope following stripping and among them, mature metaphase II oocytes were used for ICSI.

Embryos were scored based on normal cleavage rate, absence of fragmentation, and even-sized blastomeres on a scale from 4 (the best) to 1 (the worse) under a light microscope on the day of embryo transfer [13]. Two to three embryos were transferred according to embryo quality assessment and patient’s preferences. Luteal phase support was provided with 200 mg of micronized progesterone administered intravaginally three times daily from the day after egg collection onwards and serum hCG was measured 14 days after that. Clinical pregnancy was defined as the presence of a gestational sac on ultrasound at six gestational weeks.

Hormone assessments were performed in the same Lab. Ultrasound scans, oocyte retrievals, and embryo-transfers were conducted by either of the two fertility specialists of the Centre. Similarly, oocyte grading, fertilization, early embryo development, and embryo grading were conducted by either of the two senior embryologists of the Centre.

Statistical analysis

Statistics Package for Social Sciences was employed to analyze the data of the study. Two independent samples t-test was used for quantitative data and chi-square test (Fisher exact test) for qualitative data. Due to the deviation from normality, non-parametric Mann-Whitney U test was applied in order to evaluate the univariate association of demographic and biochemical factors. This test was used as a complementary statistical test due to the relatively limited number of women comprising each group and subgroup to double check the results obtained by the parametric test.

Bombarded by progressively accumulating evidence of a tentative beneficial effect of hCG when added to rFSH, fertility specialists of Fertility Institute tended to mobilize hCG in women in which they expected a rather suboptimal ovarian response to ovarian stimulation. Among them, women of more advanced reproductive age were anticipated. Thereby, given the retrospective methodology of the study, in addition to statistical analysis in the study population [control group n=124 and hCG group n=141], a subgroup analysis was performed for age ≥ 35 years [control group n= 62 and hCG group n= 98] as well as for age ≥ 36 years [control group n=44 and hCG group n=87]. Statistical significance was set at the level of 5% (p < 0.05).

Results

Indications for fertility treatment with IVF/ICSI (male factor, tubal factor, unovulatory cycles due to polycystic ovaries, other causes of infertility, complex etiology, and unexplained infertility) did not differ among the study groups (data not shown).

Taking into consideration the Centre’s latent tendency to add hCG in expected poor responders, it is not surprising that women in hCG group were statistically significant older (37.02 vs. 33.99 years) and with higher basal FSH levels (8.52 vs. 6.67) compared to control group. However, despite this and the fact that several ovarian stimulation parameters, such as peak estradiol levels, number of oocytes retrieved, number of mature oocytes, and fertilization rates

Table 1. — Analysis of clinical and laboratory results of all cases.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n=124)</th>
<th>hCG group (n=141)</th>
<th>t-test p-value</th>
<th>Mann-Whitney U p-value</th>
<th>Fisher’s exact test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.99 ± 3.73</td>
<td>37.02 ± 4.63</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Previous failed attempts</td>
<td>0.76 ± 0.950</td>
<td>1.59 ± 1.454</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>6.67 ± 2.13</td>
<td>8.52 ± 3.70</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Total rFSH dose (IU)</td>
<td>2692.92 ± 1053.99</td>
<td>2702.51 ± 1034.62</td>
<td>0.941</td>
<td>0.982</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>10.02 ± 1.33</td>
<td>9.78 ± 1.80</td>
<td>0.208</td>
<td>0.272</td>
<td>N/A</td>
</tr>
<tr>
<td>Peak E2 levels (pg/ml)</td>
<td>2152.76 ± 1104.26</td>
<td>1850.07 ± 1331.19</td>
<td>0.045</td>
<td>0.002</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>8.85 ± 2.71</td>
<td>6.31 ± 2.68</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of mature oocytes</td>
<td>7.323 ± 2.69</td>
<td>5.738 ± 2.37</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>89.4 ± 0.113</td>
<td>81.7 ± 0.141</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Quality of transferred embryos</td>
<td>2.871 ± 0.382</td>
<td>3.355 ± 0.575</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>26.6</td>
<td>39.7</td>
<td>N/A</td>
<td>N/A</td>
<td>0.027</td>
</tr>
</tbody>
</table>

N/A: not applicable; Statistical significance: p-value < 0.05.
were in favor of the control group, the quality of transferred embryos were in favor of hCG group as were pregnancy rates (Table 1). Other parameters such as height, weight, BMI, and basal hormonal profile (excluding FSH) did not differ among the study groups (data not shown). Furthermore, no difference was seen in total rFSH dose, and duration of ovarian stimulation (Table 1). However, it should be mentioned that in the hCG group, average number of previous failed IVF/ICSI attempts was statistically significant higher compared to control group (1.596 vs. 0.764 respectively). Although, it could be assumed that the higher the number of previous cycles, the more experience is gained on each individual case’s response to ovarian stimulation, which may lead to a more tailored to each patients needs approach in a future IVF/ICSI cycle, this assumption is not supported in the present study, when examining the correlation between the number of previous IVF/ICSI attempts and pregnancy rates (Table 2). Similar results were obtained in the subgroup analyses aside from peak estradiol levels measured on the day of triggering final oocyte maturation, which did not differ among the subgroups (Tables 3 and 4).

Discussion

Since the introduction of COS in IVF, the goal has been gradually shifted from “quantity” to “quality”. It is well known that the first successful IVF was conducted through a natural cycle. Thenceforth, the objective of developing as many follicles as possible in a single ovarian cycle became quite attractive. The rationale was that the more the follicles developed, the more the eggs collected and the more the embryos produced, among which the best would be selected for transfer. However, soon after increasing clinical application of this aspect, the advent of ovarian hyperstim-

Table 2. — Previous IVF/ICSI attempts and pregnancy rates.

<table>
<thead>
<tr>
<th>Previous failed attempts</th>
<th>Pregnancy rate (%)</th>
<th>n</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>175</td>
<td>1.14</td>
<td>1.31</td>
<td>0.216</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>89</td>
<td>1.35</td>
<td>1.31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical significance: p-value < 0.05.

Table 3. — Subgroup analysis of clinical and laboratory results for age ≥ 35 years.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=62)</th>
<th>hCG group (n=98)</th>
<th>t-test</th>
<th>Mann-Whitney U</th>
<th>Fisher’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.96</td>
<td>39.47</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Previous failed attempts</td>
<td>0.758 ± 1.066</td>
<td>1.755 ± 1.534</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>6.66 ± 1.88</td>
<td>8.69 ± 3.70</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Total rFSH dose (IU)</td>
<td>2711.82 ± 1110.37</td>
<td>2636.73 ± 962.06</td>
<td>0.651</td>
<td>0.702</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>9.72 ± 1.50</td>
<td>9.33 ± 1.76</td>
<td>0.472</td>
<td>0.629</td>
<td>N/A</td>
</tr>
<tr>
<td>Peak E2 levels (pg/ml)</td>
<td>1909.64 ± 1037.56</td>
<td>1754.67 ± 1365.59</td>
<td>0.446</td>
<td>0.088</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>8.33 ± 2.69</td>
<td>5.83 ± 2.64</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of mature oocytes</td>
<td>7.27 ± 2.69</td>
<td>5.43 ± 2.29</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>89.3 ± 0.137</td>
<td>83.0 ± 0.134</td>
<td>0.002</td>
<td>0.002</td>
<td>N/A</td>
</tr>
<tr>
<td>Quality of transferred embryos</td>
<td>2.952 ± 0.335</td>
<td>3.418 ± 0.555</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>19.4</td>
<td>36.7</td>
<td>N/A</td>
<td>N/A</td>
<td>0.022</td>
</tr>
</tbody>
</table>

N/A: Not Applicable. Statistical significance: p-value < 0.05.

Table 4. — Subgroup analysis of clinical and laboratory results for age ≥ 36 years.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=44)</th>
<th>hCG group (n=87)</th>
<th>t-test</th>
<th>Mann-Whitney U</th>
<th>Fisher’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.72</td>
<td>40.04</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Previous failed attempts</td>
<td>0.909 ± 0.137</td>
<td>1.759 ± 1.525</td>
<td>0.001</td>
<td>0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>6.83 ± 2.03</td>
<td>8.76 ± 3.22</td>
<td>0.000</td>
<td>0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>Total rFSH dose (IU)</td>
<td>2730.68 ± 1081.35</td>
<td>2593.39 ± 878.37</td>
<td>0.437</td>
<td>0.652</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>9.59 ± 1.54</td>
<td>9.51 ± 1.75</td>
<td>0.814</td>
<td>0.909</td>
<td>N/A</td>
</tr>
<tr>
<td>Peak E2 levels (pg/ml)</td>
<td>1847.77 ± 901.14</td>
<td>1721.12 ± 1342.65</td>
<td>0.574</td>
<td>0.128</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>8.15 ± 2.71</td>
<td>5.83 ± 2.61</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of mature oocytes</td>
<td>7.15 ± 2.65</td>
<td>5.83 ± 2.25</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>88.9 ± 0.101</td>
<td>83.1 ± 0.127</td>
<td>0.006</td>
<td>0.008</td>
<td>N/A</td>
</tr>
<tr>
<td>Quality of transferred embryos</td>
<td>2.955 ± 0.371</td>
<td>3.425 ± 0.563</td>
<td>0.000</td>
<td>0.000</td>
<td>N/A</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>11.4</td>
<td>37.9</td>
<td>N/A</td>
<td>N/A</td>
<td>0.002</td>
</tr>
</tbody>
</table>

N/A: not applicable. Statistical significance: p-value < 0.05.
Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial?

Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial?

Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial?

Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial?

Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial?
ovaries, which are less sensitive to gonadotropins [55]. Previous studies have shown that regardless of FSH, low-dose hCG can support development and maturation of larger ovarian follicles, whose granulosa cells have acquired LH/CG receptors, rendering hCG a potentially safer and more effective regimen [51]. Besides, androgen production by theca cells as a result of LH activity, hCG has been suggested to increase follicular responsiveness to FSH, implying that granulosa cells resistant to rFSH stimulation might benefit from the addition of low-dose hCG, in terms of increased peak estradiol levels, number of oocytes retrieved, and number of mature oocytes. Unfortunately, this was not the case in the present study, where, on the contrary, these parameters were in favor of the control group. A plausible explanation can be found in the retrospective pattern of the study, according to which hCG group was not consisted exclusively of poor responders, although probably more such patients might have been included in this group due to common trend of the fertility specialists of the Centre to add hCG in patients of poor prognosis to ovarian stimulation.

Reviewing the literature, the addition of hCG at daily doses of 50-200 IU have been applied so far in COS during the early or late follicular phase or throughout the follicular phase [56–59], whereas a single dose of 1,250 IU of hCG have been used in a GnRH-antagonist protocol in combination with aromatase inhibitor in early-follicular phase [60].

A study conducted by the present group showed that the addition of 200 IU of hCG in a short GnRH-agonist protocol with rFSH for the first five days of ovarian stimulation yielded statistically significant higher number of follicles and oocytes and, most importantly, higher implantation and pregnancy rates compared to the addition of 200 IU of LH [12].

A recent randomized controlled dose-response pilot study came across similar results with the present study regarding embryo quality. In that study, a fixed dose of 150 IU/day of rFSH was selected and patients were randomized to receive daily hCG doses of 0, 50, 100 or 150 IU throughout stimulation in a short GnRH-agonist protocol [61]. Peak E2 levels were twice as high after 100-150 IU/day of hCG compared with no hCG administration, although the number of follicles and oocytes retrieved did not differ substantially. However, as in the present study, embryo quality was higher in the hCG group. With regards to pregnancy rates, daily doses of hCG up to 150 IU were compatible with good pregnancy rates, although the design of the study with the small sample size did not allow the detection of differences in pregnancy and live birth rates. Finally, a positive dose-response was seen for pre-ovulatory progesterone, but concentrations remained below values for which an impairment of endometrial receptivity has been previously reported.

Another study which is in line with the present study, assessed the effect of the addition of hCG to rFSH at a dose of 50 or 100 IU/day in a GnRH-agonist protocol. Interestingly, lower total rFSH dose, fewer oocytes, and fewer embryos but higher implantation and pregnancy rates, were associated with hCG administration [62].

An obvious difference between these studies and the present study is the mean age of women, which did not differ among the study groups, whereas in the present study women in hCG group were significantly older. However, results imply that hCG-mediated LH activity may improve embryo quality.

In conclusion, the addition of hCG to rFSH in a short GnRH-agonist protocol, throughout the follicular phase, had a beneficial effect in terms of pregnancy rates. Furthermore, hCG was associated with better quality embryos. The significance of these findings was accentuated by the fact that women, who received hCG were significantly older and with higher basal FSH levels, thereby with expectant poorer ovarian reserve. Among the underlying explanations, hCG interaction with LH/CG receptors developed in granulosa cells of larger antral follicles, which can enhance follicle growth and maturation, as well as hCG properties in improving endometrial environment and subsequently implantation potential should be stressed. In fact, hCG-mediated LH activity sounds quite attractive due to its long acting profile, which can provide more prolonged and stable stimulation of LH/CG receptors compared to other means of LH activity.

Limitations of the present study include its retrospective design and the difference in the age among women, who constituted the hCG (older) and the control group (younger), although the latter may even reinforce findings of the study. Nevertheless, larger-scale prospective randomized studies in stratified age groups are welcome in order to clarify the role of hCG in contemporary ovarian stimulation protocols, given the wide availability and the low cost of this regimen.

References

Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial?


Address reprint requests to:
D. LOUTRADIS, M.D., Ph.D.
Division of Human Reproduction
1st Department of Obstetrics and Gynecology
Alexandra Hospital, Athens University Medical School
80 Vasilissis Sofias Avenue
11528 Athens (Greece)
e-mail: loutradi@otenet.gr
Serum carcinogenic antigen (CA)-125 and CA 19-9 combining pain score in the diagnosis of pelvic endometriosis in infertile women

H. Zhu, H. Lei, Q. Wang, J. Fu, Y. Song, L. Shen, W. Huang
Department of Gynecology and Obstetrics, West China Second University Hospital, Sichuan University, Chengdu (China)

Summary
Objective: To define the utility of serum carcinogenic antigen (CA)-125 and CA 19-9 combining pain score in the prediction of pelvic endometriosis in infertile women. Materials and Methods: Serum CA-125 and CA 19-9 were measured using immunoradiologic methods during the follicular phase preceding laparoscopy for infertility. Values obtained were correlated with the occurrence and severity of endometriosis. Receiver operating characteristic (ROC) curve was applied to assess the utility of serum CA-125, CA 19-9, and pain score in preoperative preparation. Cut-off value of CA-125 and CA 19-9 was defined. Results: The study enrolled 294 infertile women receiving laparoscopy between July 2010 and September 2011. Ninety-four patients were diagnosed with endometriosis and 200 patients without. Preoperative serum CA-125 and CA 19-9 levels were significantly different between the two groups. ROC curve analyses of serum CA-125 and CA 19-9 set a cut-off value of 18.25 IU/ml and 13.15 IU/ml, producing a sensitivity of 64.8% and 84.8%, a specificity of 81.9% and 51.6%, a positive predictive value (PPV) of 63.6% and 46.1%, and a negative predictive value (NPV) of 81.0% and 87.4%, respectively. Combined-analyses of CA-125 and CA 19-9 produced a sensitivity of 72.4%, a specificity of 81.9%, a PPV of 62.3%, and a NPV of 81.8%. Combined-analyses of serum CA-125, CA 19-9 and pain score produced a sensitivity of 74.0% and a specificity of 74.0%. Conclusions: Preoperative CA-125 and CA 19-9 levels combining pain score can be useful for the prediction of pelvic endometriosis and may be included in the evaluation of unexplained infertile women.

Key words: Carcinogenic antigen 125; Carcinogenic antigen 19-9; Endometriosis; Infertility; Pain score.
Table 1. — Age, pain scores, duration of infertility, and serum CA-125 and CA 19-9 levels between patients with pelvic endometriosis and controls.

<table>
<thead>
<tr>
<th></th>
<th>Age Duration of infertility</th>
<th>Pain scores</th>
<th>CA-125</th>
<th>CA 19-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic endometriosis</td>
<td>28.81±3.90± 4.00±</td>
<td>3.75</td>
<td>45.11</td>
<td>27.88</td>
</tr>
<tr>
<td>Controls</td>
<td>28.95±4.67± 1.82±</td>
<td>4.33</td>
<td>12.37</td>
<td>15.58</td>
</tr>
</tbody>
</table>

*p* 0.778 0.060 0.001 0

and CA 19-9 assays, along with the clinical pain scores in the aforementioned clinical scenario.

**Materials and Methods**

**Subjects**

Patients who underwent laparoscopy for infertility at the West China Second University Hospital Sichuan University from July 2010 to September 2011 were included. Infertility was defined as failure to achieve pregnancy after unprotected intercourse for one year and above. All patients were advised to avoid exogenous hormones for at least three months before laparoscopy. To avoid any adverse influence of anatomical factors, patients with cysts detected by preoperative ultrasonography were excluded. The study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Sichuan University. Written informed consent was obtained from all participants.

Systematic laparoscopic evaluation of all pelvic peritoneal surfaces and structures was performed on all patients. Pelvic endometriosis was quantified by the revised American Fertility Society (AFS, 1985) score. Perioperative complications were recorded.

Preoperative serological tests of serum CA-125 and CA 19-9 were performed, and the concentrations were measured with chemiluminescence immunoassay (CLIA).

A multivariate stepwise logistic regression analysis was performed to establish a predictive model, which was transformed into a scoring system. Receiver operating characteristic (ROC) curves were constructed to assess the discriminative power of each biomarker, by measuring the area under the ROC curve (AUC). An operating point was selected on the ROC curve corresponding to a specificity of 70% or higher, and sensitivity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were evaluated. All test results with a *p*-value below 0.05 were reported as significant.

**Results**

**Patients**

A total number of 294 patients with the preoperative diagnosis of infertility were recruited prospectively in the study. The age of patients ranged from 20 to 42 years, with a mean age of 33.7±7.0 years. The age, pain scores, duration of infertility, and serum CA-125 and CA 19-9 levels between patients with pelvic endometriosis and control group are listed in Table 1. There was no significant difference in the mean age and the duration of infertility between the pelvic endometriosis and control group. Most of the patients with endometriosis are stage I and II (75.4%).

**Biomarkers**

The levels of CA-125 and CA 19-9 in patients with pelvic endometriosis were significantly higher as compared to patients without endometriosis (*p* < 0.05). The pain scores of patients with pelvic endometriosis were higher than those without endometriosis (*p* = 0). The mean serum CA-125 and CA 19-9 concentrations (IU/ml) in patients with AFS-r stage I, II, III, and IV endometriosis were 17.1 ± 8.3 / 17.9 ± 9.8, 28.1 ±2 0.4 / 22.7 ± 18.1, 47.1 ± 77.0 / 50.5 ± 35.1, and 55.2 ± 29.2 / 53.9 ± 34.7, respectively. ROC curve analyses of serum CA-125 and CA 19-9 levels set a cut-off value of 18.25 IU/ml and 13.15 IU/ml (AUC 0.755 and 0.707, respectively; 95% confidence interval, 0.690-0.813) (Figure 1) and this gave a sensitivity of 64.8%/84.8%, a specificity of 81.9%/51.6%, a PPV of 63.6%/46.10%, and a NPV of 81.0%/87.4%, respectively. Combined-analyses gave a sensitivity of 72.4%, a specificity of 81.9%, a PPV of 62.3%, and a NPV of 81.8% (AUC 0.798, 95% confidence interval) (Table 2).
Table 2. — Sensitivity, specificity, Youden’s index and cut-off value of serum CA-125, CA 19-9, and combined concentration in the diagnosis of pelvic endometriosis.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Youden’s index</th>
<th>Cut-off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA125</td>
<td>0.648</td>
<td>0.819</td>
<td>0.467</td>
<td>18.25</td>
</tr>
<tr>
<td>CA19-9</td>
<td>0.848</td>
<td>0.516</td>
<td>0.364</td>
<td>13.15</td>
</tr>
<tr>
<td>Combined CA125 and CA19-9</td>
<td>0.724</td>
<td>0.819</td>
<td>0.448</td>
<td>31.42</td>
</tr>
</tbody>
</table>

Discussion

The diagnosis of endometriosis is difficult and delayed because of the non-specific symptoms and may result in social and work-related problems for the patient [12]. Two-thirds of women undergoing laparoscopy for pelvic pain or infertility are subjected to potential risks, as well as the cost associated with this procedure without actually having endometriosis [13]. The development of a simple blood test as a marker for screening of endometriosis would reduce the number of unnecessary interventions and would therefore be very useful [14]. The quest to develop a diagnostic test of endometriosis has mostly concentrated on the levels of CA125 and other cytokines [15].

Many studies have shown the usefulness of serum CA-125 assay in the detection of endometriosis. Xavier et al. [11] reported a specificity greater than 90% for a cut-off value of 22.6 IU/ml, and Kitawaki et al. [16] found that the highest accuracy (78.8%) and the maximum diagnostic value (61.2%) were achieved when 20 IU/ml was used as the cut-off value. A meta-analysis [17] evaluated the value of serum CA-125 assay in the detection of endometriosis, and the results showed a specificity of 85% and sensitivity between 20% and 50%. Mihalyi et al. [18] found that moderate–severe endometriosis was diagnosed with a sensitivity of 100% (specificity 84%) and minimal–mild endometriosis was detected with a sensitivity of 87% (specificity 71%) during the secretory phase. Meanwhile, minimal-mild endometriosis was diagnosed with a sensitivity of 94% (specificity 61%) during the secretory phase and with a sensitivity of 92% (specificity 63%) during the menstrual phase. Recently, Mohamed et al. showed that there was a statistically significant difference in serum CA-125 level in the patients with endometriosis and those without endometriosis [19]. However, detailed dissection on the study of pelvic endometriosis in infertile women is still needed. The present study showed a significant difference of the levels of serum CA-125 between the patients with endometriosis and the controls (p < 0.01). The authors also found the levels of CA-125 increased progressively with the stages of AFS-r in endometriosis and the cut-off value was 18.25 IU/ml, which gave a sensitivity of 64.8% and a specificity of 81.9%, respectively. The results were consistent with other studies, which showed that CA-125 might be considered as a reliable method to detect pelvic endometriosis in infertile women.

In former studies, the results of serum level of CA 19-9 in patients for detecting endometriosis were contradictory [9-11]. The latest study [20] reported that the mean levels of CA 19-9 were significantly elevated at all stages of endometriosis and indicted that endometriosis might be the source of high CA 19-9 levels. The present study showed the similar result, and gave a cut-off value of 13.15 IU/ml, with a sensitivity of 84.8% and a specificity of 51.6%. This indicated that serum CA 19-9 might predict the patients with endometriosis. The discrepancy in the earlier studies might be due to the differences in study designs or patients selection bias. The AUC of the combination marker is 0.798, with a sensitivity of 72.4%, and specificity of 81.9%, which means that the combination biomarker demonstrates a higher clinical utility in diagnosis of pelvic endometriosis.

In the present study, there was a statistical difference in the pain scores between patients with pelvic endometriosis and control group. This demonstrated that pain scores can be used as a valuable marker in the diagnosis of pelvic endometriosis when used in combination with CA-125 and CA 19-9.

Considering the cost-effectiveness and simplicity of the test, the present authors believe that CA-125 and CA 19-9 together with pain scores should be included in the evaluation of unexplained infertility in women. Meanwhile, considering the relatively low sensitivity of these markers, further prospective studies or multi-center studies involving larger number of patients suitable for proper comparisons are needed to explore the value of these markers in detecting pelvic endometriosis.

Acknowledgements

The authors would like to thank all of the women who participated in the study. They also would like to extend their thanks to all the staff involved at the clinics.

References


Address reprint requests to:
W. HUANG, M.D.
Department of Gynecology and Obstetrics
West China Second University Hospital
Sichuan University
No. 20, Section 3, South Remmin Road
Chengdu 610041 (China)
e-mail: weihuangcn@126.com
Impacts of maternal anxiety on non-stress test parameters

S. Nergiz Avcioğlu1, S.Ö. Altinkaya1, İ. Kurt Ömürlü2, M. Küçük3, S. Demircan-Sezer1, H. Yüksel1
1 Department of Gynecology and Obstetrics, Adnan Menderes University, School of Medicine, Aydın
2 Department of Biostatistics, Adnan Menderes University, School of Medicine, Aydın
3 Department of Gynecology and Obstetrics, Muğla Sıtkı Koçman University, School of Medicine, Muğla (Turkey)

Summary

Objective: To determine the association between antenatal maternal anxiety with non-stress test (NST) parameters, which is an indicator test of fetal well-being in the third trimester. Materials and Methods: Between January and December of 2013, 212 pregnant women, with 36-41 weeks of gestation were assessed with measures of distress and anxiety with Beck Anxiety Inventory (BAI) and with NST. The new National Institute Child Health and Human Development (NICHD) 2008 guideline criteria were used for interpretation of NST. Anxiety scores were grouped as minimal, mild, moderate, and severe. The impact of anxiety on NST parameters were investigated. Result: Anxiety scores were inversely correlated with fetal heart rate (FHR) accelerations (r = -0.631, and r = -0.855), number of fetal movements (r = -0.633, r = -0.860), FHR variability scores (r = -0.650, r = -0.877), and NST scores (r = -0.505, r = 0.729), (for all p < 0.001). NST scores were lower in severe anxiety group than the others. Conclusion: The study showed that severe form of anxiety significantly affects NST parameters in near-term pregnancies.

Key words: Anxiety; Antenatal; Non-stress test.

Introduction

Non-stress test (NST) is one of major basic components of antenatal care and nowadays it is the most dedicated test to assess the fetal wellbeing in the third trimester [1]. A normal NST test result is associated with a low probability of fetal distress [2]. It is based on an increase in fetal heart rate (FHR) in response to fetal movement. Nowadays, new standards for electronic fetal monitoring were recommended at the National Institute of Child Health and Human Development (NICHD) workshop. Most important features of NST indicating fetal well-being are heart rate variability and FHR accelerations, besides absence of FHR decelerations [2].

Anxiety is defined as the psychological result of exposure to a real or imagined stress [3]. Pregnant women may be exposed to various environmental stressors. These may include absence of social support, intimate partner violence, psychological distress, nicotine, and alcohol and drug abuse. It was also shown that women from low- and middle-income countries, especially, have high levels of psychological distress [4]. Some studies have determined that prenatal occurrence of stress factors may have also deleterious impacts on fetal and subsequently, infant development and behavior [5, 6] and for pregnant woman [7, 8]. The stress factors in pregnancy have been associated with increased risk of gestational hypertension, low birth weight, and preterm birth [9-11]. As mentioned above anxiety may have some adverse effects on pregnancy. This study was conducted to investigate impacts of anxiety on NST parameters for assessment of fetal well-being.

Materials and Methods

The present study was approved by the local ethic committee, where the study was conducted. All singleton pregnant women gestational aged between 36 and 41 weeks, who were referred to Adnan Menderes University hospital clinic between January and December of 2013, were included. All participants were informed about the study and a written consent was obtained from each participant. The study followed principles in the declaration of Helsinki. Patients with any systemic disease, twin gestations, and fetal congenital malformations were excluded from study. Materials for data collection included questionnaires, external fetal electronic monitoring instrument to monitor FHR with a marker for controlling and recording fetal movements. Questionnaires included maternal demographic information and personal characteristics such as age, educational level, economic status and income level, number of alive children, number of abortions, last menstrual period, gestational age on the basis of last menstrual period, and first trimester ultrasonography of pregnancy. Anxiety and stress levels of all participants were assessed by Beck Anxiety Inventory (BAI) prior to NST. The inventory consisted of 21 items descriptive of subjective, somatic, or panic-related symptoms of anxiety. In that inventory, answers were based on a 4-point Likert scale. All patients were asked to scale responses of ‘not at all’ to ‘severe’ in terms of the experience of that symptom over the past month. A high total score indicated more severe levels of anxiety. When levels of anxiety were classified; a score of 0-7 indicated minimal anxiety; 8-15 indicated mild anxiety; 16-25 indicated moderate anxiety, and a score of 26 and above indicated severe anxiety [12].

All pregnant women in the study underwent NST in the same environmental conditions and in the same resting position (mothers were lying on their left side in all of the tests). FHR parameters were monitored with the same fetal electronic monitors. The NST parameters considered were: time (minutes) of minimum
length of NST defined as reactive, number of fetal movements, basal FHR, number of large accelerations ≥15 beats per minute (bpm)-15 s, variability score and number of variable decelerations. The NICHD (2008) guideline [2] criteria were used for interpretation of NST.

FHR tracings were normal when the baseline FHR was between 110 and 160 bpm. Baseline FHR was defined as fluctuations in the baseline of irregular amplitude and frequency. These fluctuations were quantified in terms of the amplitude of the peak-to-trough in bpm. Bradycardia was defined by a baseline FHR less than 110 bpm. Tachycardia was defined by a baseline FHR greater than 160 bpm. FHR accelerations were defined as the minimum increase of 15 bpm for 15 seconds or more in FHR, and suggested optimum number of FHR accelerations was one to five in a period of 20-30 minutes [13]. On the other hand, assessment of variability was an important part of evaluation of a FHR pattern. Absence of variability and non-reactivity of NST was defined as no peak-to-trough changes in FHR detected. Minimal variability if amplitude was > 0 and ≤ 5 bpm, moderate variability if amplitude was 6–25 bpm, and marked variability was amplitude > 25 bpm. The occurrence of moderate and marked variability was accepted as normal fetal acid–base status. The minimum length of reactive NST was the time in minutes of trace including the second large acceleration of FHR. Therefore, NICHD 2008 guidelines classified all FHR patterns into three categories. Category I FHR pattern included the following four characteristics: baseline rate, 110–160 bpm, moderate variability (6–25 bpm), absence of late or variable decelerations, absence or presence of early, decelerations or accelerations. Patterns in Category I were almost always associated with normal fetal acid–base status. Category III was diagnosed when baseline FHR variability was absent and any one of the following was present: sinusoidal heart rate, recurrent late decelerations, recurrent variable deceleration, bradycardia. Category II comprised all FHR patterns not in Category I or III. In present study, Category I NST’s according to NICHD criteria were scored as 2, Category 2 NST’s as 1, and Category III NST’s as 0 points.

Statistical analyses were performed by using SPSS 18 version. The Kolmogorov-Smirnov test was used to assess the normality of numeric variables. Numeric variables that were not normally distributed, therefore descriptive statistics are presented as median (25-75 percentiles). Kruskall Wallis test was computed to compare NST parameters including basal FHR, number of accelerations ≥ 15 bpm-15 s, variability score, decelerations, duration of NST, NICHD NST scores, and number of fetal movements between patients with minimal, mild, moderate, and severe anxiety groups. Pearson correlation test was also computed to quantify associations between maternal anxiety score and NST parameters mentioned before. The authors used classification and regression trees (C&RT) method in order to determine parameters affecting NST variables. The p-values < 0.05 were considered statistically significant.

Results

The flow chart in Figure 1 shows the selection of the study population. Six hundred and twenty women, who were referred to the present clinic between January - December 2013 were included; however, only 212 women were accepted and available to answer the questions of BAI.

Among six hundred and twenty women, 218 women had systemic illnesses like diabetes mellitus, chronic hypertension or gestational hypertension or preeclampsia, liver diseases, neurologic illness, etc. Also, 39 patients had twin gestations and 11 women had fetal congenital malformation. Therefore, only 140 patients were really denied to answer BAI.

Mean age of all participants was 29.37 ±6.05 (16-43) years, and mean gestational age was 37.82 ±1.32 (36-41) weeks. In addition, mean number of gestations (gravity) and parity were; 2.64 ± 1.55 (1-9), and 1.19 ± 1.13 (0-7), respectively. Demographic characteristics of women in all groups are described in Table 1.

In the present study, 12 (5.7%) patients had minimal, 31 (14.6%) patients had mild, 101 (47.6%) patients had moderate, and 68 (32.1%) patients had severe form of anxiety. Also anxiety scores were inversely correlated with FHR accelerations (r = -0.855), fetal movements (r = -0.860), variability scores (r = -0.877) and NST scores (r = -0.729) (for all, p < 0.001). Number of FHR decelerations were significantly correlated with maternal anxiety scores (r = 0.327, p < 0.001) (Table 2). A significant difference was observed between moderate and severe anxiety groups in terms of number of decelerations (p = 0.028). However there was no significant difference between the other anxiety groups (p > 0.05).

The NST parameters are described in Table 3. There were no significant differences in basal FHR, duration of NST with severity of anxiety (p = 0.562, p = 0.959, re-
Impacts of maternal anxiety on non-stress test parameters

respectively). On the other hand, number of fetal movements, number of large accelerations ≥ 15 bpm-15 s, variability scores, and NST scores were low in patients with severe anxiety (p < 0.001). Difference in number of fetal movements, number of FHR accelerations, and FHR variability score were significant in severe-minimal, severe-mild, severe-moderate, moderate-mild, moderate-minimal, but no significant difference was determined between minimal and mild forms of anxiety. Furthermore, NST scores were determined lower especially in severe anxiety group when compared to mild, minimal, and moderate forms of anxiety (Table 3).

There has been no research investigating anxiety on NST parameters in literature. In the present study, the authors determined experimental power of study for indicating the relationship between anxiety and NST parameters. They have performed the study step-by-step and when they determined > 90% experimental power at α = 5%, they stopped the study. C&RT method was used in order to determine affecting NST parameters including, number of FHR accelerations, number of fetal movements, FHR variability, basal FHR score and NST scores were shown in Figures 2-5. For accelerations, fetal movements and NICHD, anxiety score proved the best predictor variable at < 27.5 (67.9% for all). However, when the anxiety scores were at the range of 27.5 to 15.5 for accelerations and fetal movement, and 27.5 to 16.5 for NICHD, they proved a very weak predictor variable (32.1%, 32.1% respectively). The best predictor proved for variability was again anxiety score variable at < 15.5 (79.7%). For baseline FHR, anxiety score proved to be non-predictor variable.

Discussion

This study showed that anxiety scores were inversely correlated with FHR accelerations, number of fetal movements, FHR variability scores, and NST scores. NST scores were determined low, especially in severe anxiety group when compared to mild, minimal, and moderate anxiety groups. Also, anxiety score variable proved to be a better predictor for NST parameters except baseline FHR. Similarly, in the literature there have been data suggesting that prenatal ma-

### Table 1. — Demographic characteristics of participants.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=12)</th>
<th>Group 2 (n=31)</th>
<th>Group 3 (n=101)</th>
<th>Group 4 (n=68)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.5 (25.25−34)</td>
<td>29 (25−34)</td>
<td>29 (25−34)</td>
<td>29 (24.25−34)</td>
<td>0.987</td>
</tr>
<tr>
<td>Gravity (n)</td>
<td>2 (1−3)</td>
<td>3 (2−4)</td>
<td>2 (1−4)</td>
<td>2 (1−3)</td>
<td>0.561</td>
</tr>
<tr>
<td>Parity (n)</td>
<td>1 (0−1.75)</td>
<td>1 (0−2)</td>
<td>1 (0−2)</td>
<td>1 (0−2)</td>
<td>0.423</td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>38 (37−38)</td>
<td>38 (37−39)</td>
<td>38 (37−39)</td>
<td>38 (37−39)</td>
<td>0.465</td>
</tr>
</tbody>
</table>

Statistical significance: p < 0.05.

### Table 2. — Correlation of NST parameters with maternal anxiety scores.

<table>
<thead>
<tr>
<th></th>
<th>Maternal Anxiety Score Correlation coefficient-r/p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (min)</td>
<td>-0.005 0.947</td>
</tr>
<tr>
<td>Basal FHR (bpm)</td>
<td>0.098 0.156</td>
</tr>
<tr>
<td>Fetal movements (n. / 20 min)</td>
<td>-0.860 &lt;0.001</td>
</tr>
<tr>
<td>Acceleration (n. / 20 min)</td>
<td>-0.855 &lt;0.001</td>
</tr>
<tr>
<td>Deceleration (n. / 20 min)</td>
<td>0.327 &lt;0.001</td>
</tr>
<tr>
<td>Variability score</td>
<td>-0.877 &lt;0.001</td>
</tr>
<tr>
<td>NICHD score of NST</td>
<td>-0.729 &lt;0.001</td>
</tr>
</tbody>
</table>

NST: non-stress test; min: minute; FHR: fetal heart rate; bpm: beats per min; NICHD: National Institute of Child Health and Human Development, r: correlation coefficient; statistical significance: p < 0.05.

### Table 3. — Comparison of NST parameters with severity of maternal anxiety

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=12)</th>
<th>Group 2 (n=31)</th>
<th>Group 3 (n=101)</th>
<th>Group 4 (n=68)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (min)</td>
<td>21 (17.25−21.75)</td>
<td>20 (17−25)</td>
<td>20 (18−22)</td>
<td>20 (18−23)</td>
<td>0.959</td>
</tr>
<tr>
<td>Basal FHR (bpm)</td>
<td>140 (130−146)</td>
<td>140 (130−147)</td>
<td>140 (130−146)</td>
<td>140 (130−150)</td>
<td>0.562</td>
</tr>
<tr>
<td>Fetal movements (n. / 20 min)</td>
<td>7 (7−8)</td>
<td>5 (5−6)</td>
<td>4 (3−4)</td>
<td>2 (1−2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acceleration (n. / 20 min)</td>
<td>8 (8−8.75)</td>
<td>6 (6−7)</td>
<td>4 (3−5)</td>
<td>1 (1−2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Variability score</td>
<td>3 (3−3)</td>
<td>2 (2−2)</td>
<td>1 (1−1)</td>
<td>0 (0−0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NICHD score of NST</td>
<td>2 (2−2)</td>
<td>2 (2−2)</td>
<td>2 (2−2)</td>
<td>1 (0−1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Min: minute; FHR: fetal heart rate; bpm: beats per min; NICHD: National Institute of Child Health and Human Development; NST: non-stress test, median (interquartile range); statistical significance: p < 0.05.

1: Statistical significance between minimal and moderate form of anxiety, p < 0.001; 2: statistical significance between minimal and severe form of anxiety, p < 0.001; 3: statistical significance between mild and moderate form of anxiety, p < 0.001; 4: statistical significance between mild and severe form of anxiety, p < 0.001; 5: statistical significance between moderate and severe form of anxiety, p < 0.001.
ternal stress, anxiety, and emotions affect fetal functioning, as evidenced by changes in FHR and movements [14]. There have been limited number of studies supporting the fact that maternal anxiety affected FHR patterns. One study performed in midwifery school with 84 pregnant women, showed that relaxation improved NST results and increased FHR accelerations [15]. Again, in another study performed with 204 pregnant women determined that listening to music had positive impact on FHR accelerations [16].

Some studies have tried to explain how maternal anxiety and stress affects the fetus. Animal studies have determined that chronic stress might inhibit the fetal cortisol barrier enzyme response resulting in increased exposure of the fetus to maternal cortisol levels [17]. High placental corticotropin releasing hormone (CRH) levels cause vasodilatation resulting in reduced oxygen and nutrient delivery to the fetus [18]. If this condition is prolonged, then disordered metabolism develops [19] predisposing to type II diabetes and obesity in later life [20]. On the other hand, anxiety or stress stimulates of the autonomic nervous system (ANS), results in secretion of catecholamines, such as noradrenaline, that causes increased uterine artery resistance and arterial pressure. Thus, uterine blood flow and oxygen delivery to the fetus decreases [21]. As a result, for example, high noradrenaline levels in pregnancy were negatively correlated with fetal head and abdominal circumferences [22]. In accordance with the literature, the results of this study showed that fewer numbers of fetal movements and fewer numbers of large accelerations ≥ 15 bpm-15 s, and low NST scores were observed in pregnant women with severe anxiety.

The results of this study revealed that the number of FHR decelerations was directly correlated only with maternal anxiety scores; however there was significant difference in number of decelerations, between only moderate and severe anxiety. Similarly it was reported that, as FHR decelerations had low specificity and might occur during reactive, as well as non-reactive NSTs. Hence, when FHR decelerations occurred, as they might signify some form of abnormal cord position, further evaluation was advised and required [23].

It is known that the NST is a useful, conventional test to measure FHR which is the most commonly used diagnostic tool to monitor fetal health, especially in the antepartum period and intrapartum periods. FHR recording also helps
Impacts of maternal anxiety on non-stress test parameters for assessment of the maturation of the fetal central nervous system (CNS) and ANS [24]. An increase in heart rate directly relates to the sympathetic and parasympathetic ANS, which may not exist normally before 26–27 weeks of gestation [13]. In determining fetal wellbeing, variability is the most important FHR characteristic alone. Normal variability is associated with intact neurologic modulation and normal cardiac responsiveness of the FHR [25]. Accordingly, we generally perform NST after 36 weeks of gestation if there is no pregnancy related complication. As decreased variability may be observed in prematurity [25] and women with signs of preterm labor may have higher amount of anxiety about her baby than women at term, preterm pregnant women were excluded in this study.

The vast majority of participants of this study had high anxiety scores whereas the number of cases with minimal anxiety is fewer. The possible explanation might be the anxiety scores of pregnant women may arise as they approach to term than the nonpregnant women or referral to tertiary center clinic. Almost all patients that apply to our clinic came from centers at periphery by the referrals. During this referral procedure, pregnant women may concern with wellbeing of their babies so anxiety level of patients might be increased. However, studies including high number of cases with different gestational weeks are needed to explain this better.

This study has some limitations. Firstly, as we have mentioned before, our center was a referral hospital for high risk pregnancies. That issue may also increase the risk of selection bias. The women probably have a high anxiety level due to the referral. So results of our study should be confirmed with multicenter studies. Secondly, limitation of our study was that, the BAI gives no indication of a woman's underlying 'trait' anxiety. So, we have tried to minimize the confounding influence of trait anxiety on our observation by excluding women with previous histories of anxiety and depression, as we have not determined trait anxiety in our cohort of pregnant women. For future research, the recently developed Beck Anxiety Inventory-Trait (BAIT) may be a useful survey instrument.

In conclusion, anxiety in pregnancy had great impact on NST parameters such as number of FHR accelerations, decelerations, fetal movements, variability scores and NICHD NST scores which were basic, useful, reliable markers of

Figure 4. — C&R tree of NICHD scores.

Figure 5. — C&R tree of FHR variability score.
fetal well-being. It is also important to emphasize that, not minimal, mild or moderate, but especially severe forms of anxiety significantly affects NST parameters. Therefore, obstetricians should emphasis on therapies reducing severity of anxiety in pregnancy that seem to be important issues for fetal well-being.

Acknowledgements

Special thanks to fellows of obstetrics and gynecology for personal assistance and contributions.

References

Management of bladder endometriosis with combined transurethral and laparoscopic approach.

Follow-up of pain control, quality of life, and sexual function at 12 months after surgery

A. Pontis¹, L. Nappi², F. Sedda³, F. Multinu³, P. Litta⁴, S. Angioni³

¹ U.O.C Obstetric and Gynecology, Ospedale San Francesco, Nuoro
² Department of Obstetric and Gynecology, University of Foggia, Foggia
³ Department of Obstetric and Gynecology, University of Cagliari, Cagliari
⁴ Department of Obstetric and Gynecology, University of Padua, Padua (Italy)

Summary

To describe the pre-surgical and post-surgical outcomes at one year in terms of recurrence of lower urinary tract symptoms, quality of life, and sexual function of a transurethral and laparoscopic combined approach in the treatment of bladder endometriosis. The authors performed a prospective observational study of 16 women affected by symptomatic bladder endometriosis at the University Hospitals of Cagliari, Padua, and Foggia. In all patients bladder nodule was excised with a transurethral and laparoscopic combined approach technique. Intensity of lower urinary tract symptoms (VAS score) were assessed pre- and post-operatively at one, six, and 12 months after surgery; quality of life (SF-36) and sexual functions (FSFI) were collected preoperatively and one year postoperatively. Operative time was 120.18 ± 15.77 minutes and mean blood loss was 65.12 ± 44.74. No intraoperative and postoperative complications and conversion laparotomy occurred. Intensity of lower urinary tract symptoms evaluated with VAS score were significantly lower after one, six, and 12 months postsurgery vs. presurgery (p < 0.001). The authors observed a significantly improvement in the quality of life and sexual functions in all patients at one year after surgery. This surgical approach is safe and simple in the treatment of bladder endometriosis, with low risks and optimal resolution of symptoms, and improvement of quality of life and sexual function.

Key words: Bladder endometriosis, quality of life, sexual function, pain control.

Introduction

Endometriosis is a disease that affects about 6-10% of the female population; it consists in the presence of endometrial tissue outside the uterine cavity. It is present in 35-50% of women with pelvic pain or infertility [1-3]. Etiology and pathogenesis of endometriosis is controversial and several theories have been proposed: according to three main theories, it may develop as a result of menstrual transtubaric reflux of endometrial cells which implant on the bladder dome, by extension of nodules adenomyosis from the uterine wall at the front bladder, or by metaplasia of Müllerian residues in the vesico-uterine septum [4-10].

The detection of endometriosis in the urinary tract is rare; an incidence of 1% in women affected by endometriosis was reported [1-3, 11, 12]. Bladder endometriosis in 30% of cases have no symptoms and the diagnosis is casual, while in the remaining 70% it is symptomatic and the symptomatology shows dysuria, tenesmus, change in urination frequency, burning, suprapubic pain, and less frequently, hematuria. The differential diagnosis includes recurrent cystitis, overactive bladder, bladder cancer, and interstitial cystitis. Bladder endometriosis is often connected with other localizations of pelvic endometriosis, hence it cannot be regarded as an independent form of the disease [13, 14]. Also, deep endometriosis is correlated to a significant impact on women’s sexuality; according to recent studies, it was observed that women with deep endometriosis have sexual function damage and to a progressive reduction in quality of life and sexual satisfaction [15, 16].

The aim of this prospective study was to evaluate urinary tract symptoms pre-surgery and one, six, and 12 months after surgery; quality of life (SF-36) and sexual function preoperatively and one-year postoperatively in patients undergoing laparoscopic surgery for bladder endometriosis.

Materials and Methods

Between January 2008 and December 2010, a prospective observational study, whose purpose was to examine the cases of 16 patients, treated at the University Hospitals of Cagliari, Padua, and Foggia, because of symptomatic bladder endometriosis.
Preoperatively patients were subjected to a thorough clinical assessment by medical history, pelvic examination, transvaginal ultrasound [17, 18], hysteroscopy and endometrial biopsy [19, 20], MRI, cystoscopy, and each patient was requested to complete a questionnaire on quality of life (SF-36) and sexual functions (FSFI). Urinary tract symptoms (pain, dysuria) were collected using VAS score. Transvaginal ultrasound was performed preoperatively and one month postoperatively. Bladder nodule volume was measured using the prolate ellipsoid formula (L x H x W x 0.53).

Presurgery and at one, six, and 12 months after surgery, lower urinary tract symptoms (VAS score) were evaluated. Quality of life and sexual function were evaluated preoperatively and at 12 months postoperatively.

Operative data such as blood loss, operative time, and complications were recorded. At one, six, and 12 months after surgery, all patients underwent a pelvic examination and transvaginal ultrasound.

Lower urinary tract symptoms (LUTS) was evaluated by using a ten-cm visual analogue scale [21]. The definition LUTS includes all conditions of irritation and pain, acute and chronic, of the genitourinary sphere, including hematuria, pollakiuria and dysuria, non-microbial cystitis, and recurrent urinary tract infections [22].

The authors assessed quality of life with the Medical Outcomes Survey Short Form 36 (SF-36) which is the most widely used generic instrument to evaluate health-related quality of life. It is a self-administered questionnaire accepted for the assessment of quality of life; it consists of 36 questions and eight subscales focusing on the evaluation of elements of physical and mental health. For the purpose of the study, the authors used four subscales related to the physical health and four inherent mental health (respectively physical functioning, role physical, bodily pain, general health vitality, social functioning, emotional role, and mental health). The total score is the sum of the scores obtained with the subscales. In all scales examined, the highest score corresponds to better physical and mental health [23].

The female sexual function index (FSFI) is a 19-item self-report instrument providing scores on six domains of sexual function. These domains include the following: desire (two items, questions 1–2), arousal (four items, questions 3–6), lubrication (four items, questions 7–10), orgasm (three items, questions 11–13), satisfaction (three items, questions 14–16), and pain (three items, questions 17–19). The sum of the 19 items provides the total FSFI score. Points given for each topic changed between 0 and 5 and the score was calculated using multipliers of the factors. A total score of 26.55 has been used as the cut-off for clinical SDF in previous studies [24]. The authors treated these patients with a partial cystectomy that was performed with combined approach - transurethral and laparoscopic according to Litta technique [25].

All patients were informed about the surgery and signed a consent.

Surgery
The surgical procedures were performed with the patient under general anesthesia. The first step of surgery consisted of a cystoscopy with a 5.2-mm continuous-flow endoscope with a 30° optic and an operative channel of 5 F that allows passage of a 0.6-mm diameter and three-mm long bipolar electrode. The cystoscopy, performed under continuous flow of saline solution, allowed the authors information about the node’s location, size, and its location with respect to the ureteral ostia. They defined just the edges of the nodule via cystoscopy, with a free margin of two to three mm, and entered the bladder wall in one lateral edge, at three or nine o’clock positions, for a length of two cm, without transfixing into the bladder peritoneum.

A. Pontis, L. Nappi, F. Sedda, F. Multinu, P. Litta, S. Angioni
symptoms. Table 3 shows quality of life results evaluated by the SF-36. At one year follow up, patients showed significant improvement in physical function (p < 0.01), in general health (p < 0.00021), in physical (p < 0.0003) and emotional roles (p < 0.03), in mental health (p < 0.004), and vitality (p < 0.0013), in comparison to baseline (pre-surgery). Six domains and total score of sexual function were all statistically significant improved at 12 months after surgery compared with sexual function before surgery (Table 4). In all 16 cases, the diagnosis of endometriosis of the bladder was confirmed by histological examination.

**Discussion**

Only less than 4% of women with endometriosis have urinary involvement mainly of the bladder [26]. The predominant symptoms of bladder endometriosis is pelvic pain, although the pathogenesis is not well understood and many women with endometriosis may have no pain symptoms. The pain is most often cyclic, but may also be chronic in nature. It usually begins just before menses and is continuous throughout the duration of menstrual flow. Dysuria is a less common symptom and is associated with bladder endometriosis. Pain may also be perceived in musculoskeletal regions, such as the flank, low back or thighs [27].
References


Is maternal Vitamin D associated with gestational diabetes mellitus in pregnant women in Cyprus?

I. Soytac Inancli, E. Yayci, T. Atacag, M. Uncu

1 Department of Endocrinology and Metabolism, Near East University, Lefkoşa; 2 Department of Obstetrics and Gynecology, Near East University, Lefkoşa; 3 Department of Biochemistry, Near East University, Lefkoşa (Cyprus)

Summary
Background: There are conflicting results about the relationship between Vitamin D deficiency (VDD) and gestational diabetes mellitus (GDM). Aim: The aim of the study was to determine the association between 25 hydroxy vitamin D levels and GDM in Turkish Cypriot pregnant women. Study design: The authors conducted a prospective case-control study. Materials and Methods: The authors analysed serum 25OHD concentrations in 230 pregnant women between 2013-2014 in the winter period (November-April). Twenty-five hydroxy vitamin D was measured at the time of glucose tolerance testing at mid-gestation (24-26 weeks of pregnancy), in a population at increased risk for GDM. Patients were then divided into two groups: pregnant women with GDM and pregnant women without GDM. Results: The mean level of 25 hydroxy vitamin D in the study group was 21.9 ±10.4 ng/ml. Pregnant women were diagnosed as having vitamin D insufficiency. The level of 25 hydroxy vitamin D in women with and without GDM were 22.3 ± 10.7 ng/dl and 20.3 ±9.3 ng/dl, respectively (p = 0.262). Conclusion: The present study showed 25OHD insufficiency in pregnant women at midgestation which was not associated with GDM.

Key words: Vitamin D deficiency; Gestational diabetes mellitus.

Introduction
Gestational diabetes mellitus (GDM) is the glucose intolerance seen in women with the onset during pregnancy. The incidence of GDM has increased in recent years partly due to the obesity epidemic and different diagnostic cut-offs taken [1, 2]. It affects 2-13% of all the pregnancies [3]. GDM has been recognized as a significant risk factor for unfavorable pregnancy outcomes. Women with GDM have a risk of developing DM in the future. In addition the children born to GDM mothers are more likely to be obese and have glucose intolerance [4, 5]. Studies are accumulating that GDM is an important contributor to the formation of Type 2 Diabetes Mellitus (DM) epidemic [6].

Vitamin D deficiency (VDD) or insufficiency have been associated with impaired glucose tolerance [7, 8]. VDD was significantly related to insulin resistance and impaired insulin secretion which was reversible with vitamin D supplementation [9, 10, 11]. Although the exact cause is unknown, a potential cause may be through the presence of vitamin D receptors in pancreatic beta cells influencing insulin secretion or through the effects of 25OHD on calcium metabolism [12, 13].

Emerging evidence suggests that Vitamin D administration can improve insulin sensitivity and glucose tolerance, but whether vitamin D supplementation can prevent GDM is unknown. Observational studies provide conflicting evidence as to whether low serum 25OHD levels are associated with GDM.

However, current evidence suggests that VDD or insufficiency may be a risk factor in the development of GDM. Some studies have shown low levels of 25OHD in patients with GDM [14,15]. Another study showed low levels of 25OHD at 16 weeks of pregnancy before the diagnosis of GDM was made [16]. It is not known whether supplementation of vitamin D can prevent GDM. The aim of this study was to evaluate whether second trimester vitamin D was associated with GDM development during pregnancy in Turkish Cypriot women.

Materials and Methods
The present study was conducted in the Department of Obstetrics and Gynecology and Department of Endocrinology and Metabolism between January 2013 and April 2014 in the months between November and April (winter). Two hundred and thirty pregnant women were consecutively enrolled to the study. All pregnant women were residing in Cyprus. The patients attended the outpatient clinic for their first prenatal visit and were consecutively included to the study. Exclusion criteria were patients who had a diagnosis of pregestational diabetes mellitus, chronic disease, and pregnant women with strict religious clothing.

At first visit, blood was withdrawn for glucose, urea, creatinine, ALT, AST, and TSH. Height was measured when the patient first attended and weight was measured at every visit. At the time of the study, it was a routine practice for obstetricians to prescribe prenatal multivitamins which contained calcium and vitamin D from the beginning of the second trimester. Medications and supplements taken by the pregnant women were recorded at every visit. All of the study participants were consuming prenatal mul-
tivities which contained 500 IU cholecalciferol.

Women underwent routine screening for GDM at 24-25 weeks gestation with a 12-hour fasting 75-gm oral glucose tolerance test (OGTT). Blood was withdrawn at 0, one, and two hours. Normal results were <92 mg/dl at baseline, <180 mg/dl at one hour, and <153 mg/dl at two hours. The participants were then divided into two groups. The first group had a normal screening test, the second group had an abnormal glucose tolerance test and were defined has GDM (with one abnormal result after the OGTT). National Diabetes Data Group criteria were used to establish the diag-nosis [17]. Blood for 25OHD was obtained at same time when 75-gm OGTT was performed (as routine practice in this clinic).

Using a standard data collection sheet, demographic charac-teristics, past medical history, and maternal characteristics (height and weight), were taken in the first visit. Maternal body mass index (BMI) was calculated for every participant.

The serum 25OHD concentration were determined by using a commercial electrochemiluminescence immunoassay method. Women were then followed through their pregnancy, and data were abstracted from medical records after delivery.

The authors categorized plasma 25 OH vitamin D concentra-tions according to the criteria Holick et al. published for vitamin D sufficiency (≥30 ng/ml), insufficiency (20-29 ng/ml), and deficiency (<20 ng/ml) [18]; <10 ng/ml was defined as severe de-ficiency of 25OHD.

Data analysis was performed by using SPSS for Windows, version 11.5. Serum glucose measurement was analyzed with the hexokinase method. Serum TSH, free T4, free T3, 25OHD, anti-thyroglobulin antibody (anti-Tg), anti-thyroid peroxidase antibody (anti-TPO) were measured by electrochemiluminescence immunoassay method. The method of measurement was carried out according to the manufacturer instructions. Informed consent was obtained from all individual participants included in the study. The study was approved by the Near East University Institutional Review Board.

All procedures performed in studies involving human partici-pants were in accordance with the ethical standards of the institu-tional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable eth-ical standards.

Statistical analysis

Because the distribution of 25OHD was approximately nor-mally distributed, the authors examined the difference in mean concentrations between cases and controls. Whether the distribu-tions of continuous variables were normally distributed or not was determined by Kolmogorov Smirnov test. The mean differences among groups were compared by using Student’s t-test and Mann Whitney U test.

**Table 1. — Demographic characteristics of women who developed GDM and women who did not.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM (n=45)</th>
<th>Non-GDM (n=185)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>31.0 ± 3.9</td>
<td>29.5 ± 4.6</td>
<td>0.053</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.7 ± 4.6</td>
<td>23.2 ± 4.5</td>
<td>0.019</td>
</tr>
<tr>
<td>Weight gain at time of OGTT (kg)</td>
<td>8.4 ± 4.8</td>
<td>8.6 ± 4.7</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Data are means ± SD. GDM: gestational diabetes mellitus.

**Table 2. — Levels of 25OHD in pregnant women with GDM and without GDM.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM (n=45)</th>
<th>Non-GDM (n=185)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25OHD</td>
<td>20.3 ± 9.3</td>
<td>22.3 ± 10.7</td>
<td>0.262</td>
</tr>
<tr>
<td>≤ 10 ng/dl</td>
<td>8 (17.8%)</td>
<td>20 (10.8%)</td>
<td>0.31</td>
</tr>
<tr>
<td>10.1 - 20 ng/dl</td>
<td>17 (37.8%)</td>
<td>57 (30.8%)</td>
<td></td>
</tr>
<tr>
<td>20.1 - 29.9 ng/dl</td>
<td>12 (26.7%)</td>
<td>72 (38.9%)</td>
<td></td>
</tr>
<tr>
<td>≥ 30 ng/dl</td>
<td>8 (17.8%)</td>
<td>36 (19.5%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are n (%).

Results

The incidence of GDM was 19.6% (45 for GDM and 185 for non-GDM). The maternal age of both groups were not significantly different. Women who developed GDM had a higher BMI (Table 1). Pre-pregnancy BMI of 230 pregnant women was 23.5 ± 4.6 kg/m². The BMI of the pregnant women with and without GDM was 24.7 ± 4.6 kg/m² and 23.2 ± 4.5 kg/m², respectively (p = 0.019). There was no difference between both groups according to the pre-pregnancy and the weight gain at six months (8.4 ± 4.8 vs. 8.6 ± 4.7, p = 0.791).

Levels of 25OHD are shown in Table 2. Mean level of 25OHD in the study group was 21.9 ±10.4 ng/ml. The study population had 25OHD insufficiency. Severe VDD was 12.2% and 44.4 of all the participants had VDD (25OHD < 20 ng/ml) and only 19.1% of the participants had a sufficient vitamin D level. Mean level of 25OHD in pregnant women with and without GDM was 20.3 ± 9.3 ng/dl and 22.3 ± 10.7 ng/dl, respectively (p = 0.262). Although not statistically significant 25OHD deficiency (25OHD < 20 ng/ml) in pregnant women with GDM and without GDM was 55.5% and 41.6%, respectively (p = 0.19).

Although not statistically significant, 17.8% of the pregnant with GDM and 10.8% of the control group had 25OHD levels below 10 ng/dl - consistent with a diagnosis of sever 25OHD insufficiency. 25OHD insufficiency was 26.7% and 38.9% in pregnant women with and without GDM, respectively (p = 0.21).

Pregnant women with sufficient levels of 25OHD were 17.8% in GDM and 19.5% in non-GDM pregnant women. Although not statistically significant in patients with 25OHD levels < 10 ng/dl, the risk of GDM was 1.784 (95% CI: 0.730 - 4.361, p = 0.2), and the risk in patients with 25OHD levels < 20 ng/dl was 1.753 (95% CI: 0.909 - 3.981, p = 0.09).

Discussion

It is not clear weather low levels of 25OHD has a causative role in the formation of GDM. In the present study, GDM was not associated with maternal plasma...
25OHD. The present results were in concordance with the results of Whitelaw et al. who found no association between 25OHD levels and GDM [19]. The mean level of 25OHD was 9.3 ng/ml and GDM was diagnosed in 9.3% of the pregnant women. Park et al. found no association between the risk of GDM, insulin resistance, and impaired β cell function in Korean women [20]. Although Farrant et al. could not find an association between 25OHD levels and GDM in women in India, they found an inverse association between 25OHD levels and 30-minute glucose concentrations after a glucose load [21]. Makgoba et al. found no association between first trimester 25OHD and subsequent development of GDM, but found a inverse correlation between 25OHD levels and glucose measurements after a two-hour fasting glucose tolerance test [22]. Among 29% of the pregnant women, pregnant women with 25OHD levels of < 15 nmol/L, the prevalence of GDM was significantly higher compared with women with 25OHD levels of ≥ 35 nmol/L. Burris et al. suggested that low levels of plasma 25OHD had an increased risk for the development of GDM and found an inverse association between glucose levels after one-hour 50-gram challenge test and low 25OHD levels [23].

Maghboli et al. demonstrated that maternal serum levels of 25OHD at 24-28 weeks of pregnancy were significantly lower in women with GDM compared with controls [14]. Clifton-Bligh et al. demonstrated an inverse association between maternal serum 25OHD and fasting blood glucose but could not demonstrate a statistically significant association between mid gestation 25OHD and GDM [24]. However Lacroix et al. demonstrated that low levels of 25OHD at first trimester was an independent risk factor for developing GDM [25].

The present gestational diabetic pregnant women were higher than expected. This may be due to the test and diagnostic cut-offs the authors used to define GDM.

The present study group had 25OHD insufficiency Although under vitamin D replacement treatment, the amount of pregnant women with severe 25OHD deficiency was higher than expected. Although this was not associated with GDM, the amount of vitamin D given was not sufficient. In previous studies there was controversy about the association of VDD in pregnancy and adverse pregnancy outcomes. 25OHD deficiency in pregnancy is likely to cause pregnancy outcomes such as preeclampsia, gestational diabetes mellitus, preterm birth, and small-for-gestational age [26]. So the amount of vitamin D that should be given to a pregnant woman must be identified by prospective studies.

Insulin resistance and β cell function may play an important role in the development of GDM. However Park et al. could not find an association between insulin resistance and 25OHD levels [20].

Overweight and obesity are known risk factors that cause diabetes mellitus. Obesity is associated with both GDM [27] and low 25OHD levels [28]. In concordance with the literature, pregnant women with GDM had a slightly higher BMI score than patients without BMI which may be due to pregnancy induced insulin resistance and impaired insulin secretion [16]. However the weight change at six months was not different in both groups.

In conclusion, this was the first study performed in Cyprus. Turkish Cypriot pregnant women have vitamin D insufficiency with a mean level of 25OHD level of 21.9 ± 10.4 ng/ml in the second trimester which is not associated with increased GDM prevalence.

References


Address reprint requests to:
S. SOYTAC INANCLI, M.D.
Hakki Boratas Sok.
Kemal Sayin Apt.
No: 8 Daire 3, Girne
KKTC Mersin 10 (Turkey)
e-mail: inancis@yahoo.com
Obstetric outcomes of intramural leiomyomas in pregnancy

R. Arisoy1, E. Erdogdu1, E. Bostancı1, R.N. Ergin2, P. Kumru1, O. Demirci1, M. Polat1, O. Pekin1

1 Zeynep Kamil Gynecologic and Pediatric Training and Research Hospital Department of Perinatology, Istanbul
2 Bahcesehir University, Department of Gynecology and Obstetrics, Istanbul (Turkey)

Summary
Aim: The authors aimed to study larger intramural leiomyoma with a size of ≥ three cm on pregnancy outcome of singleton pregnancies compared with control group. Materials and Methods: The hospital records of all pregnancies followed between years of 2009 and 2013 were searched for the diagnosis of intramural leiomyoma in the second trimester ultrasonographic screening, past medical history, demographics, pregnancy follow up, and pregnancy outcomes of pregnant women. In the data analyses, 112 singleton pregnant women with intramural leiomyoma were included in the study group and 168 singleton pregnant women without leiomyoma were included in the control group. Results: The presence of pregnancy associated leiomyoma was found to be a risk factor for abortion (odds ratio (OR): 12.6, 95% confidence interval (CI) 2.5–63.6) hospitalization for pain (OR: 19.6, 95% CI 5.8–66.5), premature rupture of membranes (OR: 6.7, 95% CI 1.4–32.4), oligohydramnios (OR: 5.3, 95% CI 1.4–20.0), preterm birth (OR: 4.7, 95% CI 1.9–11.6), and breech presentation and other abnormal presentations (OR: 9.7, 95% CI 2.8–34.2) and neonatal intensive care need (OR: 3.0, 95% CI 1.2–7.5). No correlation with the rate of intrauterine growth restriction, intrauterine fetal death, placenta previa, abruption of placenta, and cesarean section was found. Conclusions: Pregnancy associated intramural leiomyoma is a risk factor for some perinatal complications and these results may be useful for prenatal counseling.

Key words: Intramural leiomyoma; Pregnancy; Obstetric outcome; Perinatal complication.

Introduction
Abnormal overgrowth of the smooth muscle cells in the uterus causes benign soft tissue masses called leiomyoma at a histopathological level. These masses do occur at any age, however are mainly prevalent in childbearing age. The prevalence of uterine leiomyoma in pregnancy varies between 2.7% and 10.7% [1-5]. The obstetric outcomes in women with leiomyoma are conflicting, and according to studies, different and discrepant outcomes were reported in literature. Leiomyoma in pregnancy can be associated with miscarriage, preterm birth, preterm premature rupture of membranes (PROM), intrauterine growth restriction (IUGR), intrauterine fetal death (IUFD), placenta previa, placental abruption, fetal malpresentation, labor dystocia, delivery by cesarean section, and postpartum hemorrhage [1-6].

In this present retrospective study the authors aimed to study larger intramural uterine leiomyoma with size of ≥ three cm on pregnancy outcome of singleton pregnancies compared with control group.

Materials and Methods
This study was a retrospective analysis of data. The hospital records of all pregnancies followed between years of 2009 and 2013 were searched for the diagnosis of intramural leiomyoma in the second trimester ultrasonographic screening, past medical history, demographics, pregnancy follow up, and pregnancy outcomes of pregnant women.

In the second trimester ultrasonographic screening at 15-24 weeks of gestation and the dimensions of the leiomyomas were measured and recorded in three dimensions. All pregnancies with intramural leiomyoma with size of ≥ three cm were included in the study group. Ones with subserosal and submucosal leiomyoma were excluded. To exclude potential effects of systemic diseases, multiple pregnancy on pregnancy outcome, ones with systemic diseases or multiple pregnancies were excluded. Pregnancies with proven chromosomal abnormalities or structural abnormalities were excluded as well from data analyses. In the second trimester, ultrasonographic screening at 15-24 weeks of gestation, pregnant women without leiomyoma, aged 30 to 40, were included in the control group. Likewise, ones with chromosomal abnormalities or structural abnormalities, systemic diseases or multiple pregnancies were excluded in the control group.

Specific demographic and perinatal outcomes were recorded including maternal age, parity, gestational age at ultrasound screening, presence of abortion (< 20 weeks), hospitalization for pain-pseudo, fetal presentation, mode of delivery, birth week, neonatal birth weight (grams), presence of IUGR, IUFD, placenta previa, placental abruption, preterm delivery (< 37 weeks, < 34 weeks, and <32 weeks), PROM, oligohydramnios (without PROM), retained placenta, APGAR scores at one and five minutes, and neonatal intensive care need. Also all birth weights were transformed into birth weight centile according to normative references [7]. Data regarding demographic, obstetric, and perinatal outcomes were obtained from the present hospital database. In case of incomplete data, the authors contacted the patients by phone to complement their data.

Data analysis was performed by using Statistical Package for
Results

In the data analyses, 112 singleton pregnant women with leiomyoma were included in the study group and 168 singleton pregnant women without leiomyoma were included in the control group, in accordance with inclusion and exclusion criteria. Demographics related to these two groups are summarized in Table 1. Total of 144 intramural uterine leiomyoma mass were identified in 112 pregnancies. Mean diameter of the leiomyoma was 60.99 ± 30.95 mm (range 30.33–203.70). Determined locations of the leiomyoma in order of decreasing frequency was as following; anterior uterine wall (53.5%), posterior uterine wall (20.8%), isthmus (10.4%), fundus (8.3%), left lateral uterine wall (3.5%), and right lateral uterine wall (3.5%). The correlation analyses showed weak to moderate significant negative correlation between the diameter of leiomyoma and birth week (Pearson correlation coefficient: - 0.266, p = 0.006) (Figure 1). Calculated equation for birth weeks in relation to the diameter of leiomyoma was “birth weeks = - 0.016 x diameter of leiomyoma + 38.69”. Though initial correlation analyses showed weak to moderate significant negative correlation between the diameter of leiomyoma and birth weight, when controlled with regression analyses for probable effect of birth week itself, size of leiomyoma had no significant effect on birth weight (p = 0.07). When compared with control group, pregnancies with associated intramural leiomyoma had significantly higher rates of preterm birth before gestational weeks of 34 and 37. However, percentiles related growth of the fetus represented with weight in this study was indifferent between control and, pregnancies with associated intramural leiomyomas. Likewise, intrauterine growth retardation rates were similar in both groups.

When presence of pregnancy associated leiomyoma was evaluated as a risk factor for clinical parameters related to pregnancy and fetal outcome, it was found to be a risk factor for abortion, hospitalization for pain, PROM, oligohydramnios, preterm birth, breech or other abnormal presentation, and neonatal intensive care need as shown in Table 2. No correlation with the rate of IUGR, placenta previa, IUFD, retained placenta, and cesarean section was found. Perioperative complications were myomectomy (n=2), hysterectomy due to postpartum hemorrhage (n=1), and Bakri balloon (n=1) in pregnancies with intramural leiomyoma.

Discussion

Uterine leiomyoma do occur at any age, however are mainly prevalent in childbearing age. The prevalence of uterine fibroids in pregnancy varies between 2.7% and 10.7%, depending upon the trimester of assessment and the size threshold [1-5]. In one study, 4% of 12,708 pregnant patients had fibroids with a diameter > three cm [1]. In another study, 2.7% of 15,104 consecutive pregnant patients had fibroids > one cm [2]. Stout et al. [3] reported a similar prevalence of 3.2% in 64,047 women undergoing ultrasound screening in the second trimester. Laughlin et al. [4] observed 10.7% of women had a fibroid of ≥ 0.5 cm in

---

Table 1. — Demographic futures and obstetric outcomes of pregnant women with leiomyoma compared with pregnant women without leiomyoma.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Study group (n=112)</th>
<th>Control group (n=168)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.4 ± 4.9</td>
<td>34.3 ± 2.7</td>
</tr>
<tr>
<td>Parity (n.)</td>
<td>1.2 ± 1.3</td>
<td>1.3 ± 1.0</td>
</tr>
<tr>
<td>Gestational age at ultrasound screening (weeks)</td>
<td>20.0 ± 2.9</td>
<td>19.6 ± 1.8</td>
</tr>
<tr>
<td>Birth week*</td>
<td>37.7 ± 2.5</td>
<td>38.8 ± 2.0</td>
</tr>
<tr>
<td>Neonatal birth weight (grams)*</td>
<td>3077 ± 575</td>
<td>3326 ± 556</td>
</tr>
<tr>
<td>Neonatal birth weight (percentile)</td>
<td>48.9 ± 25.4</td>
<td>50.1 ± 27.1</td>
</tr>
<tr>
<td>APGAR scores at 1 min*</td>
<td>7.4 ± 1.3</td>
<td>7.8 ± 1.0</td>
</tr>
<tr>
<td>APGAR scores at 5 min*</td>
<td>8.5 ± 1.2</td>
<td>9.1 ± 0.9</td>
</tr>
</tbody>
</table>

*Statistically significant p ≤ 0.01.
Due to pregnancy, increases in estrogen and progesterone levels and uterine blood flow are believed to affect fibroid growth, but reported volumetric changes during pregnancy are in conflict [4, 8-10]. In one study, 31.6% of pregnant women with leiomyoma were reported to increase statistically between the first and the third trimesters [9]. In accordance with this study, even a higher rate of volumetric increase was reported as 71.4% between the first and second gestational periods and 66.6% between the second and third trimesters [9]. However, in another study, reduction in leiomyoma size during pregnancy was reported to be 55.1% with a mean decrease in mass volume of 35 ± 4%. Interestingly the rest (44.9%) was reported to have volume increase with a mean increase of 69 ± 11% in the same study [11]. Postpartum resolution of fibroids was reported as 36% and postpartum ultrasonographic volume reduction ratio was reported as 79%, with a median change of 0.5 cm. This volume reduction in diameter was more significant in the uterine leiomyoma located in the submucosa and ones in lower segment of uterus [4].

Leiomyoma have been associated with a number of pregnancy complications, but mechanisms by which leiomyoma increase the risk of adverse obstetric outcomes are unknown. Speculations as to distensibility of the uterus, physical obstruction, efficacy of contraction patterns, inflammation, changes in the endometrial structure, and molecular signaling all have been postulated [3]. In literature, the obstetric outcomes in women with leiomyoma are conflicting. Different studies with different cut-off diameters or methods reported various pregnancy related problems like abruptio placenta, pelvic pain, abortion, breech presentation, placenta previa, PROM, preterm birth IUGR, IUFD, cesarean section rate, and postpartum hemorrhage with varying and conflicting ratios [1-6, 11]. In the present study, pregnancies with intramural leiomyoma with size of ≥ three cm included and cases with subserosal and submucosal leiomyoma excluded. This is the most striking difference between the present study and the previously published studies.

In the present study, we showed that the presence of leiomyoma was associated with higher rates of abortion (5.4% compared with 0.6%, OR:9.4 [95% CI 1.1−79.6]), hospitalization for pain-pseudo (26.4% compared with 1.8%, OR:19.6 [95% CI 5.8−66.5]), PPROM (9.8%) compared with 1.2% [95% CI 2.8−34.2]), oligohydramnios (without PROM) preterm birth < 34 weeks (9.5%) compared with 2.4% [95% CI 2.0−13.9]), oligohydramnios (without PROM) preterm birth < 37 weeks (9.4%) compared with 4.2% [95% CI 1.9−11.6]), Intrauterine growth restriction (9.4%) compared with 1.3% [95% CI 2.7−21.6]), oligohydramnios without PROM [7.5% compared with 1.2%, OR:6.7 (95% CI 1.4−32.4)] preterm birth < 34 weeks (9.4% compared with 2.4%, OR: 4.3 (95% CI 2−13.9)] and [for < 37 weeks 17% compared with 4.2%, OR: 4.7 (95% CI 1.9−11.6)], breech presentation, other abnormal presentations [15.1% compared with 1.8%, OR: 9.7 (95% CI 2.8−34.4)], and neonatal intensive care need [13.2% compared with 4.8%, OR:3.0 (95% CI 1.2−7.5)]. Despite this the authors detected higher rate of preterm birth for < 32 weeks in pregnancies with leiomyoma, however there was no significance. In addition, we found significant negative correlation between the diameter of leiomyoma and birth week (Pearson correlation coefficient: - 0.266, p = 0.006).

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Study group (N=112)</th>
<th>Control group (N=168)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion (&lt; 20 weeks)</td>
<td>6 (5.4%)</td>
<td>1 (0.6%)</td>
<td>9.4 (1.1−79.6)*</td>
</tr>
<tr>
<td>Hospitalization for pain-pseudo</td>
<td>28 (26.4%)</td>
<td>3 (1.8%)</td>
<td>19.6 (5.8−66.5)**</td>
</tr>
<tr>
<td>PPROM</td>
<td>8 (7.5%)</td>
<td>2 (1.2%)</td>
<td>6.7 (1.4−32.4)**</td>
</tr>
<tr>
<td>Oligohydramnios (without PROM)</td>
<td>9 (8.8%)</td>
<td>3 (1.8%)</td>
<td>5.3 (1.4−20.0)**</td>
</tr>
<tr>
<td>Preterm birth &lt; 32 weeks</td>
<td>6 (5.7%)</td>
<td>3 (1.8%)</td>
<td>3.3 (0.8−13.4)</td>
</tr>
<tr>
<td>Preterm birth &lt; 34 weeks</td>
<td>10 (9.4%)</td>
<td>4 (2.4%)</td>
<td>4.3 (2.0−13.9)**</td>
</tr>
<tr>
<td>Preterm birth &lt; 37 weeks</td>
<td>18 (17%)</td>
<td>7 (4.2%)</td>
<td>4.7 (1.9−11.6)**</td>
</tr>
<tr>
<td>Intrauterine growth restriction</td>
<td>9 (8.5%)</td>
<td>9 (5.4%)</td>
<td>1.6 (0.6−4.3)</td>
</tr>
<tr>
<td>Placenta previa</td>
<td>3 (2.8%)</td>
<td>1 (0.6%)</td>
<td>4.8 (0.5−47)</td>
</tr>
<tr>
<td>Abruptio placenta</td>
<td>2 (1.9%)</td>
<td>1 (0.6%)</td>
<td>3.2 (0.3−35.6)</td>
</tr>
<tr>
<td>Intrauterine fetal death</td>
<td>-</td>
<td>1 (0.6%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Retained placenta</td>
<td>-</td>
<td>1 (0.6%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Breech or other abnormal presentation</td>
<td>16 (15.1%)</td>
<td>3 (1.8%)</td>
<td>9.7 (2.8−34.2)**</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>61 (57.5%)</td>
<td>86 (51.5%)</td>
<td>1.3 (0.8−2.1)</td>
</tr>
<tr>
<td>Primary Cesarean section</td>
<td>39 (46.4%)</td>
<td>43 (34.7%)</td>
<td>1.0 (0.9−2.9)</td>
</tr>
<tr>
<td>Neonatal intensive care need</td>
<td>14 (13.2%)</td>
<td>38 (4.8%)</td>
<td>3.0 (1.2−7.5)*</td>
</tr>
</tbody>
</table>

*Statistically significant p < 0.05. **Statistically significant p ≤ 0.01.
present study further confirms higher pain risk related to pregnancy associated leiomyoma and the need of hospitalization for pain. However, in the present study, leiomyoma was shown to have no effect on the mode of delivery, abortion, preterm birth, and PROM. In the Exacoustos and Rosati study, 32 of 492 patients had either myomectomy or hysterectomy for uterine leiomyoma [1]. A similar surgery rate at birth was present in the current study (3/112).

Qidwai et al. [2] reported that the presence of leiomyomata was associated with increased risks for cesarean delivery (OR 1.64, 95% CI 1.28–2.11), malpresentation (OR 1.64, 95% CI 1.11–2.40), malposition (OR 1.59, 95% CI 1.18–2.15), preterm delivery (OR 1.45, 95% CI 1.08–1.96), severe postpartum hemorrhage (OR 2.57, 95% CI 1.54–4.27), and placenta previa (OR 1.86, 95% CI 1.02–3.39), but they determined that PROM, placental abruption, operative vaginal delivery, chorioamnionitis, and endomyometritis were not associated with leiomyoma. Whereas Stout et al. [3] reported that breech presentation (5.3% compared with 3.1%, adjusted OR 1.5, 95% CI 1.3–1.9), placenta previa (1.4% compared with 0.5%, adjusted OR 2.2, 95% CI 1.5–3.2), cesarean delivery (33.1% compared with 24.2%, adjusted OR 1.2, 95% CI 1.1–1.4), placental abruption (1.4% compared with 0.7%, adjusted OR 2.1, 95% CI 1.4–3.0), preterm PROM (3.3% compared with 2.4%, adjusted OR 1.3, 95% CI 1.0–1.7), preterm birth less than 37 weeks (15.1% compared with 10.5%, adjusted OR 1.5, 95% CI 1.3–1.8), less than 34 weeks (3.9% compared with 2.8%, adjusted OR 1.4, 95% CI 1.0–1.8), and IUFD in women with a fetus with growth restriction (3.9% compared with 1.5%, adjusted OR 2.5, 95% CI 1.2–5.0) were significantly associated with the presence of leiomyomas. Unlike other studies, the present authors did not find an association between leiomyoma and the rate of IUGR, IUFD, placental abruption, and placenta. Similarly, Ciavattini et al. [7] reported that no correlation with IUGR and placental abruption was found.

Coronado et al. [5] reported independent associations between uterine leiomyoma and first trimester bleeding (OR 1.82, 95% CI 1.05–3.20), dysfunctional labor (OR 1.85, 95% CI 1.26–2.72), breech presentation (OR 3.98, 95% CI 3.07–5.16), and placental abruption (OR 3.87, 95% CI 1.63–9.17). In one study, increased risk of malpresentation (OR 2.9, 95% CI 2.6–3.2), cesarean (OR 3.7, 95% CI 3.5–3.9), and preterm delivery (OR 1.5, 95% CI 1.3–1.7) are reported by Klatsky et al. [6]. Similarly in another study, size of uterine leiomyoma were compared in 95 singleton pregnancies with pregnancy associated uterine leiomyoma and larger leiomyoma (> five cm) and were also found to be associated with earlier gestational age, short cervix, preterm PROM, preterm delivery, blood loss at delivery, and need for postpartum blood transfusion [12].

In a study concerning the natural growth pattern of uterine leiomyoma during pregnancy, in which 36 pregnant women with uterine leiomyoma were followed, uterine leiomyoma with the volume ≥ 200 cm³ were found to have a higher incidence of complications compared to ones with uterine leiomyoma with the volume ≤ 100 cm³ [8]. Lam et al. [13] evaluated 121 patients for their pregnancy associated uterine leiomyoma; preterm delivery was related to multiplicity of the uterine leiomyoma. Study suggested that the location of leiomyoma had no significant effect on the rate of preterm delivery. However, ones with uterine leiomyoma in the lower part of uterus had higher cesarean section rate, postpartum hemorrhage, and greater estimated blood loss compared to the body of the uterus. Presence as well as increasing size of uterine leiomyoma was associated with higher incidence of hemorrhage, estimated blood loss, and admissions for related pain. Mean estimated blood loss was found to be higher in subserosal and intramural uterine leiomyoma. Michels et al. [14] reported that women with leiomyoma were at increased risk (RR, 1.27; CI, 1.17–1.37) for cesarean birth particularly, those with larger tumor volumes. However, the present study included leiomyoma with a size of ≥ three cm that are suggested as large leiomyoma in the previous study and we did not detect any significant difference in the rate of cesarean section between ones with leiomyoma and ones without leiomyoma. Lai et al. [15] compared the rates of neonatal outcomes between the group of women with leiomyoma and the group of women without leiomyoma. They reported leiomyoma were significantly associated with preterm delivery, PROM, IUFD, and low Apgar scores, but not with IUGR, shoulder dystocia, presence of meconium, umbilical cord pH < 7, neonatal jaundice (hyperbilirubinemia), and sepsis. In the present study, we found significantly lower Apgar scores and higher rate of neonatal intensive care need in pregnancies with intramural leiomyoma but no correlation with the rate of IUGR and IUFD was found.

In conclusion, pregnancy associated intramural leiomyomas do have negative effect on pregnancy outcome and are a risk factor for abortion, hospitalization for pain, PROM, oligohydramnios, preterm birth, breech presentation, and neonatal intensive care need, but IUGR, IUFD, placenta previa, abruption of placenta, retained placenta, and cesarean section were not associated with leiomyoma. These results are important for prenatal counseling of women with intramural leiomyoma.

References
Obstetric outcomes of intramural leiomyomas in pregnancy

113, 630.


Address reprint requests to:
R. ARISOY, M.D.
S.B Zeynep Kamil Kadın ve Çocuk Hastalıkları Egitim ve Arastirma Hastanesi, Perinatoloji Kliniği
Opr. Dr. Burhanettin Üstünel Cd No:10
Üsküdar, İstanbul (Turkey)
e-mail: drresular@hotmail.com
Immunohistochemical expression of hormonal receptors, collagen, elastin, and proteoglycans in genuine urinary incontinence

E. Pantatosakis1, D. Karandrea2, E. Liapis3, A. Kondi-Pafiti4, A. Liapis5
1Aretaieion Hospital, Athens; 2Pathology Department, Aretaieion Hospital, Athens; 3Mitera Maternity Hospital, Athens
4Pathology Laboratory, Aretaieion Hospital, Athens; 5Department of Obstetrics and Gynecology, Aretaieion Hospital, Athens (Greece)

Summary
Purpose: To study the expression of hormonal receptors, collagen, elastin, proteoglycans, and VIP in the vaginal wall of women with stress urinary incontinence (SUI). Materials and Methods: Fifty-eight specimens of the anterior vaginal wall (28 women with SUI) were processed by Ventana immunostaining method. Results: Both groups were compatible for age, BMI, and obstetric history. Positive ER-α and ER-β immunoreaction was observed in 46.4% and 3.6% of SUI (43.3% and 33.3 % of controls) (p < 0.05), respectively, and PR immunoreaction in 39.3% of SUI (46.7% of controls). Collagen I and III immunoreaction was observed in 28.6% and 21.4 % of SUI (30.% and 36.7% of controls), respectively, and elastin, decorin, and fibromodulin immunoreaction in 10.7 %, 10.7%, and 10.7% of SUI (50%, 33.3%, 33.3% of controls) (p < 0.05), respectively. VIP immunoreaction was observed in 7.1% of SUI (36.7% of controls). Conclusion: Immunoreexpression of ER-β, elastin, decorin, fibromodulin, and VIP was significantly lower in SUI than controls, showing that the ER-β dependent re-modeling of the extracellular matrix of vaginal tissues is the main mechanism of SUI.

Key words: Incontinence; Hormones; Collagen; Elastin; Proteoglycans.

Introduction
Genuine stress urinary incontinence (SUI) is defined as the involuntary loss of urine in conditions that increase intra-abdominal pressure such as coughing, laughing, lifting weight, et al. [1]. The pathophysiology of genuine SUI includes deficits in the supporting tissues of pelvic floor, mainly of collagens and elastin, factors important in maintaining the elasticity of pelvic floor (hammock hypothesis) [2]. Proteoglycans, such as decorin and fibromodulin, interact with the collagen and elastic fibers to form a network supporting the fibers in place [3-5].

Vasoactive intestinal peptide (VIP) is a neurotransmitter, component of a group of molecules responsible in changing the voltage of the supporting tissues and its deficiency contributes to the pathogenesis of SUI [6].

Estrogens and progesterone are important in various functions of the lower urinary tract via their receptors. Estrogen deficiency is considered to be a main factor of the atrophic changes of epithelial and stromal tissues of vaginal wall, producing SUI. Estrogen administration is considered as a therapeutic option in these cases [7].

There is evidence that the two subtypes of ER, ER-α, and ER-β, have different actions, and because of the fact that there is little information about the ER-β function, the present authors considered it important to study separately their expression in the various vaginal wall components [8, 9].

The purpose of this study was to highlight the distribution and changes of the estrogen and progesterone receptors expression, by immunohistochemical method, and their relation with the distribution and changes of collagen, elastin, proteoglycans, and the VIP, at the vaginal wall of women with or without SUI.

Materials and Methods
Biopsy specimens from the anterior vaginal wall of 58 women were obtained: 30 specimen from women without SUI who underwent surgery for benign disease, like hysterectomy for leiomyomas, and 28 specimens from women with SUI who underwent surgery therapy with tension-free vaginal tape. Definition of SUI was according to criteria of International Continence Society [10]. Both groups were compatible for age, BMI, and obstetric history. All women were postmenopausal, aged 55-70 years and had one to three children. Their BMI was between 18.5 to 30. Cases reporting hormone therapy, history of pelvic inflammatory disease, malignancy or previous surgical procedures were excluded from the study.

This research was approved by Aretaieion Hospital Research and Ethics Committee and informed consent was obtained from patients participating to this study. All cases were protected by special codes to protect the anonymity of patients.

After routine procedure sections from paraffin embedded tissues were stained by Hematoxylin-Eosin for basic study. Additional sections were obtained for immunohistochemical study by...
Table 1. — Demographic characteristics of the two study groups.

<table>
<thead>
<tr>
<th>Age (years), mean (SD)</th>
<th>Non-SUI n=30</th>
<th>SUI n=28</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>61.5 (4.1)</td>
<td>62.6 (4.2)</td>
<td>0.317†</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parity, median (IQR)</th>
<th>Non-SUI n=30</th>
<th>SUI n=28</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (1.5 - 2)</td>
<td>2 (1.5 - 2)</td>
<td>0.827‡</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parity (n)</th>
<th>Non-SUI n=30</th>
<th>SUI n=28</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 (23.3)</td>
<td>7 (25)</td>
<td>0.974*</td>
</tr>
<tr>
<td>2</td>
<td>17 (56.7)</td>
<td>16 (57.1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6 (20.0)</td>
<td>5 (17.9)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI (kg/m²), mean (SD)</th>
<th>Non-SUI n=30</th>
<th>SUI n=28</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.7 (2.1)</td>
<td>27.3 (1.6)</td>
<td>0.212‡</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>Non-SUI n=30</th>
<th>SUI n=28</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (20.0)</td>
<td>3 (10.7)</td>
<td>0.473**</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Overweight</th>
<th>Non-SUI n=30</th>
<th>SUI n=28</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 (80.0)</td>
<td>25 (89.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Pearson’s Chi-square test; **Fisher’s Exact test; † Student’s t-test; ‡ Mann-Whitney test.

Results

The present sample consisted of 58 women (28 with SUI and 30 without SUI). Demographic characteristics for the two study groups are presented in Table 1. The mean age for the SUI group was 62.6 (SD = 4.2) years and for the non-SUI group was 61.5 (SD = 4.1) years (p = 0.317). The two study groups were similar in terms of parity and BMI. The proportion of overweight women was 89.3% in the SUI group and 80% in the non-SUI group.

The percentage of women with positive ER-α was 43.3% for women without incontinence and 46.4% for women with incontinence. The proportion of women with positive PR was 46.7% for women without incontinence and 39.3% for women with incontinence. Percentage of women positive to collagen I was 30.0% for women without incontinence and 28.6% for incontinent women. The percentages of women positive to collagen III was 36.7% for women without incontinence and 21.4% for incontinent women. Elastin was found in 10.7% of women positive to collagen III was 36.7% for women without incontinence and 21.4% for women with incontinence. Elastin was found in 10.7% of incontinent women and in 50.0% of those without incontinence (p < 0.05).

The percentage of women without incontinence positive to decorin and fibromodulin was 33.3% and 33.3%, and 10.7% and 10.7%, respectively, for incontinent women (p < 0.05) Women without incontinence who had positive VIP were 36.7% compared with 7.1% of women with incontinence. The comparison of study indexes between the SUI and non-SUI group is shown in Table 2. Graphic presentation of the results is shown in Figure 1.

Discussion

Urinary incontinence is a symptom that affects many women and especially after menopause. The pathophysiology of genuine SUI include deficits in the supporting tissues. Differences have been found in expression of estrogen receptors in vaginal wall of women with SUI according to the study of Xie et al. [11], less expression of ER-β in the present study, which explains the benefit in use of estrogen in therapy of SUI [12]. The concentration of elastin is described to be less in women with SUI, which shows the changes of the tissues that explain dysfunction of the elas-
ticity of pelvic floor, which have been described by Goepel et al. [13]. The present findings regarding the alterations of proteoglycans, decorin, and fibromodulin, show the difficulty in demonstration and remodeling which in leads to abnormal forming of the tissues and loss of their mechanical role in women with SUI, which Chen et al. also reported [14]. The role of VIP as neurotransmitter, component of a group of molecules responsible in changing the voltage of the supporting tissues, was established by the finding of less expression in the tissue of women with SUI, as Wang et al. also showed by their study [15, 16].

Conclusions

The number of women with SUI who had positive ER-β was statistically significantly lower than the group of women without urinary incontinence. Participants with incontinence who had positive reaction to elastin were statistically significantly fewer compared with those without incontinence. The samples of women without urinary incontinence with positive reaction to proteoglycans (decorin and fibromodulin) were statistically significantly more compared to samples from women with SUI. VIP was detected statistically significantly in more samples of women without stress SUI.

References


Figure 1. — Comparative expression of hormone receptors, collagens, elastin, and proteoglycans in normal subjects and women with SUI.
lapse and stress urinary incontinence with collagen status and metabolism". *Zhonghua Yi Xue Za Zhi*, 2013, 93, 500.

Address reprint requests to:
E. PANTATOSAKIS, M.D., MSc
Makedonias 2
Kifissia 14561, Athens (Greece)
e-mail: epantatosakis@hotmail.com
The assessment of maternal and umbilical cord homocysteine levels in obese pregnant women

B. Kaya¹, S. Kaya¹, H.L. Keskin¹, S. Aydoğmuş¹, G. Akçay Yeğin¹, A. Ekiz², A.F. Avşar³

¹Ankara Atatürk Education and Research Hospital, Department of Obstetrics and Gynecology, Ankara
²Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Department of Obstetrics and Gynecology, Istanbul
³Yıldırım Beyazıt University, School of Medicine, Department of Obstetrics and Gynecology, Ankara (Turkey)

Summary

Purpose: The aim of this study was to compare the maternal plasma and umbilical cord blood homocysteine levels in obese and non-obese pregnant women. Materials and Methods: One hundred-ten term pregnant women, who completed their 37th gestational weeks and were not in active labor, were enrolled in the study. While 41 out of them were obese (BMI ≥ 30 kg/m²), 69 were non-obese (BMI < 30 kg/m²). The maternal plasma and umbilical cord homocysteine levels and umbilical cord pH values were compared between the groups. The statistical analyses were performed using t-test, Mann Whitney test, and Chi-square test. A p < 0.05 value was set as statistically significant. Results: The mean of age was higher in obese group in borderline significance (26.8 ± 5.4 vs. 28.8 ± 5.1, p = 0.049). The mean of gestational weeks, birthweight, the mode of delivery, and umbilical cord pH values were similar between the groups (p > 0.05). The maternal plasma homocysteine levels [median (interquartile range); 7.6 (4.1) vs. 7.1 (4.9)] and umbilical cord homocysteine values were not statistically different [8.6 (4.2) vs. 8.8 (4.5)] between the groups (p > 0.05). Conclusion: The maternal and umbilical cord blood homocysteine levels are not different in obese and non-obese pregnant women.

Key words: Pregnancy; Homocysteine; Obesity; Body mass index.

Introduction

Obesity is a chronic process that is common all around the world with an increasing prevalence and contributing to many chronic diseases and it is defined as excessive body fat accumulation [1-3]. It is a multifactorial situation developing under genetic, behavioral, environmental, physiological, and socio-cultural factors effecting the energy balance and fat storage [4]. Body mass index (BMI) over 30 kg/m² is evaluated as obesity [5]. While an increase in miscarriage, thromboembolic events, gestational diabetes, hypertensive disorders, cesarian section, and related maternal complications is observed, fetal complications such as fetal death, congenital abnormalities, and macrosomia are also shown to increase [6-8].

Homocysteine is a sulfur-containing amino acid and it is formed during the methionine metabolism. It takes charge in two important pathways containing methylation and sulfuration and it is not used in protein synthesis. Normal values of fasting plasma homocysteine is five to 15 µmol/L [9, 10]. Although homocysteine levels are found to be lower in normal pregnant women when compared to non-pregnant women, they are found to be even higher in pregnant women with preeclampsia, placental abruption, recurrent pregnancy loss, and intrauterine growth restriction when compared with normal pregnant women and it is indicated that it plays a role in the development of these aforementioned diseases [11-14]. Hyperhomocysteinemia has many mechanisms that cause atherogenic disease development such as blood vessel wall thickening, proliferation in the smooth muscle cells in the vessel intima layer, lipid accumulation on the blood vessel wall, platelet and leukocyte activation, increase in the low density lipoprotein (LDL) oxidation, thromboxane synthesis activation, and increased oxidative damage with radicals as a result of oxidation of homocysteine itself [15-18].

Although obesity and hyperhomocysteinemia have been separately associated with poor obstetric outcomes, there is limited information in the literature about the co-occurrence of these two conditions at pregnancy. In the present study, the authors aimed to examine homocysteine levels in obese pregnant women and to ascertain if homocysteine levels contribute to the poor obstetric outcomes in obese pregnancies.

Materials and Methods

One hundred-ten term pregnant women, whose routine pregnancy follow-up was carried out at the obstetrics outpatient clinic of an educational and research hospital, were enrolled in the study. The study protocol was reviewed and approved by the local ethics committee. Informed consent was obtained from all subjects. The authors investigated 49 obese (BMI ≥ 30 kg/m²) pregnant women for the study group and 61 non-obese (BMI < 30 kg/m²) pregnant women for the control group at gestational week 37-41 of their singleton pregnancy, not engaged in active labor, with no medical
disease or fetal anomaly and regularly followed antenatally were enrolled in both study and control groups. Gravida, parity, age, BMI in early pregnancy, gestational week at delivery, birth weight and delivery method were recorded for both groups. Maternal and umbilical cord homocysteine levels and umbilical cord pH values of the patients were measured. Pregnant women with chronic disease (diabetes mellitus, hypertension, renal disease, thyroid dysfunction, cardiomypulmonary disease, and autoimmune disease), pregnancy-related diabetes, hypertension and other pregnancy complications, history of poor obstetric outcome and fetal anomaly, smokers, multivitamin users, and those engaged in active labor were excluded from the study. The last menstruation date and the first trimester ultrasonographic measures of those who did not know their last menstruation date were used to determine the gestational age. Five cc peripheral venous blood samples were taken into ethylene diacetic acid (EDTA) containing tubes following eight to 12 hours of fasting in both groups. Right after the delivery, following the cord clamping, venous blood samples were collected from umbilical cords and similarly taken into tubes with EDTA. Both maternal and fetal blood samples were placed in the refrigerator after the sampling and centrifuged at 3,000 cycles/for ten minutes for one hour and its serums were separated. Serum samples were preserved at -25°C until being analyzed and the samples were studied on in 3 days at latest. Serum homocysteine levels were measured by use of chemiluminescence method [19]. Before the study was begun, a minimal sample size was calculated using G’Power ver. 3.1.3 software, and the minimum number of samples was determined as 98 subjects (49 cases for each group) to conform to the following statistical requirements: Type I (a) error = 0.05, Type II (b) error = 90%, and effect size (f) = 0.6 difference between group proportions of subjects. At the end of the study, data of 110 subjects (61 for non-obese and 49 for obese group) to conform to the following statistical requirements: Type I (a) error = 0.05, Type II (b) error = 90%, and effect size (f) = 0.6 difference between group proportions of subjects. At the end of the study, data of 110 subjects (61 for non-obese and 49 for obese group) were evaluated; thus the actual power was carried out as 99%.

The data of the study were analyzed using SPSS Statistics 17.0 software. Before the statistical analysis was performed, the distribution of data was evaluated using the Shapiro-Wilk normality test. It was observed that, whereas the data of variables body mass index, homocysteine levels in the maternal plasma and umbilical cord blood did not distribute normally (p < 0.05), the values of age, gestational age, umbilical cord pH, and birth weight were in conformity with the normal distribution (p > 0.05). Descriptive values of the parameters distributed normally were indicated as mean ± SD, and values of the variables that did not distribute normally as median (interquartile range - IQR).

In variables with continuous data, the parametric test (t-test) was used for comparison the normal distributed data among the groups, and non-parametric test (Mann-Whitney Test) was used in comparing data that did not distribute normally. The comparison of the data in categorical variables, gravida and parity, between the groups was performed using Chi-square test. As for the evaluation of the results, p < 0.05 was accepted as statistically significant.

Results

Whereas 49 (44.5%) of 110 cases enrolled in the study were obese, BMI in 61 cases was below 30. The mean age of all the cases was 27.6 ± 5.3 years and gestational age at birth was 39.0 ± 1.2 weeks. In all the cases, the average maternal plasma homocysteine level was 8.4 ± 3.9 µmol/L and the average umbilical cord homocysteine level was 9.9 ± 4.8 µmol/L. Whereas 70 (63.6%) of the cases delivered by vaginal delivery, 40 (36.4%) of the cases delivered by cesarean section. In eight (7.3%) of the cases, fetal distress findings were detected.

Later the cases were divided into two as normal weight (n=61) and obese (n=49) and the two groups were compared. The age of obese cases was higher in borderline significance when compared to normal-weight cases (28.8 ± 5.1 vs. 26.8 ± 5.4, p = 0.049) (Table 1). Gestational week at delivery and birth weight were similar between two groups (p > 0.05) (Table 1). Gravida and parity were found to be higher in obese pregnant women (p < 0.05). Delivery methods were also similar in two groups. While the delivery rate by cesarean was 36.1% (22/61) in normal-weight cases, it was 36.7% (18/49) in obese group (p = 0.827). Whereas fetal distress developed in three (4.9%) of normal-weight cases, five (10.2%) of obese cases developed fetal distress (p = 0.264). Umbilical cord pH levels were similar in both groups (7.29 ± 0.06 vs. 7.30 ± 0.06). Maternal plasma homocysteine levels [7.6 (4.1) vs. 7.1 (4.9)] and umbilical cord homocysteine levels [8.6 (4.2) vs. 8.8 (4.5)] were similar in both groups (respectively, p = 0.947 and p = 0.675) (Table 1).

Discussion

Physiological and metabolic stress elevate due to the increasing metabolic needs in order to meet the needs of the growing fetus. Due to the fact that high homocysteine levels cause endothelial dysfunction, it is asserted that pregnant women with hyperhomocysteine are predisposed to placental vasculitis and endothelial dysfunction. High homocysteine concentration and low folate level may effect DNA synthesis and cell division that may cause deterioration of cell proliferation and normal fetal growth [20, 21].

It is known that obesity is a chronic oxidative stress creating situation [22]. In a study including 5,131 women, Kabiru et al. assessed the relationship between the increase

| Table 1. — Comparison of demographic data and homocysteine levels between the groups. |
|---------------------------------|---------|---------|---------|
| Age (years) ** | 26.8 ± 5.4 | 28.8 ± 5.1 | 0.049a |
| Gravida a | 2.0 (1.0) | 2.0 (1.0) | 0.002b |
| Parity a | 1.0 (1.0) | 1.0 (1.75) | 0.003b |
| Gestational age (weeks) ** | 38.9 ± 1.1 | 39.1 ± 1.3 | 0.477a |
| BMI (kg/m²) a | 27.0 (3.1) | 31.3 (1.8) | < 0.001c |
| Maternal plasma homocysteine (µmol/L) ** | 7.6 (4.1) | 7.1 (4.9) | 0.947c |
| Umbilical cord blood homocysteine (µmol/L) ** | 8.6 (4.2) | 8.8 (4.5) | 0.675c |
| Umbilical cord pH ** | 7.29 ±0.06 | 7.30 ± 0.06 | 0.122c |
| Birth weight (grams) | 3329 ±403 | 3332 ± 399 | 0.621c |

*Mean ± SD; ** Median (IQR), * t-Test; b Chi-square Test; c Mann-Whitney Test.
in BMIs and the pregnancy outcomes and they detected significant increase in the gestational diabetes, induction failure, perineal laceration, delivery by cesarean section, and postpartum infection ratios in normal-weight pregnant women with increased BMI during pregnancy. They also detected increase in the preeclampsia and operative vaginal delivery ratios in over-weight pregnant women with increased BMI and increase in the chorioamnionitis, induction failure, and delivery by cesarean in obese pregnant women with increased BMI [23]. The present authors did not detect significant difference between obese and non-obese groups as for the parameters such as cesarean, fetal distress, gestational week at delivery, birth weight, and umbilical cord pH. The fact that they did not find such difference in the present study, whereas in the literature, significant difference is found between obese and non-obese pregnant women as for the parameters examining the pregnancy outcomes, may be due to the limited number of the patients.

Bukhari et al. found that serum homocysteine levels are significantly high in obese subjects when compared to normal-weight subjects. They commented that homocysteine is an independent risk factor in the development and progression of obesity [24]. Andersson et al. detected that serum homocysteine levels in pregnant women begin to decrease as of the early phases of the first trimester when compared to non-pregnant women. They stated that the decrease in the homocysteine levels become even more evident in the weeks 15-17 and remain relatively stable afterwards and that the levels return to pre-pregnancy levels in the postpartum day 2-4 [25]. Similarly, Kang et al. noted that plasma homocysteine levels decrease by almost 50% around 16th gestational week [26]. As the responsible mechanism for this decrease, they supported the hypothesis of increased glomerular filtration rate, increased plasma volume and accordingly developed hemodilution, and increased homocysteine intake of the fetus [25, 27].

Hyaneck et al. detected the homocysteine levels significantly low in normal pregnancies, when compared to women in reproductive and climacteric periods. They stated that plasma homocysteine levels were slightly higher in complicated pregnancies, when compared to normal pregnancies [28]. Açlımş et al. found the maternal and fetal serum homocysteine levels significantly higher in severe preeclamptic patients when compared to mild preeclamptic patients and the control group; they underlined no significant difference between the mild preeclamptic patients and the control group in terms of serum homocysteine levels [29]. Vollset et al. detected that in women with high plasma homocysteine levels, preeclampsia risk (32%), and prematurity risk (38%), where high and low birth weight risk was even higher (101%). While they found a significant relationship between neural tube defect, club foot anomaly, and plasma homocysteine levels, they could not detect any relationship between placental abruption risk and homocysteine levels. Consequently, they correlated high concentration homocysteine with poor pregnancy outcomes [30].

Han et al. noted that pre-pregnancy BMI effects the pregnancy outcomes. While correlating high CRP and low folate levels with preterm labor, they did not find any significant difference between obese and non-obese pregnant women in terms of the homocysteine level, which is another factor associated with preterm labor [31].

In the present study, the authors also did not find any significant relationship between maternal and umbilical cord homocysteine levels in obese and non-obese pregnant women. In the literature, generally there is a positive correlation between obesity and homocysteine levels, however such a correlation was not found in this study. It is possible that the authors could not detect the elevation that they expected in the homocysteine levels in obese women due to the fact that the present patients were obese pregnant women and pregnancy has a decreasing effect on the plasma homocysteine levels as was aforementioned (hemodilution, increased glomerular filtration, and fetus using the homocysteine).

**Conclusion**

Obesity and hyperhomocysteinemia are associated with both pregnancy complications and many medical problems in non-pregnant women, however there is conflicting information in the literature regarding the effect of the co-existence of the two during pregnancy. Whether hyperhomocysteinemia also contributes to poor obstetric outcomes of obesity is not clear yet, under the light of current information.

**References**


Address reprint requests to:
B. KAYA, M.D.
Ankara Atatürk Education and Research Hospital
Department of Obstetrics and Gynecology
Ekin Street no. 21/8 Yesilyurt Bakirkoy
Istanbul 34149 (Turkey)
e-mail: kayabasak84@gmail.com
Three-dimensional transperineal ultrasound: is there a correlation among age, weight, delivery mode, and a change in the pelvic floor architecture in Korean premenopausal women?

S.J. Kim¹, M.J. Kim¹, T.H. Kim²

¹ Department of Obstetrics and Gynecology, College of Medicine, The Catholic University of Korea, Seoul
² Departments of Obstetrics and Gynecology, Soonchunhyang University College of Medicine, Bucheon (Republic of Korea)

Summary
Purpose: The purpose of this study was to evaluate the morphological characteristics and related factors of changes to the female pelvic floor architecture during rest and the Valsalva maneuver using three-dimensional (3D) transperineal ultrasonography (TPUS) in parous women. Materials and Methods: One hundred thirty-five premenopausal parous women (aged 29-50 years) were evaluated. The pelvic floor structures were measured during rest and during the Valsalva maneuver using 3D TPUS. Results: The delivery mode was an important affecting factor in determining the change of contractility of pelvic floor architecture. The factors of age and weight were associated with changes in the anus. Conclusion: The delivery mode might be a meaningful factor in the change of contractility of pelvic floor architecture. These measurements provide a baseline upon which further investigations in a larger cohort of subjects can be compared, to determine the range and change of the normal appearance of the specific pelvic structures.

Key words: Pelvic floor; Three-dimensional; Ultrasonography; Valsalva maneuver.

Introduction
The pelvic floor architecture is composed of complex structures, and changes in the muscular dimensions and arrangement vary dynamically. Pregnancy and childbirth have been shown to be major etiological factors for pelvic floor disorders, leading to connective tissue remodeling and a disruption of the normal pelvic floor function [1]. However, the effect of pregnancy and childbirth on pelvic floor anatomy has not been fully established. Active contraction plays a role in maintaining continence and it is important to study the change in pelvic floor architecture to evaluate these functions. The contractility of this area probably also plays an important role in maintaining continence and preventing prolapse. Alterations in pelvic floor muscle morphology and function have been associated with parity, age, weight, delivery mode, and other factors, but the relationship between the risk factors and pelvic floor change may be inconsistent. There have been few studies investigating changes in the contractility and distensibility of the levator hiatus dimensions during pelvic floor muscle contraction and the Valsalva maneuver.

Three-dimensional (3D) transperineal ultrasonography (TPUS), which is easily accessible, provides useful information about the morphological changes of the female pelvic floor. Using 4D ultrasonography with real-time imaging, the levator hiatus can be followed during maneuvers and provide both qualitative and quantitative information on muscle function [1, 2].

This study was designed to evaluate the female pelvic floor with high-resolution 3D-TPUS and to explore the potential covariates to the dimensions and change of female pelvic floor architecture including the impact of age, parity, weight, and mode of delivery in Korean parous women.

Materials and Methods
Subjects
This study is a prospective observational study on the association of pelvic floor changes between during rest and the Valsalva maneuver. One hundred thirty-five Korean female parous volunteers were evaluated by 3D TPUS between January 2009 and June 2011. Fifteen patients had to be excluded from analysis because of poor image quality. The volunteers underwent a semi-structured interview about their history, and continence status and the weight, height, parity, and delivery mode were recorded for each subject. The study was reviewed and approved by the Institutional Review Board of the Catholic Medical Center (HC08WZZZ0069), and written informed consent was obtained from all subjects prior to enrollment. Potential subjects were excluded if the period of delivery was within the past 12 months, if they had had any lower urinary tract or bowel symptoms within the past 12 months, if they had pelvic organ prolapse on pelvic examination, a past history of pelvic surgery, or previous use of pelvic floor muscle exercises, and an inability to perform a maximum Valsalva maneuver.

Three-dimensional -transperineal ultrasound examination
In total, 135 women underwent pelvic floor ultrasound imaging twice in the supine position and after voiding with a system with a 4-7.0 MHz transabdominal probe by one gynecologist in a tem-
perature-controlled room (25°C). The probe was covered with a sterile latex-free condom and placed on the perineum in the sagittal plane. Volume acquisition was performed at rest and during the Valsalva maneuver, and each pubovisceral muscle contraction took approximately four seconds to perform and was recorded. Measurements were performed as described by Dietz et al. [3], in the axial plane at the level of ‘minimal hiatal dimensions’. The plane of minimal hiatal dimensions was identified in the middle-sagittal plane as the minimal distance between the posterior aspect of the symphysis pubis and the anterior border of the pubovisceral muscle. The following variables were measured in the rendered images in the plane of minimum hiatal dimension: (1) the anteroposterior (AP) and transverse diameters and the area of the vagina and the levator hiatus (LH) (Figure 1), and (2) the AP and transverse diameters and area of the anus. The contractility of pelvic floor architecture during rest and the Valsalva maneuver were compared between the normal spontaneous delivery (NSD) group and cesarean section (C-section) group. Ultrasound examination and off line analysis of the stored volumes were performed by the same physician.

Statistical analysis
Statistical analysis was performed using SPSS 12.0. Descriptive statistics for measured variables were calculated. A paired t-test, Student’s t-test or Mann–Whitney U-test and Spearman correlation were used to compare differences in paired or unpaired continuous data as appropriate. A p-value of < 0.05 was considered significant.

Results
General data
The present study population included a consecutive series of 120 Korean parous premenopausal women during the period between January 2009 and June 2011. The mean age was 42.8 ± 5.2 years (range, 29-50) and mean BMI was 22.5 ± 1.7 (kg/m²) (range 19.7-25.7). Parity history included 90 patients with NSD {mean number of full term deliveries per subject: two (range, 1-5); mean number of abortion: three (range, 0-5)}, and 30 patients with C-section deliveries {mean number of full term deliveries per patient: two (range, 1-3), mean number of abortions: two (range, 0-4); labor failure: six patients, other causes were fetal position, and maternal condition without labor: 24 patients}.

3D TPUS during rest and Valsalva maneuver
Table 1 shows the change of hiatal dimensions during rest and the Valsalva maneuver. Comparing the pelvic floor architecture between the NSD group (n=90) and C-section group (n=30), (1) vagina area (cm²) was 5.33 ± 2.00 / 5.60 ± 1.14 (p = 0.704) at rest and 5.80 ± 1.94 / 5.67 ± 1.18 (p = 0.897) during the Valsalva maneuver; (2) levator hiatus area (cm²) was 11.96 ± 2.27 / 13.35 ± 2.94 (p = 0.133) at rest and 12.35 ± 2.29 / 11.73 ± 2.73 (p = 0.209) during the Valsalva maneuver; (3) anus area (cm²) was 2.69 ± 0.75 / 2.56 ± 0.79 (p = 0.662) at rest and 2.36 ± 0.83 / 2.06 ± 0.66 (p = 0.141) during the Valsalva maneuver. There were no significant differences in the pelvic floor architecture according to delivery mode during rest and the Valsalva maneuver. The authors studied the change of contractility of pelvic floor architecture between rest and the Valsalva maneuver in the NSD group and the C-section group (Table 1). In the NSD group, the AP and transverse diameters and area of vagina

![Figure 1. — Some of the commonly used measurements for assessment of the hiatus: (A) anteroposterior hiatal area; (B) transverse diameter of levator hiatus; (C) area of levator hiatus.](image)

Table 1. — The differences of pelvic architectural parameters between normal spontaneous delivery and C-section group during rest and Valsalva maneuver.

<table>
<thead>
<tr>
<th></th>
<th>Vagina</th>
<th>Levator hiatus</th>
<th>Anus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Valsalva</td>
<td>p-value</td>
</tr>
<tr>
<td>AP diameter (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSD</td>
<td>1.35±0.37</td>
<td>1.40±0.31</td>
<td>0.126</td>
</tr>
<tr>
<td>C-sec</td>
<td>1.35±0.35</td>
<td>1.52±0.40</td>
<td>0.125</td>
</tr>
<tr>
<td>Transverse diameter (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSD</td>
<td>3.30±0.38</td>
<td>3.38±0.56</td>
<td>0.109</td>
</tr>
<tr>
<td>C-sec</td>
<td>3.65±0.38</td>
<td>3.35±0.45</td>
<td>0.543</td>
</tr>
<tr>
<td>Area (cm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSD</td>
<td>5.33±2.00</td>
<td>5.80±1.94</td>
<td>0.267</td>
</tr>
<tr>
<td>C-sec</td>
<td>5.60±1.14</td>
<td>5.67±1.18</td>
<td>0.820</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD. NSD: normal spontaneous delivery. C-section: cesarean section.
and levator hiatus were larger during the Valsalva maneuver than during rest without statistical significance. In the C-section group, the transverse diameter of the vagina and levator hiatus and the area of levator hiatus were larger during rest than during the Valsalva maneuver without statistical significance. The AP diameter of levator hiatus was larger during rest than during the Valsalva maneuver with statistical significance. The AP diameter of levator hiatus was more contracted during the Valsalva maneuver in the NSD group, the pelvic floor architecture of the C-section group, the transverse diameter of the vagina and area of the anus were larger during rest than during the Valsalva maneuver without statistical significance. In the C-section group, the transverse diameter of the vagina and levator hiatus were larger during rest than during the Valsalva maneuver without statistical significance. The AP diameter of levator hiatus was larger during rest than during the Valsalva maneuver with statistical significance. The AP diameter of levator hiatus was more contracted during the Valsalva maneuver.

### Discussion

In this study, the authors utilized 3D TPUS to describe the effects of age, weight, parity, and delivery mode on levator hiatus dimensions, both in the anatomically measured values and on the contractility of hiatal dimensions during rest and the Valsalva maneuver. The female pelvic floor architecture could be affected by various factors (age, parity, delivery, hormonal change, menopause, and physical activity), and these changes could affect the woman’s life. Although ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) were used to evaluate the change in the pelvic floor architecture, there were many difficulties encountered in evaluating the dynamic changes of pelvic floor architecture. Simultaneous examinations (accurate physical examination and imaging study) during changes of pelvic floor architecture are important in understanding the change and pathophysiology of pelvic floor architecture. 3D TPUS has been important in providing a better understanding of the pelvic floor complex and superficial perineal structures and should make it possible to avoid unnecessary surgery and allow for conservative treatment [4-7].

Women who underwent vaginal deliveries had a higher urinary incontinence rate and rate of pelvic floor disorders than women who underwent cesarean deliveries [8, 9]. Levator ani muscle contraction is predominantly dependent upon pubovisceral and puborectalis components, which are at the greatest risk for stretch-related injury during labor [10]. Age, estrogen levels, genetic factors, and obesity may play a role in the pathophysiology of pelvic floor dysfunction [11, 12]. Pelvic floor reflexes can be altered by vaginal delivery [13]. There seems to be a reduction in the magnitude of reflex contraction after childbirth, and this reduction may be associated with the delivery mode [14]. Several studies have recently used US to determine the relation between the measurement of pelvic organ’s descent, and the diameter and area measurements of the levator hiatus [3, 5]. Widening of the levator hiatus has been suggested as a cause of genital prolapse and measurements of the increasing levator hiatus area taken during the Valsalva maneuver may be either the cause or effect of pelvic organ descent [15-17]. Levator avulsions have been reported to occur in 13-40% of all women who delivered vaginally and have been associated with an increased hiatal dimension postpartum [18-20]. Shek et al. [21] reported that after cesarean delivery, there was a decrease in the mean hiatal area during the Valsalva maneuver, while in vaginal delivery without avulsion injury, the hiatal area was increased. Possible explanations for the traumatic pathogenesis in the pelvic tissue include alterations in the matrix of connective tissues and damage to the pelvic fascia, which may lead to a loss of contractility of the levator ani complex [1, 22]. This may arise as a result of damage to the muscle from distension, obstetric trauma, or neuropraxia of the pudendal nerve. Also, preexisting individual differences in the biomechanical properties of the pelvic floor and the connective tissue may result in differences in tissue rebuilding during pregnancy and delivery and have an impact on the development of pelvic floor disorders [1, 23]. In the present

### Table 2. Correlations of pelvic floor architectures with age, height, and parity.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagina</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP diameter (cm)</td>
<td>0.121 (p = 0.203)</td>
<td>0.108 (p = 0.233)</td>
<td>0.007 (p = 0.772)</td>
<td>0.001 (p = 0.975)</td>
</tr>
<tr>
<td>Transverse diameter (cm)</td>
<td>0.061 (p = 0.374)</td>
<td>0.004 (p = 0.826)</td>
<td>0.064 (p = 0.362)</td>
<td>0.007 (p = 0.773)</td>
</tr>
<tr>
<td>Area (cm²)</td>
<td>0.266 (p = 0.074)</td>
<td>0.099 (p = 0.294)</td>
<td>0.053 (p = 0.451)</td>
<td>0.124 (p = 0.238)</td>
</tr>
<tr>
<td>Levator hiatus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP diameter (cm)</td>
<td>0.039 (p = 0.879)</td>
<td>0.023 (p = 0.558)</td>
<td>0.036 (p = 0.462)</td>
<td>0.047 (p = 0.402)</td>
</tr>
<tr>
<td>Transverse diameter (cm)</td>
<td>0.099 (p = 0.702)</td>
<td>0.001 (p = 0.977)</td>
<td>0.020 (p = 0.588)</td>
<td>0.013 (p = 0.961)</td>
</tr>
<tr>
<td>Area (cm²)</td>
<td>0.002 (p = 0.882)</td>
<td>0.009 (p = 0.974)</td>
<td>0.008 (p = 0.747)</td>
<td>0.003 (p = 0.839)</td>
</tr>
<tr>
<td>Anus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP diameter (cm)</td>
<td>0.170 (p = 0.016)</td>
<td>0.036 (p = 0.282)</td>
<td>0.138 (p = 0.031)</td>
<td>0.053 (p = 0.327)</td>
</tr>
<tr>
<td>Transverse diameter (cm)</td>
<td>0.003 (p = 0.985)</td>
<td>0.136 (p = 0.440)</td>
<td>0.004 (p = 0.721)</td>
<td>0.015 (p = 0.488)</td>
</tr>
<tr>
<td>Area (cm²)</td>
<td>0.127 (p = 0.042)</td>
<td>0.014 (p = 0.515)</td>
<td>0.180 (p = 0.015)</td>
<td>0.059 (p = 0.174)</td>
</tr>
</tbody>
</table>

*Statistically significant difference (p < 0.05).*
study, there were no differences in pelvic floor architecture according to delivery mode during rest and the Valsalva maneuver. Although the role of elective cesarean section in the prevention of pelvic floor disorders is currently controversial [8], vaginal delivery has been suggested as the main contributing factor to pelvic floor disorders. Pelvic floor change after delivery, aging, weight change, and multifactorial causes might be important contributing factors to pelvic floor architecture. Although there was no statistical significance, the measured parameters of pelvic floor architecture were larger during the Valsalva maneuver than at rest using the 3D TPUS in the NSD group. Vaginal childbirth can result in the enlargement of the levator hiatus, and there may be increased distensibility of the hiatus, which may represent another mechanism leading to the enlargement of the hiatus and pelvic organ prolapse [21]. When the present authors compared contractility in the NSD group with that of the C-section group, the transverse diameter of vagina and levator hiatus, the AP diameter of levator hiatus, and the area of levator hiatus were larger during rest than during the Valsalva maneuver. During the Valsalva maneuver, the contractility might be greater in C-section group than in the NSD group. The minimal hiatal area during the Valsalva maneuver decreased after cesarean delivery, but increased after vaginal delivery. Consequently, the delivery mode might be an important factor affecting the change of contractility in pelvic floor architecture. This may indicate that pregnancy itself causes permanent changes in the anatomy of the pelvic floor, leading to distensibility due to alterations in connective tissue properties.

The current ultrasound assessment of the anal sphincter is based on measurements during rest. However, active contraction plays a role in maintaining continence. In the present study, the parameters of anus were larger during rest than during the Valsalva maneuver in both groups. In the present study, the factors of age, height, weight, and parity were not associated with changes of the vagina or levator hiatus. The AP diameter and area of the anus increased with increasing age and weight independently. There may be multiple causes, rather than only anatomical change, which could be more important to the change of pelvic floor architecture. If so, then a multiplanar study is needed to evaluate the dynamic change of pelvic floor architecture during various events, such as pelvic floor contraction. Although the limitations of the present study are its small sample size and the women’s age (limited to 29-50 and included all parous woman), it is helpful to understand the factors affecting the change of the pelvic floor architecture in Korean women. The effects of parity and mode of delivery should be differentiated with long-term prospective studies which would provide the data necessary to quantify the excess risk of pelvic floor disorders that can be attributed to vaginal delivery.

In the future, the standard examination of pelvic floor architecture using 3D US, and larger prospective studies on the relation of the functional characteristics of change of pelvic floor architectures of women to age, weight, parity, and mode of delivery will be undertaken. This will help to treat women who have given birth and lost pelvic floor support, resulting in some degree of pelvic floor organ prolapse. In researching the association of change and contractility of the pelvic floor architecture, age and weight will be helpful in understanding pelvic organ prolapse and pelvic floor disorders.

Acknowledgements
This study was supported by The Korean Society of Ultrasound in Obstetrics and Gynecology Research Fund.

References


Address reprint requests to:
M.J. KIM, M.D., PhD
Bucheon St. Mary’s Hospital
College of Medicine
The Catholic University of Korea
327 Sosa-ro, Wonmi-gu, Bucheon si
Gyeonggi-do 14647 (Republic of Korea)
e-mail: poouh74@catholic.ac.kr
The role of hysteroscopy in unexplained infertility

M. Di Muzio¹, A.M.L. Gambaro², V. Colagiovanni², L. Valentini¹, E. Di Simone¹, M. Monti²

¹Faculty of Pharmacy and Medicine, “Sapienza” University of Rome, Rome
²Department of Gynecological-Obstetric and Urological Sciences, “Sapienza” University of Rome, Rome (Italy)

Summary

Purpose of investigation: To evaluate pregnancy rate after diagnostic and operative hysteroscopy in nulliparous patients with infertility of unknown cause. Materials and Methods: The authors conducted this study on 92 nulliparous patients with unexplained infertility that underwent diagnostic hysteroscopy which showed an uterine lesion (submucous fibroids, uterine septa, polyps, synechias) and underwent operative hysteroscopy to remove the lesion between 2007 and 2011. Patients’ data were retrospectively extrapolated from patient’s charts, then the patients were called to ask if they had pregnancies after hysteroscopic surgery. Results: The present study showed a significant increase in pregnancy rate after hysteroscopic surgery (85% during the two years after the surgery). The most common endocavitary lesions were endometrial polyps (21%), uterine septa (25%), and submucous myomas (18%). Conclusions: According to the present data, hysteroscopic evaluation of nulliparous women with unexplained infertility could be useful to detect lesions not diagnosed with other tests, and the treatment of these endocavitary lesions plays an important role in the diagnostic and therapeutic iter. The results show a pregnancy rate of 85% in the patients treated for endocavitary lesions, but 15% of the patients, despite a normal uterine cavity, continue to have unexplained infertility and are classified as idiopathic infertility.

Key words: Hysteroscopy; Infertility; Intrauterine abnormalities; Idiopathic infertility.

Introduction

The term infertility, replacing the word “sterility”, is indicated by the WHO (World Health Organization) as “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse”, in the presence of regular menstrual cycles [1]; it is the complete inability to conceive because of an obstacle to fertilization, due to male and/or female factors [2, 3]. The human species is considered one of the less fertile of the planet [2], since the chance of conception is to just 25% in the fertile period. To this is added the fact that 5-10% of couples need 12-24 months to obtain a pregnancy [2].

The diagnostic evaluation of infertility is conducted on both partners: must be carried out in a fast and complete fashion, to lighten the psychological overload to which the couple is subjected. At the present time the causes of infertility are represented by 30% from male problems, 15% by ovulatory failure, 10% by tubal pathology, and 25% by disorders of the uterine cavity, 15% by idiopathic infertility, and 5% by rare problems [2].

The diagnostic iter for fertile couples consists of two levels of screening: a first level, to assess clinical history and physical examination, and a second level of exams to assess the cause of infertility.

According to WHO and European Society of Reproduction and Embryology, hysteroscopy plays a secondary role in this iter, after hysterosalpingography and pelvic ultrasound, despite its well known and studied advantages in the study of the uterine cavity: higher diagnostic sensitivity and specificity, low cost, absence of exposure to radiations, and the possibility to perform a biopsy of a diagnosed lesion in the same session of the diagnostic procedure.

Studies [4, 5] show that hysteroscopy can be a complementary procedure when anomalies are detected with hysterosalpingography and pelvic ultrasound, as hysteroscopy is the gold standard in the diagnosis of endocavitary uterine lesions in patients with infertility. The direct vision of the uterine cavity allows the immediate detection of morphologic and functional anomalies, which can interfere with embryonic implantation and growth, and enables the operator to intervene and recreate the normal uterine environment [6, 7]. In addition, hysteroscopy is considered a more accurate diagnostic tool, compared with the high rates of false negatives and false positives observed with hysterosalpingography relative to the assessment of the uterine cavity [8-10]; for this reason, many diagnostic protocols of patients with infertility include hysteroscopy [11, 12].

Recent reviews [13, 14] point out that there is an open debate regarding the role of hysteroscopic surgery in the management of female infertility; as a benefit with the hysteroscopy, removal of uterine lesions has been reported in observational studies, but more randomised studies are needed to validate this procedure in women with unexplained infertility or prior to assisted reproduction tech-
The aim of this study was to evaluate the impact of diagnostic and operative hysteroscopy in the uterine assessment of patients with unexplained infertility in terms of diagnosis of otherwise not detected lesions and pregnancy rate after removal of the possible cause of infertility.

Materials and Methods

This retrospective pilot study was conducted at “Sapienza” University of Rome, in the Hysteroscopy Unit of the Department of Gynecology-Obstetrics and Urological Sciences. The authors reviewed the clinical charts of infertile, nulliparous women that underwent diagnostic hysteroscopy between January 2007 and December 2011, and the patients with uterine endocavitary lesions also underwent operative hysteroscopy in the same years.

Inclusion criteria were: unexplained infertility, nulliparous, female partner aged 28-44 years, normal hormonal blood tests (FSH, LH, PRL, TSH, FT3, FT4), absence of endocrine dysfunctions in both partners, absence of uterine lesions in both pelvic ultrasound and hysterosalpingography, bilateral tubal patency, normal karyotype of both partners, vaginal swap negative for bacterial infection and Chlamydia, and absence of male factor of infertility.

From January 2007 to December 2011, 1,875 patients underwent diagnostic hysteroscopy, and 633 of these were infertile. Among the 633 infertile patients, 92 met the inclusion criteria. All the diagnostic and operative hysteroscopy were performed by the same surgeon. Diagnostic hysteroscopy was carried out with a 2.7 mm hysteroscope in vaginoscopy, with saline solution as a distention media, under light sedation, and all removed lesions went to pathologic exam, which confirmed the diagnosis in all cases.

Among the studied patients in the two years following surgery in subgroups according to the removed uterine lesions and discovered that the pregnancy rate was different, as showed in Table 2.

Table 2. — Pregnancy rate after surgery, divided by type of uterine lesions.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Pregnant</th>
<th>Not pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial polyps</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Cervical polyps</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Uterine adhesions</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Submucous fibroids</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Uterine septa</td>
<td>19</td>
<td>5</td>
</tr>
</tbody>
</table>

Discussion

The assessment of the uterine cavity is one important step in the diagnostic iter of infertility. From this retrospective analysis emerged that in 79% of the cases of unexplained infertility, there was a missed diagnosis of an uterine lesion, despite the fact that every patient had a negative hysterosalpingography and pelvic ultrasound previous to diagnostic hysteroscopy: in these cases, hysteroscopy detected uterine lesions could be responsible for the infertility. The present authors report a rate of endometrial polyps (21%), submucous fibroids (18%), and uterine septa (25%), similar to the one reported in a recent study [15].

Regarding the pregnancy rate, the authors found that after the surgery for uterine submucous fibroids, uterine septa, and uterine polyps had respectively in 90%, 79%, and 78% of the cases a pregnancy, showing that the removal of lesions can interfere with the implantation of the embryo in the uterine cavity and can improve the pregnancy rate in this group of patients. The present findings are in line with the current literature [16-20], that shows that the pregnancy rate in patients with uterine submucous fibroids after removal improves significantly, demonstrating a role of the fibroids as a cause of infertility. The same results are obtained when the surgery is performed in patients with uterine polyps [21].

Regarding the correction of uterine septa, there are studies demonstrating the improvement of the pregnancy rate by 44-53% [22, 23], but there is still an open controversy, as most of the studies are conducted on patients with recurrent miscarriages and are not case-controlled studies, since they are performed with the same patients before and after surgery.
In this study, the authors did not collect data about the outcome of the pregnancy, because the heterogeneity of the lesions considered and the range of patients age did not allow simple conclusions about term pregnancy and miscarriage rate, and they reserve these data analysis to further studies.

The rate of patients which did not achieve a pregnancy (15%) is in line with the literature (10-16%), and these patients are classified with idiopathic infertility [2]. The incidence of idiopathic infertility should decrease if the diagnostic iter is improved, including new tests to identify all possible causes of infertility. For example, studies in women with abnormalities of certain proteins and genes (e.g. HOXA 10, HOXA 11, and Claudin-4) that could interfere with fertility are underway.

A study showed that the expression of the genes HOXA 10 and 11 in the endometrium could interfere with the implantation of the embryo and explain certain cases of idiopathic infertility [24]. Claudin 4 (CLDN-4) is a transmembrane protein, which was also observed in the endometrium. According to a study in 2013 [25], high levels of this protein may impair fertility.

Studies from Sharma et al. and Balakrishman et al. state that failure in obtaining a pregnancy, in the absence of disease, may depend on stress, as under stressful conditions progesterone turns into cortisol [26, 27], resulting in a decrease in the levels of progesterone in the body. At the same time, the prolactin level remains high because dopamine, the hormone of well being, decreases. The level of sex hormones thus lowers and this produces menstrual irregularities and anovulation. In humans, moreover, it would appear that exposure to certain endocrine disruptors (4-tert-octylphenol) would alter the functionality of sperm [28]. In the literature emerges that research on idiopathic infertility is still incomplete.

Conclusions

Infertility is, at this time, a condition that still leaves many interrogatives, and needs more validated scientific evidences. The use of standard guidelines is mandatory to identify a suitable diagnostic and therapeutic iter, to decrease the rate of idiopathic cases, and improve the pregnancy rate, and this can only be achieved with a multidisciplinary team work.

From the present study, the fundamental role played by hysteroscopy is clear in the diagnostic and therapeutic management of patients with otherwise unexplained infertility. Nevertheless, it is important to emphasize the need for new and improved diagnostic strategies to solve the problem of idiopathic infertility.

References


Address reprint requests to:
M. MONTI, M.D.
Department of Gynecological-Obstetric and Urological Sciences,
“Sapienza” University of Rome
Viale del Policlinico, 155
00161 Rome (Italy)
e-mail: marco.monti@uniroma1.it
Introduction

Hepatitis B virus (HBV) infection causes more than one million cirrhosis- and hepatocellular-carcinoma-related deaths every year. This infection is a problem in women of reproductive age [1, 2]. HBsAg-positive mothers transmit the virus to their infants primarily during the perinatal period. Receiving the virus in the neonatal period results in chronicity in many cases because the infant’s immune system is still immature [3-5]. In this period, the HBV DNA level and HBeAg-positivity are important for an infectious effect [4]. The risk of infection and resulting chronic hepatitis B (CHB) in the infants of HBeAg-positive mothers in developing countries are 90% [6]. On the other hand, the risk of contamination in the infant of an HBeAg-negative mother with HBV is 10–40%, and the risk of the development of CHB due to this contamination is 40–70% [4-6].

It is beyond dispute that routine vaccination of neonates against HBV can reduce the prevalence of HBV infections and HBV-related CHB, cirrhosis, and hepatocellular carcinoma [3]. The administration of hepatitis B immunoglobulin (HBIG) and hepatitis B vaccines to infants born to HBsAg-positive mothers within the first 12 hours is more than 90% protective against the occurrence of HBV infections ([7, 8]. Subsequently, it is important that all pregnant women are screened for HBV infection and those who are HBsAg-positive are assessed for treatment.

Treatment to the pregnant woman is intended to prevent the progression of liver damage, decrease the risk of hepatic exacerbation after delivery, reduce the risk of intrauterine contamination, and reduce HBV-related mortality. Therefore, hepatitis B treatment in pregnant women is an issue that should be assessed individually [3, 9]. Large-scale studies evaluating the care of pregnant women with CHB, beginning and discontinuation of their treatment indications, drug side effects, and prevention of transmission to their babies will be helpful when planning government policies.

In this study, 88 HBsAg positive pregnant women were assessed; 11 of them began treatment during their pregnancy and five became pregnant while receiving treatment. The authors would to stress that it is possible to protect HbsAg positive pregnant women and their babies and how important it is to closely monitor such pregnant women through appropriate care and treatment approaches.
Materials and Methods

Study population

The files of HBsAg-positive pregnant women who presented to the Infectious Diseases and Clinical Microbiology Outpatient Clinics of Adıyaman University Faculty of Medicine, Afyon Zubeyde Hanım Maternity Hospital, Konya Numune Hospital, Selçuk University Faculty of Medicine and between January 2010 and December 2013 were reviewed retrospectively.

The following information was recorded from each patient’s file: demographic data; gestational age; whether antiviral treatment was received before pregnancy and, if so, the duration of the treatment; whether antiviral treatment was started during pregnancy, the date it was started, and the antiviral used; HBV DNA levels before and during pregnancy; detailed hepatitis markers (HBsAg, HBeAg, antiHBe, antiHBcIgG, antiHCV, antiHDV, and antiHIV); and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values. The inclusion criteria comprised the following: age ≥ 18; absence of comorbid diseases; attended regular follow-ups from six to eight weeks of pregnancy; screened for syphilis, toxoplasmosis, herpes, rubella, and cytomegalovirus infections in early pregnancy; and absence of HCV, HDV, or HIV co-infections. The pregnant women included in the study were assessed in three groups: 1) those who were monitored without treatment, 2) those who began treatment during pregnancy, and 3) those who became pregnant during treatment. The postnatal files of the infants who received treatment during pregnancy were reviewed. The prophylaxis and vaccination programs administered to these infants were examined.

Ethics

This work was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Approval was obtained from the ethical committee of Selcuk University (2014/174). Written informed consent was obtained prior to the treatment from each participant.

Statistical analysis

The data were collected retrospectively for this descriptive study. Descriptive statistics, such as mean ± standard deviations and percentage distributions were used to evaluate the data.

Results

The mean age of the 88 pregnant women who were included in the study was 26.2 ± 5.5 (17–39) years. Of the 88 women, 72 (81.8%) did not receive treatment during pregnancy. Treatment was started during pregnancy for 11 (12.5%) women, and five (5.7%) became pregnant while receiving treatment. Of the 72 women who did not receive treatment during pregnancy, 55 were inactive carriers (ALT normal, HBV DNA < 10^4 copy/ml), and 17 were patients whose HBV DNA values were 10^4–10^6 copy/ml and ALT values were not more than twice the normal. Of the 11 patients who began treatment during pregnancy, four were at the immunotolerant phase (ALT normal, HBV DNA values > 10^6 copy/ml), and seven had HBV DNA values > 10^6 copy/ml and ALT more than twice the normal. The data of the 11 patients who started treatment are presented in Table 1. The monitoring of the pregnant women who began treatment showed that there was a noticeable decrease in the viral load and a drop in liver function tests. Of the five patients who became pregnant while receiving treatment (two lamivudine [LAM], two tenofovir disoproxil fumarate [TDF], and one entecavir [ETV]), the treatments of four women were discontinued and they were monitored during their pregnancies because mild-moderate (less than stage 3) fibrosis was found in their liver biopsy results. The data of the five patients who became pregnant at the onset of treatment are given in Table 2. Since the pre-treatment liver biopsy result of the pregnant woman who received ETV treatment was stage 4 and her histological activity index (HAI) was 13, her treatment was shifted to TDF, and she was advised to continue the treatment throughout her pregnancy. However, the woman opted to discontinue the treatment and was monitored by way of monthly follow-ups. The monitoring data of the pregnant women who became pregnant during treatment are presented in Table 2.
Monitoring and treatment results of 88 HBsAg-positive pregnant women

Table 3. — Follow-up values of patients who became pregnant while receiving treatment throughout their pregnancies.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Before pregnancy</th>
<th>Month 1</th>
<th>Month 3</th>
<th>Month 6</th>
<th>Before delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBV DNA (copy/ml)</td>
<td>ALT (u/L)</td>
<td>HBV DNA (copy/ml)</td>
<td>ALT (u/L)</td>
<td>HBV DNA (copy/ml)</td>
</tr>
<tr>
<td>34</td>
<td>Negative</td>
<td>23</td>
<td>531</td>
<td>24</td>
<td>4.8×10⁴</td>
</tr>
<tr>
<td>23</td>
<td>Negative</td>
<td>18</td>
<td>Negative</td>
<td>19</td>
<td>Negative</td>
</tr>
<tr>
<td>29</td>
<td>Negative</td>
<td>21</td>
<td>Negative</td>
<td>34</td>
<td>Negative</td>
</tr>
<tr>
<td>27</td>
<td>Negative</td>
<td>16</td>
<td>Negative</td>
<td>26</td>
<td>879</td>
</tr>
<tr>
<td>31</td>
<td>Negative</td>
<td>24</td>
<td>Negative</td>
<td>23</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table 4. — Data of the infants of the mothers who were started treatment during pregnancy.

<table>
<thead>
<tr>
<th>Baby number</th>
<th>Congenital anomaly</th>
<th>Birth weight (g)</th>
<th>Week of birth</th>
<th>AntiHBs value at week 12 (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>3,150</td>
<td>38</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>3,800</td>
<td>40</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>3,240</td>
<td>39</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>3,550</td>
<td>37</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>2,950</td>
<td>37</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>3,600</td>
<td>39</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>3,100</td>
<td>38</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>2,870</td>
<td>38</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>9</td>
<td>None</td>
<td>3,300</td>
<td>39</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td>3,450</td>
<td>38</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>11</td>
<td>Ex</td>
<td>1,350</td>
<td>22</td>
<td>-</td>
</tr>
</tbody>
</table>

Of the 11 patients who received treatment during pregnancy, seven underwent liver biopsies after delivery, fibrosis stage 3 was found in two patients, and fibrosis stage 2 was found in five patients. These women's treatments were continued, provided they agreed to forego breastfeeding. The other four patients who received treatment during pregnancy were in the immunotolerant phase. Because they were given treatment to prevent intrauterine transmission, these women's treatments were discontinued after delivery.

Of the 17 patients with HBV DNA values of 10⁴–10⁶ copy/ml and ALT values not more than twice the normal, 12 continued their follow-ups after delivery. Treatments were started after liver biopsies were done for eight of these patients. The biopsies were indicated according to the American Association for the Study of Liver Diseases (AASLD) criteria following the breastfeeding period. The other four patients were monitored, as liver biopsies were not indicated for them according to the AASLD criteria.

Of the infants of the 11 mothers who received treatment during pregnancy, ten were healthy and one was lost due to preterm birth. The data of these babies are presented in Table 4. Each baby was administered 200 IU of HBIG after birth and 20 µg of hepatitis B vaccine at zero, one, and six months. The physical examinations of the ten babies who returned for follow-up did not show pathology. The babies' anti-HBs values were ≥ 10 IU/ml.

Of the 72 HBsAg-positive patients, one miscarried at week 8 and one baby had Down syndrome. Of the 88 pregnant women, 29 had normal deliveries and 59 had cesarean sections.

Discussion

Approximately 5% of all women in the world are HBsAg-positive; the percentage is as low as 0.6% in regions with low endemicity and is as high as 20% in regions with high endemicity [10]. Although the risk of intrauterine transmission is low because the fetus is protected by the placenta, intrauterine transmission has been reported in the infants of mothers with high HBV DNA and/or HBeAg-positivity [5].

The decision whether to begin treatment in the HBsAg-positive pregnant woman is complex and requires consideration of both the woman's and the fetus' health. The decision should be supported by a combined assessment of many factors, such as the viral load, week of pregnancy, status of liver damage, and HBeAg-positivity. Studies have reported that when HBV DNA > 10⁸ copy/ml, the probability of intrauterine transmission increases to 43% [11]. This rate drops to 30% when HBV DNA < 10⁶ copy/ml, and decreases considerably when HBV DNA < 10⁴ copy/ml [11, 12]. For this reason, the recommended general approach is to treat pregnant women whose HBV DNA values are > 10⁶ copy/ml (HBeAg positive or negative) [9, 13-15]. The literature review showed that treatment was commenced for pregnant women usually when their HBV DNA values were > 10⁶ copy/ml [11, 16]. The authors also found that treatment was commenced for all of the 11 pregnant women whose HBV DNA > 10⁶ copy/ml (HBeAg positive or negative) in the present study. The other pregnant women were placed under follow-up throughout their pregnancies.

Antiviral drugs are effective against HBV contamination from mother to infant in pregnant women with heavy viral loads [5, 13, 16]. Of the agents to treat chronic hepatitis B in pregnant women, LAM and ETV are rated C, and TDF and telbivudine (LdT) are rated B by the FDA for safety during pregnancy. LAM, an antiviral drug with a low genetic barrier, has been used most frequently for the treatment of pregnant women with CHB [5, 17]. TDF is a safe...
drug considering its high genetic barrier and low resistance; thus, its use in pregnant women has increased in recent years. LdT has a low genetic barrier, although it prevents contamination to the infant fairly well. There are limited data on the use of ETV in pregnant women [16, 18].

Many studies have been carried out on the use of these drugs. Yi et al. [19] reported that LAM therapy was safe and effective in pregnant women. Liu et al. [20], You et al. [21], and Han et al. [10] preferred LdT therapy. Celen et al. [22] preferred TDF therapy. The authors found in the present study that nine pregnant women received TDF and two received LAM therapy.

The treatment initiation time is an important factor among pregnant patients. Studies have shown that the antiviral therapy should begin in the third trimester (weeks 28–32 of pregnancy) [8, 12-14]. However, the results have not been much different from those of the general population in the studies investigating the risk of birth defects from LAM or TDF (2.9% and 2.4%, respectively) [10, 12, 19]. The present authors found that the initiation of treatment for pregnant women was in line with the literature; however, treatment was commenced for one woman in week 16 because her previous child was HBsAg-positive. No major anomalies were found in the babies of these patients.

There is no standard guideline for managing HBsAg-positive women who become pregnant while receiving treatment. The decision to continue the treatment must be made dependent on factors such as previous liver biopsy results, viral loads, liver function tests, and HBeAg-positivity. Treatment continuation is recommended throughout pregnancy in the presence of serious fibrosis [23, 24]. If the pregnant woman is under control for CHB (HBV DNA negative, ALT normal), and there is mild/moderate fibrosis in her liver, she can be monitored without treatment. The HBV DNA and liver function tests of the pregnant women whose treatments are discontinued should be monitored closely for exacerbations that may occur during pregnancy. These patients should also be assessed for resuming treatment, if necessary [19, 25]. Kim et al. [25] investigated 12 pregnant women whose treatments were discontinued because they became pregnant. They observed viral rebound in some of the patients and severe hepatic flares in others after the discontinuation of the treatment. In the present study, the treatments were discontinued in four patients who became pregnant, and further appropriate antiviral therapy was proposed to a patient whose biopsy result showed advanced fibrosis. In the follow-ups of four patients whose treatments were discontinued and one patient who refused treatment, no indications were found for beginning treatment.

The post-delivery follow-ups and breastfeeding periods are as important as the monitoring carried out during pregnancy in HBsAg-positive pregnant women. The treatments of pregnant women who have post-delivery liver biopsy indications should be designed according to their biopsy results. If a mild fibrosis is found in the liver, the treatment can be stopped in the first month after delivery. However, if there is advanced fibrosis, continuation of the treatment is recommended. In cases where the treatment is continued, breastfeeding should be avoided [8, 23]. The present authors found that seven of 11 patients who received treatment during pregnancy were administered biopsies after pregnancy. The other four patients were in the immunotolerant phase, thus their treatments were discontinued at one month after delivery and they were placed under follow-up.

The progress of HBV infection is similar in pregnant and non-pregnant women [24]. Some studies have reported fetal complications, such as preterm births, stillbirths, and miscarriages [26]. Celen et al. [22] reported no difference in fetal complications between the groups receiving and not receiving treatment. The present authors found that one of the HBsAg-positive pregnant women had an eight-week miscarriage, one infant had Down syndrome, and one infant in the treatment group died due to premature birth. These observations were not different from the normal population.

Cesarean section is not superior to normal delivery in HBsAg-positive pregnant women [10, 27]. Han et al. [10] found that the rate of cesarean section was high in HBsAg-positive pregnant women. The present authors found that 29 of the pregnant women had normal deliveries and 59 had cesarean sections. Thirty-nine of these patients had deliveries by cesarean section due to their previous cesarean section histories, one due to preterm delivery (at week 22) and 19 due to reasons such as early membrane rupture.

The major limitation of the present study was that it was retrospective; therefore, the authors could not reach many of the infants of pregnant women who had been monitored before the onset of treatment. Hence, they could not compare the infants of the mothers who received and did not receive treatment.

In conclusion, because hepatitis B caught in the intrauterine or early childhood period has a high rate of chronicity, it is important to screen all pregnant women for hepatitis B and to assess those found HBsAg-positive. It is possible to protect both the mother and baby using appropriate approaches.

References

Monitoring and treatment results of 88 HBsAg-positive pregnant women


Address reprint requests to:
S. KOLGELIER, M.D.
Adıyaman University, Faculty of Medicine
Department of Infectious Diseases and Clinical Microbiology
Atatürk Bulvarı No:411
02100 Adıyaman (Turkey)
e-mail: servetkolgelier@hotmail.com
Clinical analysis of 95 cases with ovarian pregnancy

A.W. Le, Z.H. Wang, L. Shan, X.Y. Dai, T.H. Xiao, X.R. Li

1 Department of Obstetrics and Gynecology, Nanshan Affiliated Hospital of Guangdong Medical College, Shenzhen (China)

Summary

Background: Ovarian pregnancy (OP) is a rare ectopic pregnancy, in which it is very difficult to achieve preoperative diagnosis. Ovarian blood supply in OP increases which will lead to intra-abdominal bleeding, hazarding women’s lives. Surgical exploration should be conducted once cases of OP are suspected. Objective: To investigate clinical characteristics, diagnosis, and therapy of OP. Materials and Methods: A retrospective study was conducted in 95 patients with OP admitted to the present hospital from January 2005 to June 2014. Results: OP accounted for 1.79% of ectopic pregnancy over the same period, of which 68.4% had a history of artificial abortion, 6.3% was treated with intrauterine contraceptive device (IUD), 87.4% had abdominal pain, 84.2% had a history of menopause, and 51.6% had vaginal bleeding. All patients had no preoperative diagnosis and underwent laparoscopic wedge resection of ovary or lesionectomy and were all were cured. Conclusion: Since the cause of OP is still unknown and it has no typical clinical manifestations, the present authors adopt blood β-hCG combined with B-ultrasound to improve the preoperative diagnosis. They prefer laparoscopic wedge resection of ovary or lesionectomy, which induce higher rate of intrauterine pregnancy and lower rates of ectopic pregnancy and infertility in re-pregnancy after surgery.

Key words: Ovarian pregnancy; Clinical characteristics; Wedge resection of ovary.

Introduction

Ovarian pregnancy (OP) is a rare kind of ectopic pregnancy with natural incidence rate of about 1 / 15,000 to 50,000, which accounts for 0.15%~3.0% of ectopic pregnancies [1]. With the development of some birth control measures and assisted reproductive technologies, incidence of OP presents an increasing tendency. Since OP’s symptoms and signs are similar to those of tubal pregnancy, it is very difficult to achieve a preoperative diagnosis. However, ovarian blood supply in OP will increase which leads to intra-abdominal bleeding, hazarding women’s lives. Incidence of primary OP in patients with in vitro fertilization (IVF) is 0.3% [2]. This paper presents a retrospective analysis conducted in 95 patients with OP in the present hospital over a ten-year period, with the aim to improve awareness of the disease by analyzing the clinical data and exploring the diagnosis and treatment characteristics.

Materials and Methods

A total of 5,293 cases with ectopic pregnancy were admitted to the present hospital from January 2005 to June 2014, among which there were 95 cases with OP, accounting for 1.79%.

Clinical characteristics of the patients were the following: 1) Age range from 20 to 41 years old, (mean 27.6 ± 4.6). 2) Disease histories: 11 cases of first pregnancy, 25 cases of second pregnancy, 31 cases of third pregnancy, 23 cases of fourth pregnancy, five cases of five or more pregnancies, and one case of IVF-ET. Six cases were utilizing IUD contraception and one case had bilateral tubal ligation. Sixty-five cases had histories of artificial abortion. Thirty-six cases had history of endometriosis and eight cases had received promoting ovulation drug. 3) History of menopause: 15 cases had no history of menopause and 80 cases had history of menopause, where menopause days ranged from 29 to 62 days (mean 45.6 ± 17.8). 4) Belly ache and vaginal bleeding occurred in 83 cases and 49 cases had vaginal bleeding.; 5) Twenty-two cases had intra-abdominal hemorrhage more than or equal to 800 ml and 16 cases had unstable preoperative signs and required intraoperative transfusion of packed red blood cells or other plasma products. Intraoperative ruptured and ruptured ovaries were found in 43 cases and 52 cases, respectively. 6) B-ultrasound examination was performed in 95 cases and all presented ectopic pregnancy, of which 72 cases had ascites with maximum depth of 85 mm (mean 26.7 ± 15.5). 7) Colpoceoliotomia posterior was performed in 65 cases, of which 56 cases had extracted 2-10 ml incoagulable blood. 8) Human chorionic gonadotropin (hCG) measurement was taken as preoperative routine testing of blood and were positive, with minimum value 124.8 mIU/ml, maximum value 41,337.6 mIU/ml and mean value of 7,134.7 ± 986.6 mIU/ml. 9) Preoperative diagnosis: all were considered ectopic pregnancy. 10) Surgery and treatment: all patients received laparoscopic ovarian wedge resection or lesionectomy.

Diagnosis was made according to criteria proposed by Spiegelberg [3]: 1) Ipsilateral fallopian tube and its umbrella end are intact and are not adhered to the ovary. 2) Embryo sac is in the ovarian tissue. 3) Embryo sac and ovary are connected to the uterus through ovarian ligament. 4) There are ovarian tissues in the embryo sac wall, confirmed histologically.

Statistical analysis

Software SPSS 16.0 was utilized for statistical analyses. The measurements are presented as mean ± SD and compared by variance analysis. The significance of between-group differences was tested using analysis of variance or chi square tests. Values of
Results

All patients underwent laparoscopic ovarian wedge resection or lesionectomy. Examinations showed active bleeding in the rupture of ovarian surface in 23 patients, and hemoperitoneum in all patients with bleeding of 50–2,300 ml and mean of 682.7 ± 478.6 ml. Among the 95 patients, there were 58 cases with left OP and 37 cases with right OP. Intraoperative observation showed that nine cases were accompanied with pelvic endometriosis, 11 cases were accompanied with chronic pelvic inflammatory disease, and 51 cases presented pregnant corpus luteum, of which 43 cases were ipsilateral OP corpus luteum and eight cases were contralateral OP corpus luteum. One case with natural pregnancy had three gestational sacs in one ovary (Figure 1).

All surgical specimens were sent for pathological examination and were diagnosed as OP. All patients were cured and discharged from the hospital. The blood β-hCG reduced to normal level from two to four weeks after surgery.

Follow-up results: 42 of 95 cases were conducted three-year follow-up; 26 cases were pregnant at one year after surgery, of which 23 cases had intrauterine pregnancy (one case was preoperatively diagnosed as infertility) and three cases had tubal pregnancy (one case was preoperatively diagnosed as infertility). Four cases were pregnant at three years after surgery, of which two cases were diagnosed as secondary infertility. Ten cases were diagnosed as infertility prior to surgery and were not pregnant as well after surgery.

Discussion

Pathogenesis and etiology

OP is a rare gynecological acute abdomen with primary symptoms of abdominal pain. OP pathogenesis is still unclear and it may be related to the following factors: 1) It mainly occurs in women who have given birth, and it is associated with uterine cavity operation, pelvic inflammatory disease, and abdominal surgery [4]. Uterine cavity operation can cause intrauterine environment changes, such as intimal injury, which is not conductive to implantation and growth of fertilized eggs and easily leads to ectopic pregnancy. Pelvic inflammatory disease, abdominal surgery or endometriosis are likely to induce ovarian inflammation and ovulation disorders, which results in the eggs remaining within the ruptured follicle and get fertilized in the ovary. Many patients in this group had given birth, had histories of uterine cavity operation or pelvic surgery, which were likely to support these ideas. Studies [2, 3, 4] showed that pelvic inflammatory diseases, adverse intrauterine environment, endocrine disorders, and other factors may prevent discharge of the eggs from the ovary and the eggs may adhere to the ovarian surface. Then sperms may get into the surface of ovarian follicle and complete fertilization. In this study, the authors found in eight cases that the pregnant corpus luteum was located at the contralateral embryo, indicating that the fertilized eggs out-bound and implant in the contralateral ovary with unknown mechanism. Among the 95 cases, there were 58 cases with left OP and 37 cases with right OP, indicating that the incidence of left OP is significantly higher than that of the right OP, which is consistent with the previous reports with unknown reasons [5]. 2) It is associated with IUD. Sandvei et al. [6] indicated that the occurrence of OP in patients with IUD insertion is six times that of patients without IUD insertion, and its mechanism may be because IUD has a blocking effect on intrauterine pregnancy and tubal pregnancy, but with no blocking effect on OP. It has been reported that the blocking rates of IUD on intrauterine pregnancy and tubal pregnancy are 99.5% and 95%, respectively, and it has no effect on OP [7]. Studies [7] revealed that IUD can promote the secretion of prostaglandins, causing reverse peristalsis of tubal and resulting in fertilized eggs retrograde and implantation in the ovary. Meanwhile, prostaglandins may lead to dysfunction of tubal picking up eggs which further increase risks of OP. It is still controversial whether application of IUD will induce higher incidence of OP [8], although most of researchers believed that IUD insertion results in higher incidence of OP [9]. Six patients in the present study had IUD insertion, yet they could not be proven to be associated with OP. 3) Irregular maturation of follicle and ovulation failure. Relatively lower liquid pressure inside the follicle can cause ovulation disorders which results in eggs remaining in the ruptured follicle and become fertilized in...
the ovary. Some researchers believed that irregular maturation of follicle during ovulation process may also result in OP [10]. The possible causes may be as follows: first, excessive estrogen leads to tubal function changes, ovulation induction drugs can result in multiple fertilized eggs and fertilized eggs may remain in the tubal during the implantation in the uterine cavity which increases the opportunity of reverse peristalsis to ovary, promoting the incidence of OP. Second, rather than being ingested into the fimbria tubae uterinae, eggs adhere to ovarian surface and fertilized after discharge from the follicle or the eggs are not discharged from the ruptured follicle and complete follicular fertilization. 4) In recent years, incidence of OP increased with the popularization and application of assisted reproductive technologies. One case in the present study had received IVF-ET. Incidence of ectopic pregnancy after IVF-ET was significantly higher than that of natural pregnancy, which is not only associated with histories of pelvic inflammatory disease, chronic salpingitis, endometriosis and operation on fallopian tube, but also related to type of embryo transplantation tube, depth into the uterine cavity, liquid volume in the tube, injection pressure, number of implanted embryos, patient’s position after transplantation, development synchronization of embryo, endometrium and conditions of uterine tubal, etc [11].

**Diagnosis**

Due to lack of specific clinical manifestations and signs, diagnosis of OP is very difficult in early stage, and its diagnosis mainly relies on laparotomy or laparoscopy. Patients in this study had symptoms of abdominal pain, history of menopause, and vaginal bleeding with average volume of 682.7 ± 478.6 ml, indicating serious harm of OP. Therefore, early diagnosis of OP can avoid severe abdominal bleeding [12]. Some patients had no history of menopause and their abdominal pain occurred between 19 to 32 days of the menstrual cycle, which can be easily misdiagnosed as ruptured corpus luteum. In terms of imaging, theoretically, unruptured follicle may appear as an ovarian round-shaped gestational sac with thick wall, yolk sac, and embryo inside the sac by using B-ultrasound. For cases of embryo implantation in the ovarian surface, B-ultrasound may present abnormal sonographic protrusion and connection to the ovarian capsule. For cases of tubal pregnancy, B-ultrasound shows clear ovary echo and tubular structure of tuba next to the lesions [13]. Chang et al. [14, 15] believed that cases with gestational sac revealed in the ovarian examination by B-ultrasound can be diagnosed as OP, as well as cases with echo ball around the corpus luteum. Changes in ultrasound signs of OP are as follows: cystic mass containing part solid tissues, mixed adnexal mass accompanied by peritoneal free fluid, adnexal gestational sac, adnexal echogenic cystic mass, and gestational sac or embryo in the ovary. Foreign researchers believed that an early application of low and slow hCG combined with B-ultrasound and increased awareness are of assistance for early diagnosis of the disease [16]. Comstock et al. [17] believed that ovary has characteristics of sufficient blood supply, lack of muscle tissue, and crispy texture which causes it to easily break, generating blood clots which mixes with pregnant products, ovaries, and tuba and results in blurred ovarian contour and inaccurate positioning. Preoperative diagnosis of patients in the present study all had an ectopic pregnancy.

**Therapy**

Patients in this study all suffered from abdominal bleeding, of which 36 cases had bleeding with a volume of ≥ 800 ml, 19 cases appeared with shock symptoms, which is associated with sufficient blood supply in ovary as well as increasing vascular number during pregnancy. Therefore, surgical exploration should be conducted once cases are suspected with an OP. The first choice is the laparoscopic surgery even if there is intra-abdominal hemorrhage. The surgical method includes removal of the gestational sac while maintaining the normal ovarian tissue as much as possible. After surgery, hCG assessment is required until it returns to normal level. After surgical therapy, most patients can conceive naturally, while very few patients may have recurrence of ectopic pregnancy or infertility [18]. For patients who want to conceive, particularly for those undergoing IVT treatment, if any tubal lesions are found during surgery, it is better to treat them as well, otherwise OP may still occur after the next embryo transplantation if the ipsilateral tubal is kept intact. Furthermore, bilateral salpingectomy may be conducted when necessary. However, salpingectomy can affect ovarian blood supply and needs comprehensive consideration. In addition, patients with abnormal fallopian tube shape can be conducted with tubal ligation if there is a high risk of ectopic pregnancy or there is a history of ectopic pregnancy.

Reports have been found on conservative medical treatment of OP in recent years, but most are case reports. For example, Cabero et al. [19] applied intravenous MTX. Mittal et al. [20] adopted intracapsular injection of MTX using laparoscopy. However, Bagga et al. [21] reported that even if OP is in agreement with medical treatment of ectopic pregnancy, there still may be some failed cases and may require surgery. The American Society for Reproductive Medicine Practice Committee recommends that MTX is not the preferred method for treatment of OP and OP should be confirmed by surgery [22]. Ghi et al. [23] believed that MTX is not the preferred method for treatment of OP. The risk of ectopic pregnancy in re-pregnancy after drug therapy is seven to 13 times greater. The rate of intrauterine pregnancy in re-pregnancy is about 50% to 80%, the rate of tubal pregnancy is 10%~25%, and the remaining include infertility [24].

**Re-pregnancy results**

Pregnancy prognosis was retrospectively conducted in

---

*Clinical analysis of 95 cases with ovarian pregnancy*
42 cases with OP, and the results were considered to be reasonable. Lower rates of ectopic pregnancy and infertility were achieved in re-pregnancy, indicating that the results were not poor compared with other types of ectopic pregnancy, which is consistent with the literature results [18].

In summary, preoperative diagnosis of OP is very difficult due to lack of typical clinical manifestations, relatively low incidence, and lack of exact risk factors. Patients with a history of uterine or pelvic surgery, IUD insertion, endometriosis, ovulation drug use, abdominal effusion by ultrasound, and enlarged ovary should be highly suspected to be OP and treated with laparoscopic surgery, which induces better prognosis of re-pregnancy.

References


Address reprint requests to:
Z.H. WANG, M.D.
Nanshan Affiliated Hospital of Guangdong Medical College
89 Tao Yuan Road
Shenzhen 518000 (China)
e-mail: leaiwen@126.com
Effects of estradiol injection on outcome of in-vitro fertilization: a randomized, double-blind, placebo controlled trial

A. Samsami, A. Zarei, S. Shahrivar

Department of Obstetrics and Gynecology, Infertility Research Center, Shiraz University of Medical Science, Shiraz (Iran)

Summary

Purpose: To evaluate the effects of estradiol (E2) supplementation on pregnancy outcome in patients with unexplained infertility undergoing in vitro fertilization (IVF). Materials and Methods: A total of 100 women with unexplained infertility and candidates for IVF, were included in this study and were randomly assigned to receive E2 supplementation or placebo during the luteal phase. The E2 serum levels in the hCG administration day and third and seventh day after ovum retrieval were measured in control group. The rate of pregnancy was also quantified and compared between the two study groups. Results: There was no significant difference between two study groups regarding baseline characteristics. E2 level decreased significantly in third (1765.34 ± 680.09; \( p < 0.001 \)) and seventh (1459.66 ± 593.80; \( p < 0.001 \)) days after ovum retrieval (2411.16 ± 713.52). The authors found that the serum level of E2 was significantly lower in those who received E2 supplementation at day 3 (\( p < 0.001 \)) and 7 (\( p < 0.001 \)). However the pregnancy rate was not significantly different between two study groups (\( p = 0.849 \)). In the same way, there was no significant difference between two study groups regarding the number of retrieved oocytes (\( p = 0.563 \)) and number of MII oocytes (\( p = 0.103 \)). Conclusions: E2 supplementation during the luteal phase in patients with unexplained infertility undergoing IVF, is associated with decreased serum levels of E2 after hCG injection. However the fertility outcome was not affected by E2 supplementation.

Key words: Estradiol (E2); Infertility; In vitro fertilization (IVF); Luteal phase support; Pregnancy rate.

Introduction

Insufficiency of estradiol (E2) and progesterone after ovulation is referred to luteal phase deficiency, which is a common etiology of failure of assisted reproduction techniques [1]. Increased secretion of the steroid in early luteal phase results in luteinizing hormone (LH) inhibition which in turn decreases the secretion of E2 and progesterone resulting in luteal phase deficiency [2]. In assisted reproduction techniques, hormonal support is necessary for preventing luteal phase deficiency. Implantation is influenced by both the endometrium and the embryo itself. Endometrial capacity for implantation can be decreased due to decreased hormone production of ovaries and corpus luteum during luteal phase of induced cycles [3]. Without hormonal support, the decreased serum levels of E2 and progesterone result in decrease in the implantation and pregnancy rates [4-7]. Thus it is believed that luteal phase hormonal support increases the success rate of the assisted reproduction techniques.

Although the luteal phase hormonal support for alleviating the assisted reproduction techniques is proved, there is however controversy regarding the agents to be used for luteal phase support in stimulated cycles [8-10]. Previous reports have clearly shown that supplementation of progesterone and human chorionic gonadotropin (hCG) increase the implantation and pregnancy rates [11-14]. However there is still no consensus regarding the use of E2 for luteal phase support, as results of the studies is conflicting. Although some studies have shown favorable results with administration of E2 as luteal phase support in induced cycles [6, 15], some authors barely reported beneficial effects [16, 17]. The latter two studies indicated that their sample size population was not appropriate to draw any conclusions, thus their results are interpreted as pilot studies. The present authors therefore designed this randomized clinical trial in order to determine the effects of E2 supplementation on implantation and pregnancy rates in induced cycles for in vitro fertilization (IVF).

Materials and Methods

Study population

This was a randomized, double-blind, placebo controlled clinical trial performed in Qadir Mother and Child Hospital, a tertiary healthcare center affiliated with Shiraz University of Medical Sciences during a 12-month period from July 2012 to March 2013. The study protocol was approved by institutional review board (IRB) and medical ethics committee of Shiraz University of Med-

Revised manuscript accepted for publication September 30, 2015

A total number of 100 patients fulfilled the study criteria and were further included in the trial. The patients were randomly assigned to two study groups using a computerized random digit generator based on their registration number in order of referral. Those assigned to E2 group received oral E2 of four mg/d for two weeks after receiving IVF. Those assigned to control group received placebo with the same frequency.

Study protocol
All the patients underwent a complete history evaluation and physical examination by the attending gynecologist who was blinded to the study. The patients were scheduled for IVF/ICSI and E2 level was measured before oocyte pick up. All the patients underwent controlled ovarian hyperstimulation (COH) with standard long protocol. Primary sampling was done during COH cycle with hCG administration. After that, a second sampling was done at the third day after ovum retrieval - the day in which the patient admitted for embryo transfer to uterus. The third sampling was done seven days after ovum retrieval by going to the patient’s residence. Then the samples were transferred to the laboratory of Mother and Child Ghadir Hospital regarding the sample handling principles pointed out in protocols of lab kits of E2 level measurement, and the E2 level was quantified in these samples by use of the mentioned kits. All the measurements were performed using ELISA technique.

Follow-up and outcome measurement
All the patients were followed and the pregnancy test was requested two weeks after the ET. Pregnancy was documented by transvaginal sonography, at three weeks of gestation after obtaining a positive pregnancy test. Main outcome measurements were

Figure 1. — CONSORT flow diagram of the study.
Effects of estradiol injection on outcome of in-vitro fertilization: a randomized, double-blind, placebo controlled trial

estradiol supplementation. Infertility undergoing IVF with (n=50) or without (n=50) estradiol supplementation.

Table 1. — Baseline characteristics of 100 patients with infertility undergoing IVF with (n=50) or without (n=50) estradiol supplementation.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Estradiol group (n=50)</th>
<th>Placebo group (n=50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.4 ± 2.3</td>
<td>26.1 ± 2.8</td>
<td>0.356</td>
</tr>
<tr>
<td>Infertility duration (years)</td>
<td>5.54 ± 2.7</td>
<td>5.35 ± 1.9</td>
<td>0.125</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.1 ± 11.4</td>
<td>67.1 ± 7.8</td>
<td>0.022</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.2 ± 4.5</td>
<td>161.7 ± 2.3</td>
<td>0.395</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 ± 4.4</td>
<td>25.6 ± 3.2</td>
<td>0.047</td>
</tr>
<tr>
<td>Day 3 FSH (mIU/ml)</td>
<td>7.4 ± 6.5</td>
<td>7.3 ± 5.6</td>
<td>0.473</td>
</tr>
<tr>
<td>Day 3 LH (mIU/ml)</td>
<td>7.2 ± 3.8</td>
<td>6.8 ± 2.5</td>
<td>0.185</td>
</tr>
<tr>
<td>TSH (µg/dl)</td>
<td>3.6 ± 1.8</td>
<td>3.9 ± 2.1</td>
<td>0.215</td>
</tr>
<tr>
<td>Prolactin (mg/dl)</td>
<td>15.7 ± 6.7</td>
<td>15.4 ± 5.8</td>
<td>0.306</td>
</tr>
<tr>
<td>Basal E2 level (pg/ml)</td>
<td>40.8 ± 11.6</td>
<td>41.9 ± 9.6</td>
<td>0.652</td>
</tr>
</tbody>
</table>

BMI: body mass index; LH: luteinizing hormone; TSH: thyroid stimulating hormone.

BMI: body mass index; LH: luteinizing hormone; TSH: thyroid stimulating hormone.

Table 2. — The study outcome in 100 patients with infertility undergoing IVF with (n=50) or without (n=50) estradiol supplementation.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Estradiol group (n=50)</th>
<th>Placebo group (n=50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol level (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On hCG injection day</td>
<td>2411.1 ± 713.5</td>
<td>2532.6 ± 957.1</td>
<td>0.251</td>
</tr>
<tr>
<td>On day 3</td>
<td>1765.3 ± 680.9</td>
<td>2371.5 ± 567.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>On day 7</td>
<td>1459.6 ± 598.8</td>
<td>2311.8 ± 485.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>9.6 ± 1.3</td>
<td>9.7 ± 1.5</td>
<td>0.998</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>10.6 ± 2.3</td>
<td>9.3 ± 1.1</td>
<td>0.563</td>
</tr>
<tr>
<td>Number of MII oocytes</td>
<td>8.1 ± 0.8</td>
<td>6.9 ± 0.7</td>
<td>0.103</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>12 (24.0%)</td>
<td>13 (26.0%)</td>
<td>0.849</td>
</tr>
</tbody>
</table>

Statistical analysis

Based on 85% power and with α coefficient 0.05 to detect significant differences between corresponding variables (p = 0.05, two-sided), 43 patients were required for each study group. For compensating for non-evaluable patients, the authors included 50 patients in each study group. The statistical software package SPSS, version 16.0 was used for data analysis. The paired t-test was used to compare results within groups, the independent t-test to compare results between the groups, and the χ² test to compare proportions. Data were reported as mean ± SD. A p-value less than 0.05 was considered significant.

Results

Overall 108 patients were screened for eligibility out of whom 100 fulfilled the study criteria and were randomly assigned to two study groups (each containing 50 patients). None of the patients were lost to follow-up and all of them finished the study. Thus the final number of patients that were included in the final analysis was 100 (Figure 1). The baseline characteristics of the patients were comparable between two study groups. The baseline characteristics are summarized in Table 1. There was no significant difference between two study groups regarding the baseline characteristics.

Basal E2 levels were comparable between two study groups (p = 0.652). In the same way, the day of stimulation was not significantly different between two study groups (Table 2). The authors also found that the E2 level at day of hCG injection was comparable between two study groups (p = 0.251). The serum level of E2 decreased significantly in study group on day 3 (1765.3 ± 680.9; p < 0.001) and 7 (1459.6 ± 598.8; p < 0.001), when compared to baseline. However the changes on day 3 (2371.5 ± 567.9; p = 0.288) and 7 (2311.8 ± 485.3; p = 0.196) were not significant in control group. The authors found that the serum level of E2 was significantly lower in those who received E2 supplementation on day 3 (p < 0.001) and 7 (p < 0.001). However the pregnancy rate was not significantly different between two study groups (p = 0.849). In the same way, there was no significant difference between two study groups regarding the number of retrieved oocytes (p = 0.563) and number of MII oocytes (p = 0.103).

Discussion

Estrogen administration in follicular phase can improve endometrium preparation, but its role in the luteal phase is still controversial [18-20]. In unsuccessful cycles, the late luteal E2 levels decline which may compromise peri-implantation endometrial developments [21]. Vlahose et al. found that the addition of estrogen to progesterone in the luteal phase can increase L-selectin ligands – adhesion molecule in the endometrium during implantation [22]. All the aforementioned data raise the speculation about a potential positive correlation between luteal phase E2 levels and pregnancy outcomes. In this study, in order to evaluate the relationship between serum E2 levels and clinical pregnancy rates after IVF cycles, E2 serum levels were measured in three distinct periods of time which consisted of the hCG administration day, the third and the seventh day of ovum retrieval of patients undergoing IVF. The authors found that the E2 supplementation was associated with sig-
significant decrease in E2 levels after hCG injection when compared to placebo. However the clinical pregnancy rate and number of retrieved oocytes were not affected by the E2 supplementation, which is consistent with previous studies [23, 24].

The results showed that E2 serum levels in hCG administration day and the third and seventh day after ovum retrieval in these patients were noticeably decreased. There are many studies that reported a decline in E2 levels on the hCG administration day and on the sixth day after ovum retrieval (12 to 16), much like what was observed in this study. In order to evaluate the effects of E2 serum levels on clinical pregnancy rates in patients with infertility due to unknown etiologies or polycystic ovarian syndrome (PCOS), the decline in E2 levels in studied times was compensated by administering E2 to the patients in E2 group (n=50); then pregnancy rate was compared between control group and E2 group. The results showed that the increase in serum E2 levels did not have any effects on pregnancy rates and outcome of IVF/ICSI treatments. These results are in accordance with former studies and meta-analyses; including the studies performed by Gelbaya et al. and Papageorgiou et al. that assessed the relation between hCG administration day E2 levels and success of IVF cycles in producing pregnancy, but similar to the present study, could not find any positive relations between E2 levels and pregnancy rates [5, 25].

Histological data from endometrial biopsies of patients undergoing IVF on days 21 and 25 of cycles has shown that omitting the E2 injections from treatment cycles does not have any effects on unity of uterine endometrium, estrogen receptor numbers, and hormone profile of these patients. In these studies, it has been suggested that perhaps the high physiologic levels of progesterone compensates for loss of E2 during the midluteal phase and masks its effects on unity of endometrium, embryo implantation, and as a result the outcome of IVF [24]. In addition, it has been suggested that since the optimal dose of E2 administration during the luteal phase is still not determined and is of question, perhaps the reason for controversial results on this subject is due to ineffectiveness of E2 supporting doses during the luteal phase [5]. In a properly designed clinical study, different doses of E2 (0, 2 or 6 milligrams daily) were administered to patients already receiving six milligrams of progesterone daily. In this study, the patients who received low doses of E2 had significantly more implantation and pregnancy rates in comparison with those who received high dose or no E2. Furthermore, different studies use different methods to add E2 to the regimen of their patients. These methods include oral, transdermal, and vaginal administration of E2. That is why there is no general agreement on effective and optimal dose of E2 and the period of its consumption in each of these methods; this interferes with evaluation of the real effects of E2 on clinical pregnancy rates in IVF cycles [26, 27].

In other studies, evaluation of effects of E2 administration during the midluteal phase of IVF cycles, only in patients with very low serum E2 levels has been performed; but even in these studies, since determination of decreased E2 levels required multiple samplings, the time needed for E2 administration period and compensation of its loss in these patients was missed and subsequent evaluation of compensatory effects of E2 on outcome of IVF cycles was inaccurate [5].

Finally, it should be considered that although in this study the administration of E2 during the mid-luteal phase showed no significant effects in improvement of embryo implantation and pregnancy rate outcome during IVF cycles, further frequent and accurate studies are required in this field.

Conclusion

In conclusion, E2 supplementation during the luteal phase in women with unexplained infertility undergoing IVF, is associated with decreased serum levels of E2 after hCG injection. However the fertility outcome was not affected by E2 supplementation.

Acknowledgment

The authors would like to acknowledge all the patients and their families who patiently participated in the study. The authors would also like to appreciate the assistance of Dr. Fariborz Ghaffarpasand in editing the manuscript. The study was financially supported by a grant from Infertility Research Center of Shiraz University of Medical Sciences. This article is derived from Dr. Shahrivar’s thesis for partial fulfillment of the requirements for the degree of specialty in obstetrics and gynecology.

References


Address reprint requests to:
S. SHAHRIVAR, M.D.
Obstetrician and Gynecologist
Infertility Research Center
Qadir Mother and Child Hospital
Khalij Fars Avenue
Quran Gate, Shiraz (Iran)
e-mail: shahrivars1385@gmail.com
Using the LigaSure vessel sealing device in the large uterus at laparoscopic hysterectomy

M. Biçer¹, Z. Güner¹, C. Karas¹, A. Güclü², Mert Göl³
¹ Izmir University Medical School, Department of Obstetrics & Gynecology, Izmir
² Egepol Hospital, Department of Obstetrics & Gynecology, Izmir
³ Near East University, Faculty of Medicine, Obstetrics & Gynecology Department, Lefkosa-TRNC, Mersin (Turkey)

Summary

Objective: To compare intraoperative hemorrhage and other operative parameters between patients with large and small weighted uterus who underwent laparoscopic hysterectomy (LH). Materials and Methods: Forty-six patients intending to have LH were divided into two groups according to uterine weight (group 1 > 300 grams vs. group 2 < 299 grams). Intraoperative blood loss, operating time, perioperative complications, and duration of hospitalization were compared. Results: Intraoperative blood loss was significantly higher in the large uterus group (group 1); 350 (227–454) ml vs. 250 (182–320) ml (p < 0.001). However, it was not significantly different between the groups in the laparoscopy step. Mean operating time was 90 (77–103) minutes and 80 (62–98) minutes in groups 1 and 2, respectively (p < 0.001) revealing ten-minute delay in group 1. Similarly, this was also not significantly different in the laparoscopy step. No significant differences were found between two groups; in terms of hemoglobin concentration decrease, major and minor complications, and hospitalization duration. Conclusion: The authors conclude that LigaSure can be safely used for LH in patients with a large uterus.

Key words: Laparoscopy; Hysterectomy; Large uterine size; LigaSure.

Introduction

Hysterectomy is the most frequent of major gynecologic operations [1]. Laparoscopic hysterectomy (LH) became the first choice for hysterectomy performance of many surgeons after it was initially presented in 1989 [2]. This can be attributed to quick postoperative recovery time advantage. By the help of contemporarily achieved developments in surgical equipments and techniques with additional training, total LH (TLH) became a well-tolerated, safe, and efficient modality. The present authors consider that the most critical step during LH is securing the uterine arteries. Because if bleeding occurs during this step, there may be a higher risk of failure in completing the operation due to limited optical view or may have increased rate of adjacent organ complications. Therefore precise hemostasis is warranted in LH particularly in patients with large uterus. New energy modalities may be valuable supports to achieve this goal. In a previous study the present authors have shown that LigaSure vessel sealing system is a safe energy modality in securing uterine arteries at LH [3]. However, the aforementioned studies do not represent the safety of LigaSure in patients with the large uterus. In addition, the literature lacks clear data regarding the safety of LigaSure vessel sealing device when used for LH in patients with the large uterus. Therefore in the present study the authors aimed to assess the safety and efficiency of LigaSure in patients who underwent LH with large uterine size concerning intraoperative outcomes.

Materials and Methods

Intraoperative and postoperative data of both groups were recorded and analyzed. LH was performed under general anesthesia. In the present study the authors secured uterine arteries at their entry into the uterus with LigaSure V mm (Figure 1). This technique was different from our previous study in which uterine arteries were secured retroperitoneally [3]. In the vaginal part of the operation, LigaSure Vmax was used. Lithotomy position was preferred in all patients for operation with drain implementation only when indicated. The operational records analyzed included, total operating time (from the maintenance of pneumoperitoneum to vaginal cuff closure), durations of laparoscopic and vaginal parts separately, total estimated blood loss, mean blood loss in laparoscopic and vaginal parts of the operation separately, mean...
value of decrease in Hb concentration, uterus weights, intraoperative and postoperative complication incidence, conversion from laparoscopy to the classic abdominal approach, blood transfusion requirement, and hospitalization duration. Blood loss was measured by recording the contents of the fluid extraction device in both steps.

**Statistical analysis**

Statistical Analysis was performed using the SPSS ver. 14. Median, medium and percentages of the variables were analyzed. The differences between the two groups were analyzed by Chi-Square test or Mann Whitney U test. A $p$ value < 0.05 was considered statistically significant.

**Results**

In this cohort study, a total of 46 LHs were performed. Group 1 consisted of 22 patients whose uterus weight was $\geq 300$ grams, whereas group 2 consisted of 24 patients whose uterus weight was $\leq 299$ grams. To ensure similar demographic characteristics between the groups, the authors only included patients with only one previous abdominal surgery and similar body mass index values.

Table 1 illustrates demographic characteristics of both groups. No significant differences were found between the variables. The most common indication in both groups was uterine fibroid. Indications for LH were also similar between two groups (Table 2). Table 3 depicts intraoperative variables in both groups. Total operation time was significantly longer in group 1 [90 (77 – 103) minutes vs. 80 (62 – 98) minutes] ($p = 0.001$). This significance was due to the vaginal step of operation as it was significantly longer in group 1 [29 (22 – 35) minutes vs. 18 (14 – 23) minutes] ($p = 0.042$). However, mean duration for laparoscopy step was similar between the groups (Table 3). Mean intraoperative blood loss was also not significant between the groups (Table 3). However blood loss in the vaginal step was significantly higher in group 1 [240 (160 – 280) ml vs. 160 (100 – 210) ml] ($p = 0.035$.) There were no major intra- and postoperative complications in both groups. In group 1, one patient had trocar site infection and one patient in group 2 had postoperative urinary tract infection. There was no conversion from laparoscopy to laparotomy in any group. None of the patients in both groups had blood transfusion. All patients were discharged on the first postoperative day.

**Discussion**

The well known reported advantages of LH compared to laparotomy are less intraoperative bleeding, shorter hospitalization duration, faster recovery, less use of analgesics, and better cosmetic view of operation site [4, 5]. In addition, uterine artery sealing via LigaSure during hysterectomy is reported to be effective, safe, and fast leading to less intraoperative bleeding [6, 7]. In a study comparing two techniques, LigaSure vessel sealing device is reported to be as efficient as bipolar cautering in sealing uterine arteries [8].

LH may be a challenging operation in patients with enlarged uterus due to the distortion of the normal anatomy and limited view of the anatomic planes. In addition, increased vascularization in the large uterus makes the operation as more complicated due to increased risk of intraoperative bleeding. Less clear view of anatomic planes as a result of bleeding give rise to increased risk of adjacent organ damage until control of hemorrhage. On the contrary, despite difficulties of LH in patients with a large uterus, ad-
vantages have also been shown regarding postoperative morbidity [9, 10]. However, there are few studies on this concern and limited data regarding the safety of LigaSure use at LH in patients with large uterus. In the present study the authors did not observe any single vascular injury or other intraoperative complications in both groups.

In the present study, vaginal and laparoscopic steps are performed with the LigaSure vessel sealing system. Therefore the authors were able to assess the safety of LigaSure separately in both steps. Accordingly, they have demonstrated that LigaSure is an effective option in the control of bleeding during laparoscopic step. A previous study by Mistrangelo et al. [11] investigated the safety of LigaSure in patients undergoing vaginal hysterectomy. These authors confirmed that vaginal hysterectomy with LigaSure is a safe method in the large uterus with regards to intraoperative complication rates and postoperative recovery. However, the amount of intraoperative bleeding was not mentioned in that study. In contrast, the present authors have found an increased amount of intraoperative bleeding in group 1 at the vaginal step. They consider that increased amount of bleeding is due to increased traction of large uterus during the vaginal step, as they have seen while opening the uterine artery pedicles in some of their patients in group 1. Accordingly, they suggest gentle traction or morcellation of the uterus during vaginal step to reduce the amount of intraoperative bleeding.

A different study by Kriplani et al. [8] investigated the safety of LigaSure in patients with uterus weighing more than 250 grams who underwent TLH. They found longer operation time and higher amount of intraoperative bleeding in these patients compared to patients with smaller sized uterus. However, these authors concluded that LigaSure was a demandable energy modality as they found no difference in complication rates between the groups. A previous study by Hanlan et al. [12] also stated that TLH is a safe method in the large uterus. However, in this study energy modality used was not mentioned. A similar study by Alperin et al. [13] compared the safety of TLH and supracervical LH in regards to the uterus weight. These authors found that the mean operation time was 27 minutes longer in patients who had uterus weight more than 500 grams compared to patients with smaller sized uterus. Similarly, energy source was also not mentioned in this study.

In the present study, the authors have also found that laparoscopy step was longer in group 1 (uterine weight ≥ 300 grams) compared to group 2 (uterine weight ≤ 299 grams). They believe that longer operation time in group 1 was due to the limited mobilization of the uterus. Limited mobilization results in difficulties of dissecting the anatomic planes and securing the uterine arteries. Therefore at this stage the authors spent more time to safely seal the uterine arteries which added additional minutes to the operation time. However, in the present study there was no significant difference in terms of intraoperative bleeding and complication rates between the groups. The authors believe that these findings show the safety of LigaSure in the large uterus at LH. They conclude that new energy modalities should be used in such cases and LigaSure is a good option for this.

References


Address reprint requests to:
M. BİÇER, M.D.
Izmir University Medical School
Department of Obstetrics & Gynecology
Izmir (Turkey)
e-mail: mervel19742001@gmail.com
Analysis of the reason of abnormal uterine bleeding induced by copper corrosion of IUD Cu

L. Li¹, J. Li¹, N. Li², Y. Zhang¹, X. Feng³

¹Department of Obstetrics and Gynecology, Hebei General Hospital, Shijiazhuang
²Department of Neurology, Hebei General Hospital, Shijiazhuang
³Department of Anesthesiology, Hebei General Hospital, Shijiazhuang (China)

Summary
Objectives: This study aimed to analyze relationship of the copper corrosion of copper intrauterine device (TCu220 IUD) and abnormal uterine bleeding. Materials and Methods: Sixty-four patients of abnormal uterine bleeding (too much blood volume, shorten cycle, lengthen period or irregular vaginal bleeding) and 72 cases of normal menstrual cycle and quantity in the present hospital, which were removed of IUD due to non-medical reasons, were enrolled, and 36 regular menstruation cases without placing IUD were selected as control group, in which had assessed in vitro copper ion release of TCu220 IUD and content of copper ions and VEGF in endometrial tissue of each group of women. Results: Daily Cu I UD copper dissolution quantities of abnormal uterine bleeding women was significantly higher than that of regular menstruation women (p < 0.05). Copper ion content and the expression of VEGF in endometrial tissue of abnormal uterine bleeding women was significantly higher than that of regular menstruation women endometrial tissue (p < 0.05), and the endometrial VEGF expression had a positive correlation with copper ion concentration in endometrial tissue. Conclusion: High dissolution quantity of Cu IUD may lead to increase of copper ion content in endometrial tissue and may cause VEGF secretion in the endometrium, and then the occurrence of abnormal uterine bleeding.

Key words: Intrauterine device; Uterine bleeding; Copper corrosion quantity; Vascular endothelial growth factor.

Introduction
Intrauterine device (IUD) is the most widely used method of contraception among women of reproductive age [1, 2], which has advantages, such as simple, economic, safe, reliable [3], practical, effective, and reversible [4]. IUD is divided into two categories: one category is the inert IUD, and the contraceptive effect of inert IUD is poor, which has been gradually phased out; the other is the active IUD, because the copper erosion in the uterine cavity may release copper ion, the contraceptive effect is improved obviously, which has now basically replaced the inert IUD [5]. However after using IUD, some women may have an abnormal menstrual period, excessive menstruation, increased vaginal bleeding combination with dripping wet symptom [6], which has seriously influenced the lives of women and reproductive health, and has aroused widespread concern, and the literatures also include many solutions [7, 8]. Placement of a levonorgestrel IUD is recommended for women with idiopathic abnormal uterine bleeding [9]. The reason why there are such symptoms, studies have shown that after placement of Cu IUD, endometrial microvascular commonly undergoes abnormal changes in structure and in function, and changes of endometrial vascular abnormalities are more significant in those with IUD bleeding [10], but the understanding of the pathogenesis still requires more study. There was one study which suggests that the angiopoietin/Tie-2 system promotes vascular remodelling in the endometrium and the changes of Ang-1, Ang-2, and Tie-2 may contribute to abnormal uterine bleeding in some IUD users [11]. TCu220I UD is the clinical commonly used IUD in recent years. Based on the TCu220 IUD as an example, this research performed an in vitro assessment of release of copper ion, and detected content of copper in endometrial tissue and vascular endothelial growth factor (VEGF) in IUD women; an analysis of the role of Cu IUD copper dissolution quantity as the cause of uterine abnormal bleeding, at the same time, provided the theory basis for development of a new type of IUD.

Materials and Methods
Subjects
From August 2009 to December 2011, the women of gynecological clinic in Hebei People's Hospital were enrolled, including 64 patients with abnormal uterine bleeding (study group 1) (copious blood volume, shorten cycle, lengthened period or irregular vaginal bleeding) and 72 cases of normal menstrual cycle and quantity in the present hospital (study group 2), which had removed IUD due to non-medical reasons, and the wearing device time ranged from seven months to eight years. All subjects had the IUD removed at three to seven days after menorrhea and un-
derwent curettage of the endometrium. Thirty-six normal menstrual cycle cases without placing IUD were selected as control group, which underwent curettage of endometrium three to seven days after menstruation. The collected specimens were sealed and stored in a -20°C refrigerator. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Hebei People’s Hospital. Written informed consent was also obtained from all participants.

**Determination of copper ion content**

Using the electronic balance to weigh the wet weight of endometrium, adding 1.5 ml nitric acid for complete digestion, and adding deionized water to the volume of ten ml, the copper ion content in the endometrium was determined by atomic absorption spectrophotometer.

**In vitro release test**

The removed TCu220 10 ml IUD was placed in a brown bottle with cover containing ten ml simulated uterine solution, which was places in the 37 ± 1°C constant temperature water bath oscillator, with speed of 120 ± 1 rpm/min. Changing fresh simulated uterine solution once every three days for a total of three times, the replaced liquid was collected, the absorption value was determined by atomic absorption spectrophotometer, and the average value was taken.

**VEGF detection**

Immunohistochemical SP method was used for detection and tissue sections were dewaxed routinely with anti VEGF antibody according to the instruction’s of the kit. Result determination: the cells with the brown yellow reactants in the cytoplasm, which staining was significantly higher than that of the background, were VEGF positive cells. Each specimen was randomly taken at five high power fields (*400 times), with application of image analysis system, and the average optical density of intracellular positive products was measured.

**Statistical analysis**

The experimental results were expressed as the mean ± standard deviation, measurement data were assessed with t-test, and data were analyzed by correlation analysis. A p < 0.05 indicated significant differences.

**Results**

**Content of copper ion**

The endometrial copper ion content of study group 1 and group 2 women were, respectively, 9.05 ± 1.54 μmol and 6.73 ± 0 /L 92U mol/L, and the difference was statistically significant (p < 0.05). The copper ion content in endometrial tissue of women in the control group was 1.72 ± 0.46 μmol/L, which was less than that of the study group (p < 0.05). The copper ion content in endometrial tissues of all study group patients had nothing to do with the insertion time (Table 1).

**In vitro release of Cu IUD**

Most of the removed Cu IUD had integrity of copper wire, with shades of black surface; the surface of some copper wire included peeling and a lackluster copper color exposed at the peeling site; most copper wire was visible with uneven white or grey white sediments, and there was rare phenomenon, such as copper wire fracture, and copper wire falling off. Daily Cu IUD copper dissolution quantity of group 1 and group 2 was 18.02 ± 3.6 g and 15.17 ± 31 g, respectively, with significant difference (p < 0.05, Table 1).

<table>
<thead>
<tr>
<th>Items</th>
<th>Cu²⁺ content in endometrium (μmol/L)</th>
<th>Daily copper dissolution quantity of Cu IUD</th>
<th>VEGF expression in endometrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n = 36)</td>
<td>1.72 ± 0.46</td>
<td>-</td>
<td>0.283 ± 0.046</td>
</tr>
<tr>
<td>Study group 1 (n = 64)</td>
<td>9.05 ± 1.54</td>
<td>18.02 ± 3.6</td>
<td>0.553 ± 0.116</td>
</tr>
<tr>
<td>Study group 2 (n = 72)</td>
<td>6.73 ± 0.92</td>
<td>15.17 ± 3.1</td>
<td>0.425 ± 0.149</td>
</tr>
</tbody>
</table>

**Figure 1.** VEGF expression of the three groups. A: control group; B: study group 1; C: study group 2.
Expression of VEGF
The endometrium of each group all had expression of VEGF. Expression of VEGF (0.553 ± 0.116) of study group 1 (Figure 1B) was significantly higher than that of study group 2 (Figure 1C) (0.425 ± 0.149) and the control group (Figure 1A) (0.283 ± 0.046), and the difference was statistically significant \( (p < 0.05) \). VEGF expression of study group 2 was significantly higher than that of the control group \( (p < 0.05) \), Table 1).

The relationship between VEGF and copper content
The endometrial VEGF expression was positively correlated with the content of copper ion \( (r = 0.885, p < 0.05) \).

Discussion
Bleeding irregularities, such as intermenstrual spotting or heavy or prolonged menstrual bleeding, are common among Cu-IUD users and are one of the leading reasons for method discontinuation [12]. The study found that the concentration of copper ion in endometrium of women with IUD was higher than that in the women without IUD. After insertion, the daily dissolution quantity of copper in the in vitro simulated uterine fluid of uterine bleeding women was higher than that of without abnormal bleeding women, and the copper ion content in endometrial tissue of the former was significantly higher than the latter, suggesting that high concentration of copper ion in endometrial tissue may be the cause of abnormal uterine bleeding after insertion of IUD.

TCu220 IUD has a full length 29 mm, a width of 19 mm, a diameter vertical arm 1.8 mm, with 0.4 mm diameter copper wire around the copper, copper surface area being 220 mm², and there is nylon tile fiber in the end of the longitudinal rod. No difference was found whether the insertion was immediate or delayed [13]. However, immediately after the insertion, a burst release of copper ions occurs, which may be associated to a variety of side effects [14]. Under normal circumstances, the human endometrium rarely has copper ion content, and the copper concentration may increase after insertion of Cu IUD. Cu IUD may dissolve and release the copper ion in the uterine cavity, leading to destruction of endometrial enzyme system activity, thus affecting glycogen metabolism and the formation of endometrial DNA, estrogen, and progesterone, and leading to interference of endometrial cell, thus playing the contraceptive effect [15]. After insertion of IUD in the uterus, due to its wide contact area with endometrium or larger support, uterine bleeding may be caused, combined with mechanical compression of the device, which may lead to the damage of uterine endothelial cells, the release of a large number of kinins, prostaglandins, and plasmin activity factor, an increase of uterus fibroinolytic system activity, and the vascular permeability, resulting in menorrhagia and menstaxis [16]. Studies have found that the higher the Cu²⁺ concentration, the better the contraceptive effect, but the side effects are more serious, which can easily cause phenomena such as bleeding and menstaxis [7].

There have been reports in the 80's of the last century that, in the no vascular corneal of rabbits, Cu²⁺ can attract the migration of endothelial cells, and hence cause the formation of new capillaries to promote angiogenesis [17]. Later, a large number of experimental studies have confirmed this finding [18]. Human uterine cavity is a complex environment, because of the difference of race and age, composition of the uterine fluid also has a very large variance. The study results of Bastidas et al. and Cai et al. showed that the kind and concentration of protein in the uterine fluid has a great influence on the dissolution behavior of Cu in IUD, however, the influence of other components in the simulated uterine fluid, such as inorganic chloride salt, salt, glucose, and so on, on the release of the Cu²⁺ in the Cu-IUD need further study [19, 20].

VEGF is considered to be the most specific angiogenic factor. In 1989, Ferrara et al. [21] first isolated and purified this kind of glycoprotein in a bovine pituitary follicle astrocytes culture liquid. VEGF is polypeptide growth factor and combines with heparin, after high affinity binding with KDR and flt-1 in endothelial cells, which can be taken as the specific mitogen of the endothelial cells to induce hyperplasia of the endothelium, the formation of the capillary loops, and at the same time, increase the permeability of micrangium, through inducing the generation of the mesenchyme, to promote neovascularization [22, 23]. The expression level of VEGF can reflect the level of vascular endothelial cell proliferation and vascular construction. There are studies considering that hypervascularity may be the reason of abnormal uterine bleeding after insertion of IUD [24]. The results of this study suggest that, after insertion of IUD, the change of the microenviroment in the uterine cavity can lead to the enhanced expression of VEGF, while the expression of VEGF may be stronger, indicating that the abnormal bleeding after insertion of IUD may be related to the increased secretion of VEGF in the endometrium and increased density of microvascular. There was one reason which was considered as significantly higher immunoreactivity for VEGFR-1 and VEGFR-3 [25]. The associated research in the study showed that compared to after insertion of IUD and before insertion of IUD, the density of microvascular and the expression of VEGF had all increased [26], which was consistent with the present study. The result of this study also showed that in the endometrium, the expression of VEGF is positively correlated with the content of Cu²⁺, indicating that the high concentration of Cu²⁺ may cause the high expression of VEGF, causing abnormal bleeding after insertion of IUD. Studies also found that Cu²⁺ is closely related to the expression of VEGF [27, 28], and through influencing the regulatory factor, hypoxia-inducible factor 1 (HIF-1), Cu²⁺ can stimulate
the endothelial cells to upregulate the expression of VEGF [28, 29], and through the HIF-1, which can influence the expression of multi vasculogenesis related gene. Therefore, Cu²⁺ can promote the formation of the new vessels and VEGF can promote the proliferation of endometrial cells and induce angiogenesis, but the new vessels are not mature and can cause blood vessels leakage [30].

This study only assessed the TCu220 IUD; for the IUD of different surface area, the relationship between the copper erosion and the abnormal uterine bleeding need further study.

References


Address reprint requests to:
LI LI, M.D.
Department of Obstetrics & Gynecology
Hebei General Hospital
No. 348 HePing West Road
Shijiazhuang 050051 (China)
e-mail: lljbcn@163.com
Bulky fibroids discovered during pregnancy can become acutely complicated and can pose a problem in choosing the more suitable management [1].

Case Report

In view of the increasingly unbearable nature of the pain, continuation of the pregnancy did not appear possible. After reviewing the literature and then informing the patient about the risks of a procedure during pregnancy, surgical excision was proposed. At 11 weeks' gestation, the fibroid, extending from the xiphoid process to the pubis, was excised by laparotomy, with a blood loss that reached 1,100 ml. The fibroid weighed 3,000 grams and measured 27 cm in its largest diameter; it was sessile, with a base of eight cm in width (Figure 1). Obstetric follow-up was thereafter unremarkable. At 40 weeks, with local conditions unfavorable 24 hours after rupture of the membranes, a 4,020-gram child in good health was delivered by cesarean.

Fibroids are present in approximately 2-3% of pregnancies and are complicated in 10% of them [1]. The pain they cause, often due to necrobiosis, can be intense and sometimes justifies use of analgesic or anti-inflammatory treatments. Their surgical treatment during pregnancy is not recommended because of the potential risks of hemorrhage, preterm premature rupture of the amniotic cavity, late abortion, or preterm delivery.

Few cases of intervention during pregnancy have been reported. The published cases report that myomectomy has always been performed before 24 weeks, almost always by laparotomy [2-4]. Laparoscopic surgery was performed but for smaller fibroids from 4-15 cm. Thus, for large fibroids, laparotomy seems more appropriate. A late miscarriage occurred in three cases (6%) [2-4] and a hemorrhage with blood loss exceeding 500 ml in another [5], but no other fetal or neonatal complications have been reported [2-5]. Vaginal delivery was possible in one-third of the cases [2-5]. The risks of this intervention must therefore be weighed together with those of keeping in place a fibroid, as initial symptoms are likely to increase, with a risk of hemorrhage at delivery [1]. A prospective study of 106 cases, 18 of which underwent surgery, supports the hypothesis that intervention reduces the risk of late miscarriage.

Figure 1. — Pelvic and abdominal T2-weighted MRI, sagittal plane of a fibroid measuring 24×18 cm.
riage, hysterectomy, growth restriction, and threatened preterm delivery [4]. In the present case, the patient’s poor tolerance and the large size of the fibroid caused fear and substantial difficulties for the present authors during pregnancy and at delivery.

**Conclusion**

The present case confirms that some bulky fibroids can be excised during pregnancy, with a favorable maternal and neonatal prognosis.

**References**


Address reprint requests to:
J. LEPAGE, M.D.
Hôpital Jeanne de Flandre
Université Lille Nord de France
1 rue Eugène Avinée
59037 Lille Cedex (France)
e-mail: lepagejulien@msn.com
A case of a 42-year-old patient with anomalous origin of the left main coronary artery from the pulmonary artery who delivered three times with no complications: presentation, diagnosis, and review

M.S. Arnaout¹, M. Serhan¹, C. Saade²

¹ Department of Internal medicine, American University of Beirut Medical Center, Beirut
² Accreditation and Technical Officer, Diagnostic Radiology Department, American University of Beirut Medical Center, Beirut (Lebanon)

Summary
An anomalous origin of the left coronary artery (LCA) from the pulmonary artery or Bland-White-Garland (BWG) syndrome is a rare congenital cardiac anomaly, which is unusual to survive to adulthood if left uncorrected. The authors report an unusual case of a multiparity patient who delivered several times without any complications presenting at the age of 42 years with recurrent dyspnea on exertion in which echocardiographic findings of diastolic flow near the origin of the pulmonary valve were suggestive of anomalous origin of the LCA. A CT angiography confirmed that the left main coronary artery arises from the undersurface of the pulmonary flow close to its origin and gives rise to a left anterior descending (LAD) and left circumflex arteries.

Key words: Congenital heart disease; Uncomplicated pregnancy; Coronary anomalies; Echocardiography; Computed tomography angiography.

Introduction
An anomalous origin of the left coronary artery (LCA) from the pulmonary artery or Bland-White-Garland (BWG) syndrome is a rare congenital cardiac anomaly, which is unusual to survive to adulthood if left uncorrected. Collaterals to the left system are the key point for the survival to adulthood. CT angiography remains the best modality for diagnosis.

The authors report an unusual case of a multiparity patient who delivered several times without any complications presenting at the age of 42 years with recurrent dyspnea on exertion in which echocardiographic findings of diastolic flow near the origin of the pulmonary valve were suggestive of anomalous origin of the LCA.

Case Report
History dates back to the year 2003, where a 31-years-old female that was previously healthy, except for a history of mitral valve prolapse (MVP) and regurgitation corrected with a mitral ring repair in year 2000, presented for a routine follow up where echocardiography showed a suspicion for a coronary ventricular fistula. Patient was admitted for cardiac catheterization and CT angiography that showed patent right coronary artery (RCA) with prominent vessels in the anterior mediastinum that may be related to the RCA; images were suggestive of a stenosis at the origin of the Left coronary artery (LCA). This was followed by a magnetic resonance angiography (MRA) of the LCA which showed at that time, normal origin of the LCA from the aortic cusps with severe stenosis at its origin. Due to the fact that the patient had normal exercise tolerance and no angina symptoms, no surgical intervention was done and patient was stabilized on medical therapy.

Preceding this symptomatic period patient became pregnant twice with an additional pregnancy after that at the age of 34; all delivered with normal vaginal delivery, with no complications during gestation, labor or postpartum.

Patient remained well without functional limitation and no symptoms until 2014, at the age of 42 where she complained of palpitations and progressive dyspnea in a periphery hospital, EKG showed rapid rate atrial fibrillation that was electrically cardioverted to sinus rhythm.

Patient then presented to the present hospital complaining of recurrent dyspnea on exertion with no symptoms of angina. Echocardiography was done and it showed moderate dilatation of the left ventricle (LV) with mild global LV hypokinesia and an ejection fraction of 45-49%. During diastole, evident Doppler signals were seen near the origin of the pulmonary valve suggestive of aberrant origin of the left main coronary artery from the pulmonary artery (PA) (Figure 1). A CT angiography of the coronary arteries was performed and demonstrated that the left main coronary artery arose from the undersurface of the pulmonary flow close to its origin and gave rise to an LAD and left circumflex arteries (Figures 2, 3). Also it showed the anastomosis between the right posterior descending CA and the LAD (Figure 4).
Cardiac CT angiography technique

Scanner acquisition

With the patient positioned supine and arms placed above his head, ECG-gated cardiac/coronary CTA was performed using a 256-MDCT scanner. Antero-posterior and lateral scout scans were performed, with a scan range from the apex of the chest to the costophrenic angle. Scan parameters were: detector width 256 × 0.625 mm, pitch 0.2:1, rotation time 0.27 sec, 100 kVp, 200 mA, with z-axis modulation, and scanning time of 2.1 seconds.

Image reconstruction

The following parameters were set: standard image reconstruction of axial images at 0.625 mm slice width, reconstruction interval of 0.5 mm, 180 × 180 mm field of view, and iterative reconstruction technique software (iDose4) with a window width and level of 420 and 65, respectively. The ECG gated scan reconstruction interval with the least motion artifacts was determined by reconstructing a slice at the mid segment of the right coronary artery in 2% steps from 35% to 75% of the R-R interval. For diagnostic interpretation, reconstruction of the CTA images was used, with a time point with the least motion artifact located at the mid segment of the ascending aorta (68%).

Discussion

An anomalous origin of the coronary arteries from the pulmonary artery (PA) is usually an isolated abnormality, it occurs in 0.4% of all patients with heart disease [1]. The most common defect is the anomalous origin of left main coronary artery (LMCA) from the pulmonary artery, known as BWG syndrome [2].

During fetal life the condition can be tolerated due to equal pressure between the PA and the systemic circulation. Shortly after birth, the PA pressure drops and the flow in the LMCA decreases and then reverses leading to a chronic myocardial ischemia and maybe infarction. During this period collateral circulation begins to develop between the RCA and the LMCA; the extent of these collaterals is important in determining the degree of myocardial ischemia, the timing of clinical presentation, and the chances of long term survival [3, 4]. Ninety percent of cases present during
infancy with symptoms of heart failure and mitral regurgitation due to progressive myocardial ischemia, with a bad prognosis and an increased mortality risk during infancy and early childhood [4]. In the adult population it usually presents with a wide range of non-specific symptoms, including syncope, dyspnea on exertion, and angina or cardiac arrhythmias [5]. Very few reported cases were asymptomatic and diagnosed accidentally when following up for other conditions.

Although normal physiologic changes occurring during pregnancy, such as increased oxygen consumption, cardiac output (50%), heart rate, and aortocaval compression in the supine position might increase the burden on the heart [6], yet some uncomplicated cases of anomalous left coronary artery from the pulmonary artery (ALCAPA) in pregnancy have been reported [7].

The anomalous origin of the LMCA from the PA is usually suspected when transthoracic echocardiography shows a dilated RCA arising from the aorta, diastolic blood flow from the LCA into PA, diastolic blood flow from the inferior portion of the inter-ventricular septum to its superior portion, and mitral regurgitation. However these findings are not specific and are shared with other diseases such as Kawasaki’s disease and arteriovenous fistula. In order to establish a firm diagnosis cardiac catheterization is used and it shows dilated and tortuous RCA, collaterals, and shunting to the PA [8, 9]. In addition CT angiography is a non-invasive procedure that can help in diagnosis as it can show coronary arteries with a good image quality and high diagnostic accuracy [10].

Many treatment options have been suggested for adults presenting with anomalous origin of the LMCA from the PA yet no optimal surgical technique have been defined. Treatment options include LMCA ligation, re-implantation of the LMCA to its original site in the aorta, baffle creation through the PA (Takeuchi procedure), and a combination of LMCA ligation and coronary artery bypass graft (CABG). Re-implantation of LMCA to the aorta is the first choice of treatment because it restores normal anatomy and circulation; if not possible ligation and CABG are preferred because it also provides a dual coronary flow system [11, 12].

Conclusion

In conclusion, the presented patient is one of the rare cases who survived a deadly disease up until the age of 42 with three pregnancies. A relatively preserved left ventricular systolic function could be explained by having a very large and dominant right coronary artery with adequate retrograde supply to the left system. Again the authors still be-
lieve that the ultimate treatment of this disease is surgery.

References


Address reprint requests to:
M.S. ARNAOUT, M.D., FESC, FACC
American University of Beirut Medical Center
AUB-MC, PO BOX 11-0236
Riad El Solh Beirut 1107 2020 (Lebanon)
e-mail: sarnaout@aub.edu.lb
Ectopic pregnancy is the development of the embryo outside the uterine cavity. In recent years an increase in the incidence of ectopic pregnancies has been observed. It is mainly related to the increased number of IVF procedures. The most frequent localization is the ampulla of the fallopian tube. In cases of intramural or cornual ectopic pregnancies, many patients have a history of ectopic pregnancy on the same side or underwent partial salpingectomy in the past. Treatment of such pregnancies involves laparotomy and removal of the uterus or cornu of the uterus. This case concerns a patient operated because of intramural pregnancy. She had a salpingectomy in the past because of tubal ectopic pregnancy on the same side. This case shows that even after removal of the fallopian tube there is still a risk of ectopic pregnancy in the intramural part of this tube.

Key words: Ectopic pregnancy; Intramural pregnancy; Surgical techniques.

Introduction

Ectopic pregnancy is the development of the embryo outside the uterine cavity. The most frequent localization is the ampulla of the fallopian tube, where 80% of ectopic pregnancies are located. Much rarer location is the interstitial part of the fallopian tube (2-4%). Some authors emphasize that an interstitial ectopic pregnancy should not be confused with cornual pregnancy. Though the term of interstitial ectopic pregnancy is often used as synonym with cornual pregnancy, according to the definition, cornual pregnancy develops in the upper and side part of the uterus and concerns double uterus, bicornuate or unicorne uterus, and interstitial ectopic pregnancy implants in the proximal segment of the fallopian tube covered by myometrium.

The intramural part of the fallopian tube retains histological structure characteristic for the whole fallopian tube. The inner layer is the mucous membrane from columnar epithelium with secretory and ciliated cells and under it is a layer of mucous membrane without the glands. Typical mucosa of the uterine cavity is covered with a layer of columnar epithelium with single ciliated cells and under it is a thick layer of mucous membrane containing the glands. These differences in tissue structure allows in postoperative specimen to identify the location of intramural or cornual ectopic pregnancy [1-4].

In recent years an increase in the incidence of ectopic pregnancies has been observed. It is mainly related to the increased number of in vitro fertilization procedures, which have become one of the risk factors for this pathology. Diagnostic and treatment methods of ectopic pregnancies have improved significantly and are more effective [3-5].

It is believed that the intramural portions of fallopian tube can have a tortuous pattern. By analyzing the risk factors of ectopic pregnancy in the intramural part of fallopian tube, we can come to the conclusion that there will be a greater risk of interstitial ectopic pregnancy in women with a tortuous pattern of the intramural portion than those with a straight or curved one [6].

Currently, diagnosis of early ectopic pregnancy besides interview and physical examination is achieved using a combination of transvaginal ultrasonography and serial measurement of serum β-hCG concentrations [1].

The primary treatment for intramural ectopic pregnancy includes surgical treatment. Traditionally, treatment of such located pregnancies involved laparotomy and removal of the uterus or cornu of the uterus. In a patient who is haemodynamically stable, surgical management can involve laparoscopy or laparoscopy in combination with hysteroscopy. It is also permissible expectant and pharmacological treatment with methotrexate [7-11].

An intramural ectopic pregnancies sometimes occurs in patients that underwent a partial salpingectomy because of ectopic pregnancy on the same side in the past [1, 3-5].

Case Report

A 29-year-old patient was admitted to the Department of Gynecology and Urogynaecology of Pomeranian Medical University in Szczecin on October 10, 2012 with complaints of severe pelvic pain. Last menstrual period was on August 21,
2012. In 2006 patient had cesarean delivery and in February 2012 underwent left salpingectomy due to tubal pregnancy. She menstruated regularly every 32 days. On admission patient was in a good general condition. Blood pressure was 90/60 mmHg with a heart rate of 68/minute. On examination, the abdomen was painful with peritoneal signs expressed. In gynecological examination there was no vaginal bleeding. Uterus was ante-flexed and painful during motion. The area of left adnexa was tender on palpation. Transvaginal ultrasound examination revealed an anteflexed uterine corpus, uterine dimensions of 46×38 mm, normal myometrium, and 15-mm-thick decidua-endometrium with no visible intrauterine pregnancy. Right ovary measured 25×18 mm and left ovary measured 28×20 mm, both with small ovarian follicles. In a region of left uterine cornus a hyperechogenic mass with dimensions of 36×29 mm was found; there were blood clots and gestational sac with a diameter of 20 mm with fetus of crown-rump length (CRL) of 8 mm which corresponded to six weeks five days of pregnancy with visible cardiac activity. Behind the uterus a small amount of fluid in the Douglas pouch was found. Laboratory tests indicated that serum β-hCG concentration was 22308 mlU/ml. The laboratory blood count investigations were within normal limits, such as electrolytes and coagulation parameters.

After the admission patient's condition suddenly worsened, with symptoms of peritonitis and signs of bleeding into the abdominal cavity were detectable. The patient qualified for emergency laparotomy. During surgery haemoperitoneum of approximately 700 ml blood with clots and right adnexa without changes were detected. In the intramural part of the left fallopian tube, an ectopic pregnancy was visible with bleeding. Tissues of pregnancy was completely removed from the fallopian tube and uterus was sutured with hemostatic stitches. Due to the significant blood loss during the operation, the patient received two units of packed red blood cells. Hematological examination of the first postoperative day showed white cell count 10.62 thousand/ul, hemoglobin 9.5 g/dl, and hematocrit 27.5%. Postoperative follow-up was without complications and the patient was discharged on the seventh postoperative day. The result of the histopathological examination confirmed the presence of trophoblast and the embryo in tissues from the fallopian tube (Figures 1 and 2).

Discussion

The presence of spontaneous pregnancy in the intramural part of fallopian tube after salpingectomy on the same side is very rare and single cases are described in the literature [1, 8]. The main risk factor for intramural pregnancies are adhesions within the proximal part of fallopian tube and tubal surgery [1, 2, 5]. Interstitial pregnancies occur frequently in patients after assisted reproductive techniques. Implantation of the embryo in the fallopian tube after transfer may be caused by the flow of embryo to the fallopian tube after its transfer in the medium into the uterine cavity or because of transfer of embryos directly into the fallopian tube.

In spontaneous pregnancies, as in the present case, the implantation in an intramural part of the fallopian tube could be result of reflux of the embryo with the mucosa secretions from the uterine cavity [9]. Although surgical techniques have evolved over the years, most authors confirm the possibility of sparing surgery with leaving the fallopian tube undamaged by ectopic pregnancy. It carries a greater risk of a subsequent pregnancy in the operated fallopian tube or implantation in the intramural part of this tube. Therefore, some authors postulate to remove the fallopian tube with wedge cut-out of its intramural part and with stitches on the uterine horn [1, 2, 10-12]. Such action may result in the risk of uterine rupture during pregnancy or delivery [13, 14]. For this reason the choice of surgical technique is very important. When resection of the intramural part of the fallopian tube is performed, it is very important to properly stitch this area, and not only with coagulation.

Treatment of intramural tubal pregnancy includes its removal from its uterine horn. This includes the necessity of
resecting the uterine cornu or even removal of the uterus. The operation can be performed by laparoscopy or laparotomy. Laparoscopic operations are connected with shorter hospitalization and faster return of patient to everyday living than after laparotomy [15].

In the reported case, the patient underwent laparotomy because of active bleeding and haemoperitoneum. Ectopic pregnancy was removed and the site was sutured haemostatically.

The present case includes some useful clinical information and shows that even after removal of the fallopian tube, there is still a risk of ectopic pregnancy in the intramural part of this tube.

References

Address reprint requests to:
J. NAWROCKA-RUTKOWSKA, M.D.
Department of Gynecology and Urogynecology
Pomeranian Medical University
2 Siedlecka Street
72-010 Police (Poland)
e-mail: jolanaw@poczta.onet.pl
Asymptomatic isthmico-cervical uterine perforation with IUD – our experience and literature review

R. Sparic¹, J. Dotlic¹,², L. Mirkovic¹,², J. Stamenkovic¹,², B. Kastratovic Kotlica¹,², L. Nejkovic³, I. Babovic¹,², A. Malvasi⁴,⁵, A. Tinelli⁶,⁷

¹ Clinic for Obstetrics and Gynecology, Clinical Centre of Serbia, Belgrade
² Medical Faculty, University of Belgrade, Belgrade; ³ Clinic for Gynecology and Obstetrics "Narodni Front", Belgrade (Serbia)
⁴ Department of Obstetrics and Gynecology, Santa Maria Hospital, Bari (Italy)
⁵ International Translational Medicine and Biomodeling Research group Department of Applied Mathematics Moscow Institute of Physics and Technology, Moscow State University, Moscow (Russia)
⁶ Division of Experimental Endoscopic Surgery, Imaging, Technology and Minimally Invasive Therapy of Centre for Interdisciplinary Research Applied to Medicine Department of Obstetrics and Gynecology Vito Fazzi Hospital, Lecce (Italy)

Summary

Purpose: The study aim was to report an unusual case of a misplaced IUD in isthmico-cervical region causing partial uterine perforation and discuss literature data regarding such a condition. Case Report: A 50-year-old women was referred to the present institution for IUD extraction. She was diagnosed with spontaneously misplaced IUD located in isthmico-cervical region of the uterus causing partial perforation. The time of dislocation was unknown, as she was completely asymptomatic for ten years after IUD application. Moreover, she had no risk factors for device misplacement. The removal of IUD was uneventful. Conclusion: Isthmico-cervical misplacement of IUD, although rare, can occur at any time and can be asymptomatic. Thus, women with IUD should be annually checked-up in order to prevent possible IUD complications.

Key words: Intrauterine device; Secondary perforation; Asymptomatic uterine misplacement.

Introduction

Intrauterine device (IUD) is one of the most popular and widely used (14% of women worldwide) methods of contraception. IUD is confirmed as a safe and reliable [1, 2]. Rare complications of IUD contraception are device misplacement and uterine perforation. Misplacement and perforation and can occur at any time. They are mostly followed by pelvic pain and bleeding but can also be completely asymptomatic [3, 4].

The aim of this paper was to report an unusual case of a misplaced IUD that caused asymptomatic partial uterine perforation in the isthmico-cervical region diagnosed at the time of extraction ten years after application and to discuss literature data regarding such conditions.

Case Report

A 50-year-old woman (secundipara, secundigravida) was referred to the present institution for IUD extraction. At the time of referral she was menopausal for two years. The copper device was inserted ten years ago, two years after her last delivery. Following IUD application, patient did not have irregular bleeding, pelvic pain, or any other symptom of possible complications. Therefore, she did not go to regular annual gynecological check-ups. On gynecological examination IUD was suspected to lie in oblique position through isthmico-cervical region of the uterus, thus deforming the cervix (Figure 1). The arms of the IUD were located on the left, while the rest of the IUD was embedded in the right uterine wall, reaching just below the mucosa, causing the partial uterine perforation (Figure 1). Ultrasound examination confirmed the clinical findings. As the part of the institutional protocol, pelvic X-ray scan was made. It revealed the presence of T-shaped IUD in the pelvis inferior to the pelvic brim. However, IUD seemed to be dislocated and positioned almost horizontally (Figure 1). The IUD was grasped with a forceps and applying arcuate movement gently pulled out of the uterine wall and extracted through the cervical canal.

Control ultrasound scan after intervention showed partial uterine perforation that was made by the IUD (Figure 1). There were no other pathological findings in the uterus or adnexal regions. Moreover, there was no free liquid in the Douglas pouch. The intervention was uneventful and the patient was discharged from the Clinic on the same day. Regular annually gynecological check-ups were suggested.

Discussion

Perforation of the uterus by IUD is uncommon, but when it occurs it can cause potentially serious complications. After penetrating the uterine wall, IUD can migrate to the peritoneal cavity, intestines, bladder, ureter or fallopian tubes causing chronic pelvic pain, dyspareunia, bleeding, stone formation, infection, and other visceral complications [1,5]. IUD perforation can be primary or secondary [6].
perforations are primary and occur at the time of insertion. The forces required to perforate the uterus are higher than the forces needed to insert an IUD [6]. Therefore, severe pain at insertion might indicate uterine perforation, but as some pain during application can be expected, this factor is not reliable for differentiating successful placement from complications [7].

The secondary perforation also regarded as “migrating IUD” denotes that an IUD, which was in the uterine cavity for eight weeks or more after insertion, is found in an ectopic position [6]. The IUD remains in place due to the equilibrium between downward fundal force and upward forces of the myometrial promontory [6]. However, uterine muscle can also provide the force responsible for IUD misplacement. In some women, IUDs can be driven by the myometrial force alone to dislocate form its adequate intrauterine position [6].

Women experiencing IUD perforation are usually multiparas in their early 30’s [7]. No clinically significant differences in incidence of IUD perforations or patient characteristics have been registered between different types of IUD (copper or levonorgestrel-releasing) [7]. Several risk factors for uterine perforation have been described such as atrophic uterus, multiparity, history of cesarean delivery and myomectomy, uterine anomalies or posture with a steep flexion angle (hyperanteflexed or retroflexed), insertion performed promptly after curettage, as well as an inadequate insertion technique [1,8]. Some authors have hypothesized that IUD users who experience secondary perforation have abnormally arranged myometrial fibers [6]. Contributing factors to IUD perforation also include the immediate the post-partum period (six months after delivery) and lactation (regardless of the IUD insertion time) due to uterine involution and increased contractility [7, 9].

Most IUDs remain in situ, but if there is a disproportion between IUD size and volume of the uterine cavity, device can be expelled through cervix or perforate some of the uterine walls [6, 8]. IUD misplacement of different extent occurs in up to 18% of IUD users. It is more common in women with smaller fundal endometrial diameters [9]. IUD misplacement is variable, ranging from embedment into the endometrium or myometrium to complete transuterine perforation through the serosa with migration of the IUD into the peritoneal cavity [9]. IUD will be expelled through the path with least resistance. The penetration of the uterine wall is usually slow until finally IUD protrudes through the serosa [10]. Majority of perforations occur in the uterine corpus and fundus. There are only few cases reported in the literature with cervical perforation caused by an IUD [11].

According to the literature, the median time from insertion to diagnosis of misplaced IUD is on average five months [8]. So far, only few cases of IUD inserted a cou-
ple of years prior to perforation have been reported [11]. Studies regarding IUD-associated perforations indicate that the majority of perforations present with mild symptoms. Symptoms usually begin immediately after perforation or within next five days. Nevertheless, one-third of patients are asymptomatic [1, 12]. The most common symptoms are abnormal bleeding, pelvic pain or both, together with missing threads or unintended pregnancy [1, 2]. While sudden heavy bleeding may indicate IUD expulsion or perforation, inter-menstrual spotting is more common [10, 13]. Signs and symptoms mostly force women to seek medical care. Asymptomatic patients are usually diagnosed during routine check-ups or because of unintended pregnancy [14].

After IUD insertion, its position should be confirmed via its threads. A misplaced IUD is usually suspected by shortening or disappearance of its retrieval threads. Still, threads may break off or retract into the cervical canal or uterus. Missing or shortened threads need detailed diagnostics. Cytobrush or IUD hook can be used to locate retracted threads in the cervical canal, but if the threads are not located, pelvic ultrasound or abdominal X-ray should be undertaken [10]. Prevention of IUD misplacement and its complications include regular annual gynecological check-ups [5, 7].

The primary diagnostic examination of IUD misplacement or uterine perforation should be a vaginal ultrasound. If diagnosis is not confirmed by ultrasound, the pelvic and abdominal X-ray should be performed [2, 9, 15]. Computerized tomography or magnetic resonance imaging are good alternatives for further investigation if visceral complications are suspected [9, 15]. Moreover, hysteroscopy or curettage can be used for both diagnosis and treatment. The traditional form of treatment has been laparotomy, but nowadays laparoscopy, as less invasive and safer procedure, is the treatment of choice for abdominal misplacement [5, 9].

Intramurally embedded IUDs as well as partially perforating IUDs could be removed by hysteroscopy [1]. In patients with partial expulsion of the IUD into the cervix, IUD removal can usually be achieved by traction with alligator forceps or an IUD hook. The IUD should not be reinserted. If there is resistance to removal or the IUD breaks, operative hysteroscopy should be undertaken [9]. The majority of patients can be treated on outpatient basis, while only those with suspected infection need inpatient treatment [10].

The present reported case is distinct due to a completely spontaneous misplacement of the IUD with a partial perforation of the isthmico-cervical region. The time when the dislocation occurred remains unknown, as the patient was completely asymptomatic for ten years following IUD application. Moreover, the patient had no risk factors for IUD-related uterine perforation. Besides all the complications, the removal of IUD was uneventful.

**Conclusion**

Secondary cervical perforation by IUD is extremely rare, but can happen at any time and be asymptomatic. Thus, women with IUD need to be advised regarding the possibility of asymptomatic IUD misplacement and benefits of regular annual gynecological examinations.

**References**


Address reprint requests to: J. DOTLIC, M.D., Ph.D.  
Clinic for Gynecology and Obstetrics  
Clinical Center of Serbia  
Dr Košte Todorovica, 26  
11000 Belgrade (Serbia)  
e-mail: enadot@yubc.net
Successful transvaginal aspiration of interstitial pregnancy after failed methotrexate treatment

I.F. Yang¹, J.L. Hwang², H.J. Chen¹, L.W. Huang³

¹ Department of Obstetrics and Gynecology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei City; ² Department of Obstetrics and Gynecology, Shin Kong Wu Ho-Su Memorial Hospital School of Medicine, Taipei Medical University, Taipei City; ³ Department of Obstetrics and Gynecology, Shin Kong Wu Ho-Su Memorial Hospital School of Medicine, Fu-Jen Catholic University, New Taipei City (Taiwan)

Summary

Interstitial pregnancy is an uncommon condition that is challenging, not only in making an accurate diagnosis, but also in the choice of treatment. Systemic methotrexate (MTX) treatment has been favored to prevent scarring of the uterus. Nevertheless, surgery is generally indicated when this treatment fails. Transvaginal aspiration of the gestational tissue has been proposed as an alternative to surgery. The authors present a case of interstitial pregnancy in which the patient failed to respond to multidose MTX treatment and was successfully treated with transvaginal sonography-guided transvaginal aspiration of the gestational tissue, thereby bypassing the risk associated with undergoing major surgery. Transvaginal aspiration of conceptive tissue may be a novel treatment for patients with unruptured interstitial pregnancy.

Key words: Transvaginal aspiration; Interstitial pregnancy.

Introduction

Because of the trend of women postponing child-bearing until an advanced age and the increasing incidence of pelvic inflammatory disease, the need for artificial reproductive technology is inevitable [1]. Ectopic pregnancy is a complication of in vitro fertilization and embryo transfer [2]. The incidence of interstitial pregnancy, a rare and life-threatening condition, is rising, accounting for 2%–4% of tubal pregnancies [3]. Because of the rich vasculature of the implantation site, accentuated by anastomosis of the uterine and the ovarian arteries in this region [4], rupture can lead to a life-threatening hemorrhage. Recently, early use of transvaginal sonography prompted interstitial pregnancy to be diagnosed before a rupture occurred, enabling conservative treatment. Both surgical intervention and conservative treatment with methotrexate (MTX) are considered effective choices for treating interstitial pregnancy [5]. Although the success rate of systemic MTX therapy of interstitial pregnancy was reported at 94% (including cases with fetal heart beat activity) [6], interstitial pregnancy may be more difficult to treat with MTX secondary to deep implantation and protection by the myometrium [7] compared with other types of tubal gestation. If systemic MTX treatment fails, then surgical intervention is generally adopted as a rescue method.

Conservative treatment prevents surgical scarring of the uterus, which can compromise future fertility, and the risks associated with surgery. Interstitial pregnancy following failed systemic MTX treatment in infertile woman poses a clinical dilemma because surgery may cause uterine scarring and disrupt the uterine architecture. The authors present a case of a 30-year-old nulliparous woman who became pregnant after embryo transfer. The woman presented with interstitial pregnancy and failed to respond to multidose MTX treatment. To preserve the entirety of the uterus, a needle was used to aspirate the contents of the gestational tissue under the guidance of transvaginal sonography. Early interstitial pregnancy was rescued after failed systemic MTX management. Based on the present authors’ research, this is the first report of this approach to treatment of interstitial pregnancy.

Case Report

The woman was a patient at the present infertility clinic with primary infertility for four years because of tubal factors. She received laparoscopic bilateral salpingectomy because of bilateral hydrosalpinx and was enrolled in the authors’ in vitro fertilization program. Controlled ovarian stimulation was initiated with multiodose gonadotropin releasing hormone antagonist protocol, and eight oocytes were retrieved. Three four-cell stage embryos were transferred into the uterine cavity two days after oocyte retrieval. A pregnancy test was positive 14 days after embryo transfer. At 5 weeks’ gestation, transvaginal sonography revealed an absence of an intrauterine gestational sac, while the serum β-human chorionic gonadotropin (hCG) level was 2,008 mIU/mL. One week later, transvaginal ultrasound revealed an empty uterine cavity and an eccentric gestational sac located at the interstitial site. The β-hCG level was 10,825 mIU/mL. The patient reported no discomfort such as lower abdominal pain or vaginal bleeding. A tentative diagnosis of interstitial pregnancy was made. Because of the he-
modynamically stable status of the patient, conservative multidose systemic MTX treatment was applied. After three doses of MTX, transvaginal ultrasound revealed a live fetus with a crown-rump length of six mm (Figure 1). The β-hCG level was 19,714 mIU/ml. Furthermore, the patient experienced MTX-related side effects including nausea, vomiting, and dizziness.

The patient and her partner were informed that MTX treatment of the interstitial pregnancy had failed, and they were counseled regarding other options for this pregnancy, including surgical cornual resection and needle aspiration. To preserve the uterine architecture, the patient chose to undergo needle aspiration. Under the guidance of transvaginal ultrasound, a 16-gauge needle was introduced into the interstitial gestational sac through the vaginal fornix. The tissue was aspirated and the sac disrupted. The patient tolerated the procedure well and was discharged on the same day.

One week after the procedure, the serum β-hCG level dropped to 8,997 mIU/ml, and ultrasound revealed an echocomplex mass measuring approximately 2.4 × 3.0 cm over the interstitial site. The β-hCG level reduced to 4.76 mIU/ml 63 days after needle aspiration of the interstitial gestational sac, and the echocomplex mass gradually decreased in size and completely resolved five months later. The patient’s recovery course was uneventful during the follow-up period.

Discussion

Previous studies have described successful systemic medical treatment for interstitial pregnancy with extremely elevated β-hCG [6, 8]. Various regimens of systemic MTX have been proposed with an equivalent success rate. Dibaz et al. reported three cases of cornual pregnancy successfully treated with a single-dose MTX injection [9]. However, Barnhart et al. showed that although a single-dose protocol is simpler than a multidose regimen, it is associated with a higher failure rate and is less effective [10]. Because of the possibility of catastrophic outcomes associated with failed medical management, surgery remains the standard treatment [11], and for patients who fail to respond to conservative management, surgery is generally performed for rescue.

Local injection of MTX or KCl under transvaginal sonogram guidance in cases of interstitial pregnancy, including one case of failed systemic MTX treatment, have proven to be equally effective compared with traditional conservative management [5, 12]. Successful dilatation and evacuation under laparoscopy guidance with postoperative MTX rejection have also been reported [11]. Cai et al. proposed treating interstitial pregnancy with transcervical suction under laparoscopic and hysteroscopic guidance [13]. Hysteroscopic removal of cornual pregnancy has also been reported [14]. The transcervical approach appears to be promising, but the major drawback is the potential for uterine perforation.

Oyawoye et al. described the successful aspiration of a gestational sac under transvaginal ultrasound guidance and instillation of a small dose (12.5 mg) of MTX in managing a cornual heterotopic pregnancy [15]. Prorocic et al. also treated a cornual heterotopic pregnancy by transvaginal ultrasound-guided aspiration and instillation of a NaCl solution into the cornual sac. The cornual pregnancy was aborted and the intrauterine pregnancy resulted in a full-term healthy newborn [16]. Wang et al. successfully managed five cases of interstitial heterotopic pregnancy by transvaginal ultrasound-guided local aspiration and instillation of hyperosmolar glucose [17]. Without additional local injection of MTX or other hyperosmolar solutions, our case was managed solely by transvaginal ultrasound-guided aspiration.

Interstitial pregnancy warrants accurate diagnosis and immediate treatment. Transvaginal aspiration could be an option for patients who wish to bypass major surgery and the side effects of MTX treatment.

Acknowledgement

The research was not funded but we give great thanks to the Department of Obstetrics and Gynecology of Shin Kong Wu Ho-Su Memorial Hospital.

References

Successful transvaginal aspiration of interstitial pregnancy after failed methotrexate treatment


Address reprint requests to:
J.L. HWANG, M.D.
Department of Obstetrics and Gynecology
Shin Kong Wu Ho-Su Memorial Hospital
No.95 Wen Chang Road, Shih Lin District
Taipei City 105 (Taiwan)
e-mail: frank.fanny@msa.hinet.net
P450 oxidoreductase deficiency with maternal virilization during pregnancy

K. Nakanishi1,2, A. Yamashita3, T. Miyamoto1, R. Takeguchi2, A. Furuya4, K. Matsuo4, Y. Tanahashi4, M. Kawamura1, K. Sengoku3

1Department of Obstetrics and Gynecology and 2Department of Pediatrics, Wakkanai City Hospital, Wakkanai
3Department of Obstetrics and Gynecology and 4Department of Pediatrics, Asahikawa Medical University, Asahikawa (Japan)

Summary

Purpose: The authors report on a rare case of maternal virilization during pregnancy caused by autosomal recessive P450 oxidoreductase (POR) deficiency. Materials and Methods: A 24-year-old primigravida developed a deepening voice and hirsutism in the second trimester. Prenatal ultrasonography failed to detect any fetal abnormality and fetal growth was normal. POR deficiency was suspected, but the mother declined fetal genetic testing. A female neonate was delivered by cesarean section at 41 weeks’ gestation. Results: The neonate had skeletal abnormalities. Mutational analysis of the POR gene demonstrated homozygosity for c.1370 G>A and p.R457H in the patient and heterozygosity in her parents. POR deficiency was confirmed in the neonate. Conclusion: POR deficiency should be suspected in cases of maternal virilization. Maternal urinary estriol, fetal magnetic resonance imaging, and parental genetic testing should be performed. Parental consent for fetal genetic testing should be sought to ensure prompt diagnosis and early treatment.

Key words: Congenital adrenal hyperplasia; Genetic diagnosis; Maternal virilization; P450 oxidoreductase deficiency.

Introduction

Maternal virilization during pregnancy is rare. This condition may be caused by P450 oxidoreductase (POR) deficiency [1-4], which is an autosomal recessive genetic disease classified as congenital adrenal hyperplasia. Maternal virilization is associated with skeletal malformations, disorders of adrenal steroid synthesis, abnormal sexual development, and maternal virilization during pregnancy [1,2]. The authors describe a case of POR deficiency with maternal virilization during pregnancy.

Case Report

A 24-year-old primigravida with a spontaneous pregnancy was referred to the present hospital with a positive pregnancy test. Her personal and family histories were unremarkable. The expected delivery date was calculated from the fetal crown–rump length at ten weeks’ gestation, based on transvaginal ultrasonography. The woman was followed up at our hospital. Deepening voice and hirsutism started to develop in the second trimester and were worse at 38 weeks’ gestation. Maternal virilization is generally caused by POR deficiency, aromatase deficiency, luteoma, or Sertoli-stromal cell tumors. Female fetuses are more severely affected by maternal virilization than male fetuses. However, prenatal ultrasonography failed to detect any anomaly or determine the sex in the current case. The patient adopted a wait-and-see approach, but the present authors also consulted pediatricians in their hospital because of the possibility of the above-mentioned diseases. They explained the possibility of POR deficiency to the patient, but noted that it was difficult to make an accurate diagnosis based on diagnostic imaging alone. They suggested performing a genetic diagnosis by aspiration of umbilical blood and explained the potential risks of this procedure, but the patient declined and insisted that she wanted to deliver at the present hospital, even in the event of fetal anomalies. Measurement of serum androgen levels could aid diagnosis with no risk to the fetus. Although the current patient declined to undergo this blood test, it should be considered in future cases. The estimated fetal body weight was 2,795 g (+0.66 SD) at 36 weeks’ gestation, which was within the normal range. No abnormalities were detected prior to birth. Delivery was induced at 41 weeks and one day of gestation because of prolonged pregnancy, but an emergency caesarean section was performed because of arrested delivery. The neonate weighed 3,245 g with Apgar scores of 8 and 9 after one and five minutes, respectively. The neonate was diagnosed as female by transabdominal ultrasonography, based on the presence of clitoromegaly and a uterus. Abnormalities including craniosynostosis, joint contractures, arachnodactyly, vesicoureteric reflux, rhinopharyngeal stenosis, an abnormal foot shape, and cephalic angima were detected at the same time. POR deficiency was suspected from maternal virilization, as indicated by her deepening voice and hirsutism, and the physical findings of the neonate (Figure 1A, B).

Mutational analysis of the POR gene demonstrated homozygosity for c.1370 G>A and p.R457H in her parents and homozygosity for c.1370 G>A and p.R457H in the patient (Figure 1C). Genetic diagnosis confirmed that the neonate suffered from POR deficiency (Figure 1C).

Discussion

Maternal virilization during pregnancy is rare but can be caused by hyperandrogenemia. Maternal virilization is...
POR deficiency is associated with several diseases, including POR deficiency, aromatase deficiency, luteoma, Sertoli-stromal cell tumors, 21-hydroxylase (CYP21A21) deficiency, and 17α-hydroxylase deficiency [4]. Based on expert clinical advice and previous reports, the present authors diagnosed POR deficiency in the present patient with maternal virilization during pregnancy [1, 5, 6].

POR deficiency is an autosomal recessive genetic disease classified as congenital adrenal hyperplasia [1, 2]. The general incidence of POR deficiency has not been reported, although it is known to be rare in patients with congenital adrenal hyperplasia, and only approximately 80 cases have been reported [3, 7, 8]. POR is involved in electron transfer in microsomes and is required for the activity of all microsomal P450 enzymes, including CYP21A21, 17α-hydroxylase/17,20 lyase, and aromatase, as well as some non-P450 enzymes, including squalene epoxidase. Mutation of the POR gene results in abnormal complex formation and subsequent deficiency of POR [1, 2]. POR deficiency was thought to be embryonically lethal, based on mouse experiments; however, POR mutations were detected in humans in 2004 [1]. Mutational analysis in 35 Japanese patients demonstrated homozygosity and heterozygosity for c.1370 G>A and p.R457H [8]. A287P is the most common mutation in Caucasian patients, while R457H is the most prevalent founder mutation in Japanese patients [8]. Genetic analysis of the current patient showed similar results, with heterozygosity for c.1370 G>A and p.R457H in the patient’s parents (Figure 1C).

POR deficiency is often overlooked in patients with maternal virilization during pregnancy [9], and its prenatal diagnosis may be difficult in the absence of fetal ultrasonographic findings. However, POR deficiency should be suspected in the event of maternal virilization during pregnancy. The authors were able to diagnose POR deficiency in the present case on the basis of expert clinical

Figure 1. — The patient had a hypertrophic clitoris (A) and spider-like fingers (B). Genealogy of the patient and genetic analysis (C). Sequence analysis showed heterozygosity for c.1370 G>A and p.R457H in the POR gene in the patient’s parents (C), but homozygosity for c.1370 G>A and p.R457H in the patient (C).
advice and descriptions in previous reports [1, 5, 6].

Ultrasonography is useful for evaluating fetal anatomy and is usually the first technique used to detect abnormalities. However, recent developments in magnetic resonance imaging technology indicate that this may also be useful for identifying equivocal prenatal cases. Because estriol is produced by aromatization of fetal androgen precursors, POR deficiency is associated with low levels of estriol during pregnancy [3], and measuring maternal urinary estriol is therefore recommended in patients with suspected POR deficiency [3]. However, the current patient declined this test. Early detection is critical for timely treatment of this condition, allowing affected fetuses to be delivered in fully-equipped hospitals.

Conclusion

The authors report a patient with POR deficiency who developed maternal virilization during pregnancy. Early treatment of this condition is associated with improved prognosis, especially in relation to skeletal malformations. When maternal virilization is detected, maternal urinary estriol, fetal magnetic resonance imaging, and genetic analysis of the parents should be performed with patient consent. Additionally, informed parental consent should be sought for fetal genetic analysis after aspiration of amniotic fluid.

Acknowledgement

The authors would like to thank Dr. Kenji Fujieda (Department of Pediatrics, Asahikawa Medical University) for expert clinical advice.

References


Address reprint requests to:
T. MIYAMOTO, M.D.
Department of Obstetrics and Gynecology
Asahikawa Medical University, 2-1-1-1
Midorigaokahigashi, Asahikawa
Hokkaido 078-8510 (Japan)
e-mail: toshim@asahikawa-med.ac.jp
Growing teratoma syndrome after ovarian immature teratoma: a case report and review of the literature

O. Lapuente-Ocamica1, L. Ugarte1, M. Cuadra1, A. Lopez-Picado2, L. Maestro3, I. Lete1
1 Obstetrics and Gynecological Unit. Araba University Hospital, Alava
2 Araba Research Unit. Araba University Hospital, Alava
3 Pathology Department. University Hospital Araba, Vitoria-Gasteiz (Spain)

Summary
Growing teratoma syndrome is an uncommon complication of malignant germ cell cancer, characterised by the development of large tumours during or after chemotherapy, despite normalisation of tumour marker levels and metastasis, which contain only mature teratoma. Given its low incidence, little data is available. The authors present the case of a 15-year-old girl with a growing teratoma and the literature review outlines the current knowledge of its pathogenesis, common sites, diagnosis, natural course, treatment, and prognosis.

Key words: Growing teratoma syndrome; Immature teratoma; Malignant germ cell tumours.

Introduction
Malignant germ cell tumours (MGCTs) represent approximately 1-2% of malignant ovarian tumours [1] These tumours are classified into two main categories: dysgerminomas and non-dysgerminomas [1, 2]. The latter group includes immature teratoma, which accounts for 35.6% of all ovarian malignant germ cell tumours [3].

Immature teratoma, a tumour that arises from pluripotent germline stem cells, is composed of organoid and embryonic structures with variable levels of differentiation [4]. Usually, it occurs most commonly in girls and young people, and grows rapidly, with early metastasis development [4]. Growing teratoma syndrome (GTS) is a rare complication of immature teratoma, defined by the presence or development of large tumours during or after chemotherapy, normalisation of tumour marker levels, and metastasis that contains only mature teratoma [5-7]. It affects 1.9-7.6% of patients after treatment for non-seminomatous testicular cancer [6, 8-10]. GTS is less common in females, and hence most data available and the management of the syndrome are based on studies on non-seminomatous testicular cancer [9].

In this paper, the authors describe the case of a 15-year-old girl with a large immature teratoma in the left ovary who underwent fertility-sparing surgery and during chemotherapy, she was found to have an increase in residual tumour tissue, as well as new tumours, despite normalisation of alpha-fetoprotein (AFP) levels.

Case Report
The authors present the case of a 15-year-old girl referred by her general practitioner for a two-week history of pain and abdominal distension. Her gynaecological and obstetric history included menarche at 12 years of age and regular periods, and she had never had sex. During abdominal examination, the authors found abdominal distension, and pain with a palpable mobile mass, extending to four fingers above the level of the umbilicus. Endorectal ultrasound revealed a tumour with a lack of clear margins and heterogeneous content (solid-cystic) that seemed to be associated with the left ovary. A computer tomography (CT) scan of the pelvis revealed an abdominal mass of 18.6 x 12 x 25 cm extending upwards to the epigastrium and downwards to the pelvis, where it lay close to the left adnexa, with calcification foci and macroscopic fat tissue, suggestive of teratoma (Figures 1a, b). In addition, the authors observed cardiophrenic lymph node involvement and subcapsular perihepatic fluid collection in the liver segment VII. Tumour marker testing found AFP levels of 679 UI/ml, cancer antigen 125 (CA125) levels of 338.7 UI/ml, carbohydrate antigen 19-9 (CA19-9) levels of 352.6 UI/ml, and normal carcinoembryonic antigen (CEA) levels.

Given these results, the authors decided to perform infraumbilical midline laparotomy and found a large (20 x 12 cm) tumour associated with the left ovary with multiple peritoneal and epiploic implants. They proceeded to carry out left adnexectomy, peritoneal lavage for cytological analysis, partial omentectomy, appendectomy, and resection of most of the implants, except for those only millimetres in diameter in the pouch of Douglas. Histopathology showed grade 3 ovarian immature teratoma in Stage III according to the International Federation of Gynecology and Obstetrics (FIGO) system, with some mature teratomatous elements and gliomatosis peritonei (Figure 2).

A CT scan performed 23 days after surgery showed residual disease in the pouch of Douglas (58 x 67 x 20 mm) and the perihepatic region (60 x 40 x 80 mm), as well as an increase in bilateral cardiophrenic lymph node involvement. Subsequently,
Chemotherapy was initiated with three cycles of bleomycin, etoposide, and cisplatin (BEP). At three months the authors detected an increase in AFP levels: from 300 UI/ml after surgery to 623 UI/ml after the first cycles, together with radiological progression on CT with growth of the peritoneal implants in the pouch of Douglas and perihepatic region, as well as enlargement of cardiophrenic lymph nodes. After these findings the treatment was changed to paclitaxel, ifosfamide, and cisplatin (TIP).

After five cycles of chemotherapy with TIP, AFP levels normalised (4.4 UI/ml). However, a positron emission tomography (PET)-CT scan revealed progressive neoplastic disease with further increase in lymph node involvement; growth of the aforementioned implants, that of the pouch of Douglas reaching 73 x 33 mm, and the perihepatic growth 120 x 80 mm; and appearance of new implants in the retroperitoneal space and the right cardiophrenic angle (Figures 3a, b). Due to the suspicion of recurrence of immature teratoma, the authors decided to carry out rescue cytoreductive surgery, with peritoneal lavage and sample collection for cytological analysis, removal of the retrohepatic implant by peritonectomy with wide safety margins (Figures 4a, b), removal of the implant on the right kidney, opening of the right diaphragm, and cytoreductive surgery, with peritoneal lavage and sample collection for cytological analysis, removal of the retrohepatic implant by peritonectomy with wide safety margins (Figures 4a, b), removal of the implant on the right kidney, opening of the right diaphragm,
allowing the identification and removal of two implants (Figure 5), pelvic peritonectomy with en bloc resection of the uterus, right adnexa and implant in the pouch of Douglas, after dissection from the anterior part of the rectum, resection of the implant in the retropubic space, bilateral pelvic and aortocaval lymph node dissection, infragastric dissection of the greater omentum, opening of the left diaphragm allowing removal of cardiophrenic lymph nodes, and removal of the round ligament of the liver. The histopathological report, however, indicated that only mature teratoma had been found in all the samples sent for analysis (Figure 6). This clinical presentation is not attributable to a relapse of an immature teratoma, but rather chemotherapeutic retroconversion with the subsequent development of GTS.

In the immediate postoperative period, the patient was kept in the intensive care unit, and was transferred to the gynaecology ward and then discharged 14 days after surgery. At five months, the patient had no related symptoms, with no signs of disease on the follow-up CT and AFP levels of 1.1 UI/ml.

Discussion

Immature ovarian teratoma

Immature teratomas are large ovarian MGCTs, between six cm and 35 cm in diameter, encapsulated, with a smooth, nodular or lobulated margins [4]. In general, they affect young women, although a case of immature teratoma and subsequent GTS has been described in a five-year-old girl. Given that they grow rapidly and to large sizes, they tend to present with pain and abdominal distension. Further, they may be associated with elevated AFP levels [1], and for this reason, it is important to measure tumour markers in all women with this type of adnexal masses.

Histologically, they are composed of elements from all three germ layers: ectoderm, mesoderm, and endoderm [4]. Immature neural tissue is the main type of tissue in immature teratoma, the proportion of this tissue type being the basis for establishing the degree of differentiation, from grade 1 (well-differentiated) to grade 3 (poorly differenti-
Growing teratoma syndrome

This syndrome was first described by Logothetis et al. in 1982 in testicular mixed germ cell tumours [5]. Before then, in 1977, DiSaia et al. defined a similar phenomenon in ovarian MGCTs, which they called “chemotherapeutic retroconversion” [15]. More recently, Amsalem et al. concluded that these were the same phenomenon [6]. According to the original description, three criteria must be met to diagnose GTS: 1) an increase in clinical signs or radiological growth of the residual tumour or development of new tumours during or after chemotherapy for an MGCT; 2) normalisation of previously elevated levels of tumour markers (AFP, beta-human chorionic gonadotropin); and 3) histological analysis finding that metastases contain only mature teratoma [5-8, 16].

This syndrome, which is rare clinical entity, generally occurs during chemotherapy or up to two years after initiating the therapy [17], though some cases have been reported that occurred more than five years after the start of treatment [18,19].

Pathogenesis

The pathogenesis of GTS remains poorly understood, but two hypotheses have been proposed [6-8, 15]. According to the first theory, chemotherapy may induce the differentiation of malignant cells in the immature teratoma into mature teratoma; in this way, cells acquire a benign phenotype refractory to chemotherapy and grow on their own accord. The second hypothesis argues that chemotherapy may induce a selective destruction of immature elements, while mature elements, resistant to chemotherapy, persist and give rise to the syndrome. Several researchers claim that it is more likely that the underlying mechanism is the second hypothesis, given that is common to find mature teratomatous elements in primary tumours [6-8, 16].

Location

GTS tends to occur at the same sites as the primary tumour. However, cases of metastasis of the mature teratoma have also been described. The development of distant lesions of mature teratoma might be explained by the growth of small pre-existing lesions [8]. However, mature teratomas have a known tendency to metastasize, and therefore, a metastatic process cannot be ruled out. The retroperitoneum is the most common area of metastasis from testicular germ cell tumours, followed by the lung, and it is also the most common area of GTS [16, 20]. In the case of GTS arising from an ovarian MGCT, metastases tend to be confined to the pelvis, abdomen, and retroperitoneum, and distant metastasis are more rare [17]. Despite this, the present patient had metastasis in the pleural cavity and pericardium, which has not previously been described.

Diagnosis

Concerning predictors of the development of GTS, on the one hand, André et al. [8] described the following factors: 1) the presence of mature teratomatous elements in the primary tumour; 2) no reduction in the size of metastases during chemotherapy; and 3) the presence of mature teratoma in post-chemotherapy residual masses. This means that close monitoring by CT is warranted in high-risk patients, permitting early diagnosis of GTS and enabling complete tumour resection. Further, Moskovic et al. [21] described the radiological findings on CT that are suggestive of maturation and hence the development of GTS: an increased density of mass lesions, whose margins became better circumscribed in relation to adjacent tissues and the onset of internal calcification, with fatty areas of tissue and cystic changes. However, as conventional CT can underestimate tumours of less than one to two cm in diameter, in particular for those within the mesenterium and omentum, it may be useful to perform
18F-fluorodeoxyglucose (FDG) PET/CT to provide further information, to be assessed together with CT findings and tumour marker levels [22]. With GTS, there may be either positive [22] or negative [23, 24] FDG uptake. In summary, it is important to assess images and tumour marker levels together.

On the other hand, Mrabti et al. [25] considered gliomatosis peritonei in the initial laparotomy to be a predictive factor. Gliomatosis peritonei is a very uncommon entity defined by mature glial implants in the peritoneal cavity, omentum, and abdominal lymph nodes in patients with ovarian teratomas of any size. If implants have immature elements, however, they should not be classified as gliomatosis peritonei but rather as metastases of immature teratoma [25, 26].

**Disease course**

Despite the benign phenotype of GTS, two types of complications have been observed in these patients: mechanical complications and malignant transformation [7, 8, 27]. Mechanical complications are secondary to the pressure on neighbouring organs due to tumour growth: pain, intestinal obstruction, kidney failure due to ureteral compression, thrombophlebitis, and tissue necrosis. Concerning malignant transformation, Andre et al. [8] reported an incidence of 3% whereas Shigeta et al. [28] indicated a figure of 5.4%. The malignant potential of mature teratomas has been described, including their transformation to MGCTs, sarcoma, squamous cell carcinoma, adenocarcinoma, carcinoid tumours, and primitive neuroectodermal tumours [8]. There have been reports of the malignant transformation of GTS to sarcoma [29] and to carcinoid tumours [17]. This potential for malignant transformation supports complete resection of this type of tumour, given a greater risk of malignant transformation with incomplete resection [8]. Andre et al. [8] described that testicular MGCT developed in 50% of patients who underwent incomplete resection, compared to just 8% of those who underwent complete resection.

**Treatment and prognosis**

GTS, like mature teratoma, is refractory to both chemotherapy and radiotherapy; henceforth surgical treatment is the only available curative treatment. Complete surgical resection is recommended or, when this is not possible, maximal cytoreductive surgery [6, 7, 9].

In general, the long-term prognosis is good and some successful pregnancies have been described [10]. However, it is possible that further lesions appear after the treatment, especially in patients with incomplete resection [8, 22, 30, 31]. Cases of recurrence up to 19 years after the initial treatment of GTS have been described [17], and therefore it is essential to perform regular follow-up assessments for several years, using appropriate imaging techniques and measuring tumour marker levels [7, 17, 32]. Shigeta et al. [28] reported a 12.7% rate of recurrence, with a mean recurrence interval of 24 months. In their review of 48 cases of GTS, Kikawa et al. found that four out of the 48 patients who did not undergo complete resection had recurrence [22], indicating the importance of making as much effort as possible in the initial surgery. The rate of recurrence in cases of incomplete surgery is similar in GTS with a non-ovarian source, as in the series of André et al., who found recurrence in 83% of the patients with incomplete surgery compared to 4% in cases of complete tumour resection [8].

The appearance of new lesions requires more surgery, and such subsequent surgical interventions are increasingly radical. For this reason, it is important to assess the operability and weigh the risks and the benefits [9].

It is difficult to predict the potential growth of GTS. Cases of long-term stabilisation have been described, but there have been no reports of spontaneous regression. Cytokines, growth factors, and steroid hormones seem to be involved in the growth potential of these tumours [8]. For this reason, though no medical treatment has been established, when surgery is not possible, treatments have been based on agents including interferon alpha (α INF), bevacizumab, and cyclin-dependent kinase (CDK) inhibitor, PD0332991. Van der Graast et al. [33], Kattan et al. [16], and Inonue et al. [34] have described treatment with α INF in refractory cases of GTS. This seems to be a safe and well-tolerated treatment, but, although it is believed to delay cell growth, its mechanism of action remains unknown. On the other hand, Mego et al. [35] published a case of a patient with inoperable GTS treated with bevacizumab. This is a humanized monoclonal antibody that inhibits angiogenesis (a vascular endothelial growth factor inhibitor). Again, in this case, tolerance to the treatment was excellent. Finally, Vaughn et al. [36] used PD0332991, a selective inhibitor of CDK4/6, in patients with inoperable GTS. This treatment is based on the fact that mature teratomas express high levels of retinoblastoma protein and that CDK4/6 stimulates cell growth by means of phosphorylation of this protein. These three treatments achieved tumour growth stabilisation, with some cases of slight remission. However, these treatments are long term, given that their cessation would lead to tumour progression.

**Conclusions**

To conclude, GTS is a rare complication of MGCTs after chemotherapy that clinicians should be aware of to avoid it being mistaken for progression or recurrence, and to enable early diagnosis and immediate complete resection; thereby, preventing malignant transformation, problems secondary to compression of neighbouring structures, and the recurrence of GTS.
References

[37] O. LAPUENTE-OCAMICA, L. Ugarte, M. Cuadra, A. Lopez-Picado, L. Maestro, I. Lete

Address reprint requests to:
O. LAPUENTE-OCAMICA, M.D.
c/ Jose Atxotegi s/n, 01009 Vitoria-Gasteiz, Alava (Spain)
e-mail: oihane.lapuenteocamica@osakidetza.net
Introduction

In China, intrauterine device (IUD) is the most used long-acting reversible contraceptive method, with recent estimates indicating a percentage of 52.3% in the contraceptive prevalence rate [1]. In general, an IUD should be removed within 12 months, or preferably within six months of the last menses. Many women, however, ask to have their IUDs removed after one year or more after the last menses because of fear of pain or because they have forgotten it for a variety of reasons [2]. The authors report two cases of postmenopausal women who had a retained IUD for over 30 years. Written consent was obtained by both patients.

Case Report

Case 1
The 77-year-old female patient had a menopausal period of 27 years and a history of using IUD for 39 years. She was admitted due to complaint of pain in lower abdomen. With aggravation of the paroxysmal dull pain in lower abdomen, repeated medical and surgical diagnosis and treatment received no response. Ultrasonic examination detected multiple obvious echogenic spots inside myometrium and obvious IUD-like echo at local posterior wall of myometrium. Hysteroscopy found smooth, hard, yellowish-white and solid “mass” with irregular shape and multiple connections with uterine wall, while the IUD was not observed. The uterus section conducted after bilateral adnexectomy found irregular grayish-yellow hard “mess” inside the uterine cavity. Cutting out the “mess”, the circular metallic IUD was found completely encapsulated in an armor-shaped way with several incarcerations into deep myometrium. Postoperative pathology indicated proliferation of fibrous tissue around IUD accompanied by hyaline degeneration and calcification (Figure 1).

Case 2
The 76-year-old female patient had a menopausal period over 20 years and a history of using IUD for over 30 years. She was admitted due to postmenopausal vaginal bleeding for two days. The result of gynecological examination indicated uterine atrophy without positive findings. Ultrasonic examination detected obvious irregular echogenic spots around IUD inside the uterine cavity and fluid sonolucent area. Hysteroscopy found smooth, hard, yellowish-white and solid “mess” with irregular shape and diameter of about two cm as well as multiple connections with uterine wall. Metallic wire of the IUD was observed indistinctly at local position. Cutting out the “mess” gradually under uteroscope, the IUD encapsulated in the exposed mess was withdrawn. Postoperative pathology indicated proliferation of fibrous tissue around IUD accompanied by hyaline degeneration.

Discussion

As an essentially foreign matter for human body, IUD can induce mechanical damage, chronic inflammation of local tissue, and fibrosis lesion [3]. IUD with withdrawing difficulty in clinical practice may be incarcerated submucously or into superficial myometrium due to occasional embedding of fibrinoid tissue on the surface, however, extensive embedding of such scale, that the IUD is completely invisible, is an unusual situation [4, 5]. The suspected
causes include that the patient’s IUD was metallic and circular with large area and poor deformability. During postmenopausal period, the atrophic uterus becomes smaller while the IUD becomes relatively larger, which leads to press-in and incarceration of IUD into myometrium due to contracted uterus, and results in abdominal pain and vaginal bleeding. In addition, long-term stimulation by foreign matter induces chronic inflammatory response, forms foreign body granuloma that is surrounded by collagenous fiber, and eventually the IUD becomes encapsulated by the tumor-shape-liked neoplasm developed from hyaline degeneration of collagenous fiber layer upon layer.

The fibrous tissue encapsulating IUD was observed to be irregular tumor-shaped neoplasm under uteroscope. The tissue was hard with limited amount of blood vessels, yellowish-white to porcelain-white color, smooth surface, and appearance similar to a uterine fibroid with large proportion of fibrous tissue. Due to the encapsulation, diagnosis of the IUD requires combined ultrasonic or X-ray technology. Withdrawing the IUD encapsulated by fibrous tissue under uteroscope requires certain technique because premature dissociation would result in operation difficulty. The present authors’ experience of electric resection is to operate with increased power, remove the fibrous tissue without adhesion to uterine wall as well as that in the center first, namely to remove the encapsulating fibrous tissue to the largest extent with precondition of maintaining the IUD stable because a dissociated IUD would result in incision difficulty. Moreover, due to the hardness and deformation difficulty of the fibrous encapsulation, the dissociated IUD would be difficult to withdraw. Identification of the IUD in Case One was interfered due to more concomitant calcifications, which were
observed as obvious echo in ultrasound. In addition, due to a lack of awareness and multiple incarcerations nearly through serosa, hysterectomy was conducted to prevent perforation of uterus. In terms of incision for Case Two, the authors focused on withdrawing the dissociated IUD and pulled out the IUD without removing the fibrous encapsulation at central position. This operation resulted in dissociation of the IUD. Since the fibrous encapsulation was hard, smooth, and difficult to immobilize, incise, and clamp, there was a residual fiber ball with a diameter of approximately one cm left in the uterine cavity. Even though severe encapsulation of this type and serious clinical symptoms are unusual situations, some females did not have the IUD removed during postmenopausal period. It is recommended to remove the IUD within one to two years after menopause to avoid abdominal pain, bleeding, and postmenopausal intrauterine occupational disease, as well as the consequential surgical treatment.

Conclusions

The severe encapsulation by fibrous tissue cause difficulties in the retained intrauterine device removal. However, the removal is entirely possible with a good understanding of the pathogenetic condition and a skilled operation under hysteroscopy.

Acknowledgement

This study was financially supported by the National Natural Science Foundation of China (81202057).

References


Address reprint requests to:
H.Q. WANG, M.D.
Department of Obstetrics and Gynecology
Shandong Provincial Hospital
Affiliated to Shandong University
324 Jingwu Road, Jinan (P. R. China)
e-mail: 13969163910@163.com
Large pedunculated angiomyofibroblastoma of the vulva with concomitant anemia: a case report and mini review of the literature

O. Birge¹, A. Merdin², E.G. Ozbey³, D. Arslan³

¹ Department of Gynecology and Obstetrics, Nyala Sudan Turkey Training and Research Hospital, Nyala
² Department of Internal Medicine, Nyala Sudan Turkey Training and Research Hospital, Nyala
³ Department of Urology, Nyala Sudan Turkey Training and Research Hospital, Nyala (Sudan)

Summary

Angiomyofibroblastoma is a rare myxoid tumor and is often seen in vulvar and vaginal area. Patients usually present with a mass on the vulva or labium majus at the premenopausal ages. The lesions are usually between 0.5 cm and ten cm. Lesions have quite cellularity with regular borders under the microscope. Lesions contain numerous veins and bulging stromal cells. Treatment of angiomyofibroblastoma is simple surgical resection. Angiomyofibroblastoma should be differentiated from the other vulvar neoplasms, which need radical surgical treatment, especially from the aggressive angiomyxoma. Hereby, the authors aimed to report a premenopausal women presented with vulvar mass and accompanying anemia. She was diagnosed with angiomyofibroblastoma.

Key words: Angiomyofibroblastoma; Large pedunculated; Vulva.

Introduction

Angiomyofibroblastoma is a rare tumor of the vulva and it was first described by Fletcher et al. in 1992. It is a benign mesenchymal tumor, that usually arises in the genitai area, in particular in vulvar area of middle-aged premenopausal women [1]. It was initially conceived as a vulva specific neoplasm, but then was also shown to occur in the vagina, groin, rarely in the fallopian tube and rarely in the urethral area [8-10]. Angiomyofibroblastoma is rarely seen in men and occurs particularly in the scrotum, spermatic cord, and testicular tissue [11-14]. Its histopathology is not exactly clear, but the origin of the cells have been shown to be mesenchymal cells altered to the fibroblastic and myofibroblastic cells in the immunohistochemical analysis [15]. Benign solid tumors of the vulva are rarely seen. Hemangioma, lipoma, leiomyoma, myxoma, fibroma, and other pelvic masses showing myxoid changes should be considered in the differential diagnosis. Bartholin’s cysts (seen frequently in the vulvar area), vaginal cysts, vulvar abscess, Gardner cyst, and Nuck duct cysts should also be kept in mind in the differential diagnosis. It is particularly important to distinguish aggressive angiomyxoma from angiomyofibroblastoma, because of its aggressive and invasive pattern. Angiomyofibroblastoma differs from aggressive angiomyxoma by regular borders, high cellularity, containing plenty of blood vessels, minimal stromal mucin, and rarely erythrocyte extravasation. Angiomyofibroblastomas are treated with local excisions. On the other hand, aggressive angiomyxomas tend to relapse and require deeper resections because of their infiltrative characteristic [16].

Case Report

A 44-year-old female patient was admitted to the present clinic because of a palpable vulvar mass, which do not produce any symptoms and existed for the last two years. The mass had grown rapidly in the recent months. Physical examination revealed a mobile, soft, right labium majus originated and sessile mass with regular borders. The mass was ten cm in diameter, and had a five-cm stalk. In addition, the patient had a type 2 female genital circumcision history. There were some lesions, which might possible due to friction, observed on the mass (Figure 1). Ultrasonography showed a well-circumscribed mass with internal vascular areas and heterogenous echogenicity. The mass was totally excised under general anesthesia. The cut surface of the mass was grey in color and homogenous (Figure 2). Vulvar anatomy became normal after surgical excision (Figure 3). Pathological examination of the lesion revealed a lesion containing thin-walled dilated blood vessels in a variable cellular connective tissue. Oval bulging stromal cells with eosinophilic cytoplasm were detected around the vessels of the stroma. Meanwhile, stuck mucosal glands or nerve fibers were not observed. There were also no mitosis and no multinucleated giant cells (Figure 4). As a result, she was diagnosed with angiomyofibroblastoma. She had also anemia. Laboratory tests revealed 8.3 mg/dl of hemoglobin level with low MCV level. There were hypochromic microcytic erythrocytes without any blasts on the peripheral smear.
Benign solid tumors of the vulva are very rare. Other pelvic tumors like fibroma, lipoma, leiomyoma, hemangioma, and myxoma should be considered in the differential diagnosis. Fletcher et al. presented a ten case series of cellular variant angiomyxoma and named these cases as angiomyofibroblastoma [1]. Patients' ages ranged from 25 to 54 years (mean 36.3) [1]. The size of the vulvar masses in all patients ranged from 0.5 to 12 cm [1]. All had regular borders with myxoid homogeneous appearance under pathological examination [1]. Microscopic examinations revealed hypocellular hypercellular areas containing large number of thin-walled vessels without any interstitial hemorrhage [1]. All patients were treated with simple excision [1]. Many researchers have published studies with similar clinical and histological features of angiomyofibroblastoma after Fletcher et al. [2-7]. It is important to differentiate angiomyofibroblastoma from aggressive angiomyxoma because of different surgical approach and different prognosis. Angiomyofibroblastomas are generally smaller than five cm diameter, while aggressive angiomyxomas tend to be giant lesions. Angiomyofibroblastomas are lim-

**Discussion**

Benign solid tumors of the vulva are very rare. Other pelvic tumors like fibroma, lipoma, leiomyoma, hemangioma, and myxoma should be considered in the differential diagnosis. Fletcher et al. presented a ten case series of cellular variant angiomyxoma and named these cases as angiomyofibroblastoma [1]. Patients’ ages ranged from 25 to 54 years (mean 36.3) [1]. The size of the vulvar masses in all patients ranged from 0.5 to 12 cm [1]. All had regular borders with myxoid homogeneous appearance under pathological examination [1]. Microscopic examinations revealed hypocellular hypercellular areas containing large number of thin-walled vessels without any interstitial hemorrhage [1]. All patients were treated with simple excision [1]. Many researchers have published studies with similar clinical and histological features of angiomyofibroblastoma after Fletcher et al. [2-7]. It is important to differentiate angiomyofibroblastoma from aggressive angiomyxoma because of different surgical approach and different prognosis. Angiomyofibroblastomas are generally smaller than five cm diameter, while aggressive angiomyxomas tend to be giant lesions. Angiomyofibroblastomas are lim-
Angiofibromatosis of the vulva with concomitant anemia: a case report and mini review of the literature

Large pedunculated angiomyofibroblastoma of the vulva and have regular borders, but aggressive angiomyxomas invade into deeper tissues and therefore carry the risk of recurrence. Although mitosis are generally not seen in angiomyofibroblastoma, Takeshima et al. reported a mitotically active variant of angiomyofibroblastoma [3]. Nielsen et al. reported an angiomyofibroblastoma with sarcomatoid transformation and named that as angiomyofibrosarcoma [4].

Anemia is common in Sub-Saharan Tropical Africa. The major causes of anemia are iron deficiency anemia, vitamin deficiency anemia, sickle cell disease, and chronic disease anemia in this region. The authors do not think that anemia and angiomyofibroblastoma have a special relation and must be a coincidental situation.

In conclusion, angiomyofibroblastoma should be kept in mind in the differentiation of vulvar masses. Discrimination of angiomyofibroblastoma from aggressive angiomyxoma should be carefully done because of the need of aggressive surgical approach in the aggressive type.

References

LETTER TO THE EDITOR
Comment on “vaginal bilateral cervical lips suture in combination with intrauterine Foley catheter to arrest postpartum hemorrhage” - S. Matsubara - Tochigi, JAPAN ................................................................. 7

REVIEW ARTICLES

ORIGINAL ARTICLES
Reproductive Biology Section
Intravenous intralipid therapy is not beneficial in having a live delivery in women aged 40-42 years with a previous history of miscarriage or failure to conceive despite embryo transfer undergoing in vitro fertilization-embryo transfer - J.H. Check, D.L. Check - Camden, NJ, USA .......................................................... 14

General Section
Laparoscopic surgery improves pregnancy outcomes in women with suspected endometriosis with or without pathological confirmation - P.B. Miller, R.F. Savaris, D.A. Forstein, C.E. Likes, C. Nichols, L.J. Cooper, B.A. Lessey - Greenville, SC, USA ................................................................. 31
Predictive value of biochemical marker ADAM-12 at first trimester of pregnancy for hypertension and intrauterine growth restriction - C. Kasimis, N. Evangelinakis, M. Rotas, M. Georgitis, N. Pelekanos, D. Kassanos - Athens, GREECE ............................................. 43
Pregnancy after heart surgery – challenges - S. Plešinac, I. Pilić, I. Babović - Belgrade, SERBIA .......................... 48
Experience of assisted reproductive technology at King Abdulaziz University Hospital - H.S.O. Abduljabbar, S.T. Djamil, N.N. Sahly, D.S. Sawan, G.S. Ashour, A. Abduljabbar - Jeddah, KINGDOM OF SAUDI ARABIA .......................... 52
Fetal ventriculomegaly during pregnancy course, outcome, and psychomotor development of born children - J. Dukanac Stamenkovic, M. Steric, L. Srbinovic, T. Janjic, S. Vrzic Petronijevic, M. Petronijevic, A. Cetkovic - Belgrade, SERBIA ...................... 63
Correlation of twisting motion phase and infantile spasms in high risk infants - Y.Q. Wang, Z.X. Yang, P. Zhu, G.X. Gu - Xuzhou, CHINA ................................................................. 70
Expression and significance of ERβ and TrkB in endometriosis - X. Yu, H. Ren, T. Liu, M. Yong, H. Zhong - Dalian, CHINA ........................................................................................................ 75
The impact of chorionicity and type of conception on maternal-neonatal outcome in twin pregnancies - S. D’Arpe,
Comparison of mechanical artificial shrinkage methods in mouse blastocyst vitrification - J.K. Joo, J.E. Jeong, S.C. Kim, C.W. Kim, G.R. Ko, K.S. Lee - Busan, KOREA ........................................ 93

The possible role of serum leptin in preeclampsia - Y. Doster, B. Cetinkaya Demir, M.A. Atalay, E.E. Durusoy, S. Kucukkomurcu - Bursa, TURKEY .................................................. 98


CASE REPORTS
Chronic unremitting lower abdominal pain quickly abrogated following treatment with amphetamine - J.H. Check - Camden, NJ, USA .................................................. 109

Increased tissue permeability and sympathetic nervous system hypofunction may be the common link between dysmenorrhea, chronic pelvic pain, Mittelschmerz, and Crohn’s disease - J.H. Check - Camden, NJ, USA .................................................. 112

Comparison of efficacy of different embolic agents on uterine leiomyoma - Y. Mu, Y. Wang, M. Li, Y. Hu, Z. Hao - Baotou, Inner Mongolia, CHINA ........................................ 114

Dextroamphetamine sulfate treatment eradicates long-term chronic severe headaches from temporomandibular joint syndrome – a case that emphasizes the role of the gynecologist in treating headaches in women - J.H. Check - Camden, NJ, USA .................................................. 119

Failure of laparoscopic Vecchietti procedure in a woman with androgen insensitivity syndrome - D. Sawan, N. Sahly, H. Abduljabbar, A.A. Rouzi - Jeddah, KINGDOM OF SAUDI ARABIA ........................................ 123


Laparoscopic temporary clipping of uterine and ovarian arteries for the treatment of interstitial ectopic pregnancy - C. Kart, S. Guven, E.S. Guvendag Guven - Trabzon, TURKEY ........................................ 128

Incarceration of gravid uterus by growing subserosal myoma: case report - S.C. Kim, Y.J. Lee, J.E. Jeong, J.K. Joo, K.S. Lee - Busan, KOREA ........................................ 131

Laparoscopic subtotal hysterectomy due to giant uterine fibroids: a case report - J.Y. Ruan, H.Q. Chen, Y.H. Gong, G. Shi, H. Wang - Chengdu, CHINA ........................................ 134


Medical management of cesarean scar pregnancy at advanced age: case report and literature review - Ö. Birge, C. Karaca, D. Arslan, E. Kinali - Nyala, SUDAN ........................................ 140

Urinary catheterization as a successful treatment option for post-cesarean section vesicouterine fistula - A.A. Rouzi, N. Sahly, N. Mansouri, K. Khashoggi, L. Ashkar - Jeddah, KINGDOM OF SAUDI ARABIA ........................................ 143

Acute intestinal obstruction due to a non-involuted uterus after cesarean section: case report - K. Karaman, M. Ercan, H. Demir, M. Yener Uzunoglu, S. Bostanci - Sakarya, TURKEY ........................................ 146

Correlation of serum albumin with the clinical features and prognosis of preterm neonates in the neonatal intensive care unit - C.Y. Yang, B.Y. Li, P. Xu, Y.J. Yang, Q.Z. Yang - Liaocheng, CHINA ........................................ 149

Combination of selected biochemical markers and cervical length in the prediction of impending preterm delivery in symptomatic patients - M. Hadži-Lega, A. Daneva Markova, M. Stefanovic, M. Tanturovski - Skopje, REPUBLIC OF MACEDONIA ........................................ 154


No. 2, March-April

ORIGINAL ARTICLES
Reproductive Biology Section
Mid-luteal phase injection of subcutaneous leuprolide acetate improves live delivered pregnancy and implantation rates in younger women undergoing in vitro fertilization-embryo transfer (IVF-ET) - J.H. Check, C. Wilson, J.K. Choe - Camden, NJ, USA ........................................ 173

Correlation of ImmunoBead® and ImmunoSphere™ Immunoglobulin G (IGG) tests on detecting antisperm...
Sperm with an abnormal hypo-osmotic swelling test–normal fertilization, normal embryo development, but

EDITORIAL ARTICLE
Pregnancy rate and mid-luteal hormonal and sonographic parameters - J.H. Check, J.R. Liss, G. DiAntonio, D.

ORIGINAL ARTICLES

Reproductive Biology Section
A prospective comparison of outcome following cryopreservation using vitrification vs. a modified slow-freeze protocol of 2 pronuclear (2PN) and day 3 multi-cell embryos - D. Summers, J.H. Check, J.K. Choe - Mt. Laurel, NJ, USA ........................................... 271

Failure to increase the thickness of thin endometria with intrauterine infusion of granulocyte colony stimulating factor (G-CSF) - J.H. Check, J.K. Choe, D. Summers-Chase - Camden, NJ, USA ........................................... 274

General Section
Effect of mifepristone in the different treatments of endometriosis - Y. X. Zhang - Yantai, CHINA .................. 277

Endometriosis allergic or autoimmune disease: pathogenetic aspects - a case control study - D. Caserta, M. Mallozzi, F.M. Pulcinelli, B. Mossa, M. Moscarini - Rome, ITALY ........................................... 279

The chemosensitizing effect of aqueous extract of sweet fennel on cisplatin treated HeLa cells - W.S. Ramadan, Camden, NJ, USA ........................................... 282


Rectovaginal fistula caused by retained colpotomy cup after surgery - Y.S. Kwon, S. Ki You, K. Shil Im, A. Suk Kim - Ulsan, KOREA ........................................... 287


Chronic renal failure, diabetes mellitus type-II, and gestation: an overwhelming combination - E.N. Kontomanolis, S. Panagoutsos, P. Pasadakis, Z. Koukoulí, A. Liberis- Alexandroupolis, GREECE .................. 294


Idiopathic massive fetomaternal haemorrhage in the third trimester of pregnancy causing neonatal death - X. Peng, C. Liu, B. Peng - Chengdu, CHINA ........................................... 300

A rare occurrence of three consecutive autosomal trisomic pregnancies in a couple without offspring - F. Burada, S. Sosoi, D. Iliescu, M. Ioana, D. Cernea, S. Tudorache - Craiova, ROMANIA ........................................... 304

Successful conservative treatment of a cervical ectopic pregnancy at 13 weeks - S. Kadija, A. Stefanovic, K. Jeremic, M. Radojevic, R. Cerovic-Popovic, M. Srbinovic, I. Likic-Ladjevic - Belgrade, SERBIA ........................................... 307

Abdominal laparotomy formation with 30 years of evolution: report of a case - Yali Yang, Hong Zhou, Jin Guo, Wei Su, Hong Shen - Kunming, CHINA ........................................... 310

A rare complication of colporraphy anterior procedure: vesicovaginal fistula due to foreign body - O. Birge, D. Arslan, E. Kinali, C. Karaca - Nyala, SUDAN ........................................... 313


Uterine rupture in pregnancy: two case reports and review of literature - A. Pontis, C. Prasciolu, P. Litta, S. Angioni - Cagliari, ITALY ........................................... 320

No. 3, May-June

EDITORIAL ARTICLE

ORIGINAL ARTICLES
Reproductive Biology Section

A prospective comparison of outcome following cryopreservation using vitrification vs. a modified slow-freeze protocol of 2 pronuclear (2PN) and day 3 multi-cell embryos - D. Summers, J.H. Check, J.K. Choe - Mt. Laurel, NJ, USA ........................................... 271

Failure to increase the thickness of thin endometria with intrauterine infusion of granulocyte colony stimulating factor (G-CSF) - J.H. Check, J.K. Choe, D. Summers-Chase - Camden, NJ, USA ........................................... 274

General Section
Hemostatic effects of two desogestrel-containing combined oral contraceptive regimens: a multinational, multicenter; randomized, open-label study - K. Peters, N. Gordon, N. Ricciotti, J. Hsieh, B. Howard, H. Weiss - Hamburg, GERMANY ........................................... 281


Effect of mifepristone in the different treatments of endometriosis - Y.X. Zhang - Yantai, CHINA ........................................... 291

Endometriosis allergic or autoimmune disease: pathogenetic aspects - a case control study - D. Caserta, M. Mallozzi, F.M. Pulcinelli, B. Mossa, M. Moscarini - Rome, ITALY ........................................... 294

The chemosensitizing effect of aqueous extract of sweet fennel on cisplatin treated HeLa cells - W.S. Ramadan, Camden, NJ, USA ........................................... 297

No. 3, May-June

EDITORIAL ARTICLE

ORIGINAL ARTICLES
Reproductive Biology Section
Efficacy of a single injection of human chorionic gonadotropin at peak follicular maturation in natural cycles on pregnancy rate and mid-luteal hormonal and sonographic parameters - J.H. Check, J.R. Liss, G. DiAntonio, D. Summers - Mt. Laurel, NJ (USA) ........................................... 328

A prospective comparison of outcome following cryopreservation using vitrification vs. a modified slow-freeze protocol of 2 pronuclear (2PN) and day 3 multi-cell embryos - D. Summers, J.H. Check, J.K. Choe - Mt. Laurel, NJ, USA ........................................... 330

Failure to increase the thickness of thin endometria with intrauterine infusion of granulocyte colony stimulating factor (G-CSF) - J.H. Check, J.K. Choe, D. Summers-Chase - Camden, NJ, USA ........................................... 334

General Section
Hemostatic effects of two desogestrel-containing combined oral contraceptive regimens: a multinational, multicenter; randomized, open-label study - K. Peters, N. Gordon, N. Ricciotti, J. Hsieh, B. Howard, H. Weiss - Hamburg, GERMANY ........................................... 341


Effect of mifepristone in the different treatments of endometriosis - Y.X. Zhang - Yantai, CHINA ........................................... 358

Endometriosis allergic or autoimmune disease: pathogenetic aspects - a case control study - D. Caserta, M. Mallozzi, F.M. Pulcinelli, B. Mossa, M. Moscarini - Rome, ITALY ........................................... 361

The chemosensitizing effect of aqueous extract of sweet fennel on cisplatin treated HeLa cells - W.S. Ramadan, Camden, NJ, USA ........................................... 365
The incidence and risk factors of venous thromboembolism following elective gynecological surgeries without systemic thromboprophylaxis – an observational cohort study in a Chinese tertiary hospital - Z. Li, Z. Zhang - Beijing, CHINA

N terminal-pro brain natriuretic peptide in fetal umbilical cord meconium-stained amniotic fluid: a prospective case control study - M. Kaba, Y. Engin-Ustun, G. Kaba, E.G. Yaprak Eyi, S. Uysal, L. Mollamahmutoglu - Ankara, TURKEY


Clinical pathological features of endometriosis in abdominal wall – clinical analysis of 151 cases - J. Zhang, X. Liu - Shanghai, CHINA


Robot-assisted versus conventional laparoscopic surgery in the treatment of advanced stage endometriosis: a meta-analysis - Shao-Hui Chen, Zhao-Ai Li, Xiu-Ping Du - Taiyuan City, CHINA


Clinical importance of placental membrane microscopic chorionic pseudocysts in preeclampsia - K. Özkerkan, B.C. Demir, S. Baykara, K. Aslan - Bursa, TURKEY


Effects of apelin on proliferation and apoptosis in rat ovarian granulosa cells - L. Shuang, W. Jidong, P. Hongjuan, Y. Zhenwei - Jinan, CHINA

Expression and clinical significance of tumor markers in ovarian mature cystic teratoma - J.M. Chen, H.Y. Gao, Q. Wang, Q. Li - Changzhou, CHINA

CASE REPORTS

Abdominal scar endometriosis: case report - M. Miccini, M. Gregori, D. Ferraro, A. Ciardi, S. Cassibba, D. Biacchi - Rome, ITALY


Cardiac arrest: an unexpected complication during laparoscopic bilateral tubal ligation - a rare case report - Z. Kamalak, N. Köşüş, Ü. Isaoğlu, E. Ç. Tanrverdi, N. Isaoğlu - Sakarya, TURKEY


Diagnosis of antenatal Bartter syndrome - R. Narayan, M. Peres, G. Kesby - Sydney, NSW, AUSTRALIA

The improvement of hirsutism and the decrease of testosterone concentration in the peripheral blood serum after removing an endometrioid ovarian cyst - R. Słopień, M. Pawlak, A. Warenik-Szymankiewicz, S. Sajdak - Poznań, POLAND
Isolated subdiaphragmatic extralobar pulmonary sequestration: masquerading as suprarenal mass with spontaneous resolution - N. Obeidat, B. Sallout, W. ALAAli - Al-Riyadh, SAUDI ARABIA ......................................................... 457

Acardiac twin pregnancy: successful intrauterine ablative treatment with alcohol at 14 weeks of gestation - M.A. Guven, O. Koc, H. Bodur, S. Erkanli, T. Bagis, D. Usal - Istanbul, TURKEY .......................................................... 460

The role of the adhesion molecule Nectin-4 in the pathogenesis of endometriosis - R. Bedir, I. Sehitoglu, G. Balik, M. Kagici, H. Gucer, C. Yurdakul, P. Bagci - Rize, TURKEY ......................................................... 463

Digyneic partial hydatidiform mole with increased fetal nuchal translucency and ovarian hyperstimulation syndrome - C.R. Gaggero, S. Bogliolo, P. Sala, C. Molinari, M. Motzo, E. Fulcheri, P. Anserini, P. De Biasio - Genoa, ITALY ................................................................................ 467

Effect of different luteal support schemes on clinical outcome in frozen-thawed embryos transfer cycles - Z. Zhang, D. Su, P. Zhu, X. Bi, G. Qi, X. Wu - Taiyuan, CHINA .............................................................................. 486

Very unusual symptoms consistent with a possible migraine immediately following the injection of recombinant follitropin beta - J.H. Check - Camden, NJ, USA ............................. 484

Effect of different luteal support schemes on clinical outcome in frozen-thawed embryos transfer cycles - Z. Zhang, D. Su, P. Zhu, X. Bi, G. Qi, X. Wu - Taiyuan, CHINA .............................................................................. 486

A combination of ultrasound-guided rectus sheath and transversus abdominis plane blocks is superior to either block alone for pain control after gynecological transumbilical single incision laparoscopic surgery - R. Shimizu, R. Kawahara, R. Hanada, S. Okuno, K. Yamasaki, Y. Tamai, H. Kawahara - Osaka, JAPAN ......................................................... 501

Role of mean platelet volume and neutrophil/lymphocyte ratio to predict single-dose methotrexate treatment success in tubal ectopic pregnancy - Y. Cekmez, A. Gocmen, F. Sanilkan, S.B. Turkmen - Istanbul, TURKEY ........ 509

Diagnosis of endometriosis in women with chronic pelvic pain - R.M.F. Daher, J.C. Rosa-e-Silva, O.B. Poli-Neto, F.J. Cândido-dos-Reis, A.A. Nogueira - Ribeirão Preto, BRAZIL ......................................................... 512


Screening of sexual dysfunction in Saudi women before and after the age of 40 years - H.S.O. Abduljabbar, M.A. Alkasih, S.W. Khayat, R.M. Qotbi, M.T. Alkhatieb, A.H. Abduljabbar - Jeddah, SAUDI ARABIA ......................................................... 526

Effects of aloe vera gel on the induction of endometriosis and regression of endometrial explants in a rat model - M.S. Bostanci, M. Bakacak, O. Kizilkale Yildirim, G. Yildirim, R. Attar, F. Ozkan, F. Inanc Tolun - Istanbul, TURKEY ......................................................... 529

Effect of antenatal betamethasone administration on rat cerebellar expression of type 1a metabotropic glutamate receptors (mGlur1a) and anxiety-like behavior in the elevated plus maze - R. Pascual, M. Valencia, C. Bustamante - Valparaiso, CHILE ............................................................................. 534

Is there an association between serum vitamin D levels and endometrial polyps? - G. Ozaksit, A. Tokmak, H. Kalkan, E. Sarkkaya - Ankara, TURKEY ............................................................................. 539

Depressive symptoms’ pattern in postmenopausal women - R. Slopiew, A. Slopiew, M. Pawlak, A. Warenik-Szymankiewicz, S. Sajdak - Poznan, POLAND ............................................................................. 544
Effect of cervical conization on pregnancy outcome of in-vitro fertilization/intracytoplasmic sperm injection treatment: a retrospective cohort study - Lingling Ding, Miao Li, Lei Yan, Rong Tang, Zi-Jiang Chen - Jinan, CHINA .................. 546

The comparison of EMG-biofeedback and extracorporeal magnetic innervation treatments in women with urinary incontinence - N. Özengin, Y. Bakar, Ö. Cinar Özdemir, B. Duran - Bolu, TURKEY .................. 550

Effect of a low glycemic diet in patients with polycystic ovary syndrome and anovulation - a randomized controlled trial - L.H. Sordia-Hernández, P. Ancer Rodríguez, D. Saldivar Rodríguez, S. Trejo Guzmán, E.S. Servin Zenteno, G. Guerrero González, R. Ibara Patiño - Monterrey, MEXICO .................. 555

Perinatal and neonatal outcomes of maternal heart diseases - N. Aka, Ş. Arpacı, F. Vural, G. Köse - Istanbul, TURKEY .................. 560

Electromyographic activity of the pelvic floor muscles in the third trimester: comparison between primigravidae and secundigravidae - A.S. Moccellin, M.T. Rett, P. Driusso - São Carlos, SP, BRAZIL .................. 565


Attitudes towards abortion in Italian women: socio-economic trends and epidemiological features - D. Caserta, E. Matteucci, E. Ralli, F. Moscarini - Rome, ITALY .................. 578


CASE REPORTS

Uterine arteriovenous fistula after perforation during the placement of an intrauterine device – Minimally invasive treatment using uterine artery embolization - W. Kondo, M. Tessmann Zomer, F.L. Erzinger - Parand, BRAZIL .................. 602

Vasa previa rupture in velamentous insertion of the umbilical cord: an analysis and report of a case - Z. Zhang - Shanghai, CHINA .......................... 606

A case of discordant monochorionic diamniotic twin with umbilical cord entanglement after spontaneous rupture of the dividing membrane - Kwan Young Oh, Sang Kyu Kang, Chan Hee Jin, Yun Suk Yang - Daejeon, REPUBLIC OF KOREA .......................... 609


Appendectomy for asymptomatic appendicitis during caesarean section - an interesting case report - N. Panteleris, A. Danilidis, A. Stamatkopoulos, S. Kogeorgos, P. Chatzis, E. Assimakopoulos - Thessaloniki, GREECE .......................... 614

A laparoscopic surgery for deep infiltrating endometriosis and the review of literature - S.J. Liu, W. Lv - Hangzhou, CHINA .......................... 616


No. 5, September-October

REVIEW ARTICLES

The feasibility of establishing classification system for ovarian function - Wei Wang, Yan-Qing Wang, Ying-Ying Qin, Yu-Jie Dang, Ming-Di Xia, Ying Ma, Yu-Lan Mu - Jinan, CHINA .......................... 631

Dental management in pregnancy: recent trends - S.G. Vitale, S. Privitera, F.A. Gulino, A.M.C. Rapisarda, G. Valenti, D. Rossetti, M. Zigarelli, A. Cianci - Catania, ITALY .......................... 638

ORIGINAL ARTICLES

The predictor markers of ovarian response in poor responders under 40 years of age - B. Vural, Y. Çakıroğlu, F. Vural - Kocaeli, TURKEY ................................................................. 650

Colposcopy today - S. Dexeus, D. Dexeus - Barcelona, SPAIN ................................................................. 654

A novel laparoscopic surgical technique for severe adenomyoma - S. Liu, R. Shi, Y. Xie, H. Sun - Changzhou, CHINA ........................................................................................................ 656

Migraine management in pregnancy - Y. Ehi, L. Şahin, M.F. Mutlu - Kars, TURKEY ........................................... 661

Bulking agents – an analysis of 500 cases and review of the literature - Y. Zhao, X. Guo, K. Lobodasch, B. Liu, S. Wang, Q. Lin, Y. Yu, F. Su - Shenzhen, CHINA ...................................................... 666


Abdominal wall endometriosis occurring after cesarean section: an underestimated complication - W.Y. Wang, B. Wei, Y.X. Cao, X. Xie, C.Q. Li, Y.J. Xu - Hefei, CHINA ................................................................. 678

Application of fluorescence in situ hybridization (FISH) as a tool to aid cytogenetics in 1,409 fetal samples - A.C. de Moraes-Malinverni, F.R.S. Oshima, A.F. Moron, I.D.C.G. da Silva, M.M. de Souza - São Paulo, (Brazil) ........................................................................ 685

Evaluation of frequency of nausea and vomiting as well as depression level in pregnant women - S. Sahin, K. Ozdemir, A. Unsal, A.S. Cevrioglu, K.D. Beydag - Sakarya, TURKEY ................................................................................ 691

Predictive value of transvaginal ultrasound score for detection of endometrial malignancy - S. Mihajlovic, M. Vasiljevic, A. Jurisic, D. Kisic-Tepavcevic, D. Dimitrijevic, L. Nejkovic - Belgrade, SERBIA ...................... 698


Does amnioreduction increase success of emergency cervical cerclage in cases with advanced cervical dilatation and protruding membranes? - Y. Cakıroğlu, E. Doger, S. Yildirim Kopuk, A. Gunlemez, D. Oguz, E. Caliskan - Kocaeli, TURKEY ........................................................................ 708


Analgesia: effects on the first and second stages of labor - G. Carta, A. D’Alfonso, V. Franchi, P. Catana, S. Necozione, F. Patacchiola - L’ Aquila, ITALY .................................................................................... 718

Elective cervical cerclage versus no treatment in women with the history of cervical insufficiency: retrospective analysis of pregnancy outcomes - S. Kaya, S. Kayatas, A. Boza, M. Eroğlu, M. Api - Istanbul, TURKEY .................................................................................. 723

Effects of adding different doses of clonidine to intrathecal bupivacaine for spinal anesthesia in cesarean section - Zhi-Gao Pan, Bin Qian - Yancheng, CHINA ........................................................................ 727

Pelvic arterial embolization for postpartum hemorrhage: long term results of a single center experience in 29,091 deliveries - D. Rossetti, S.G. Vitale, F.A. Gulino, A. Biondi, P. Cignini, A.M.C. Rapisarda, S. Privitera, L. Frigerio - Catania, ITALY .................................................................................. 733

The protective effect of the proteasome inhibitor bortezomib on the uterus of ovariectomized rats - İ. Can, B. Büyük, S. Can, B. Karakaş, M. Bozkurt, S.A. Karameteş, S.S. İnalöz - Kars, TURKEY .................................................................................. 737


Couple-related factors of ART outcome? - P. Korovits, E. Lapp, R. Mändar - Tartu, ESTONIA .................................................................................. 747

Comparative analysis of perinatal clinical problems in early and late preterm infants - Z. Jin, X. Liu - Hwu, CHINA .................................................................................. 751

The association of impaired gestational glucose tolerance with maternal and fetal outcomes - E. Kasap, M. Genc, G.A. Turan, F. Eskicioğlu, A. Saklamaz, N. Sahin, S. Güçlü - İzmir, TURKEY .................................................................................. 755

Evaluation of ten years of intrauterine insemination results at a tertiary center - E.S. Güngör, C. Dane - Istanbul, TURKEY .................................................................................. 759

CASE REPORTS


Iatrogenic parasitic myoma on the peritoneum of the right pelvic wall - X. Yang, K. Ma, Shuang Zhang, He
Wang, Wenpei Bai - Beijing, China .................................................. 769


A case of placental mesenchymal dysplasia with one year follow-up - M. Cheng, B. Peng, Y. You - Chengdu, China .................................................. 777

No. 6, November-December

EDITORIAL ARTICLE


ORIGINAL ARTICLES

Flemish obstetricians’ personal preference regarding induction of labor and mode of delivery in term births - H. Sonnemans, A. Schmid, J. Muys, Y. Jacquemyn - Edegem, Belgium .................................................. 792

HELLP syndrome is still a serious, life-threatening complication of pregnancy: admission of 34 women to an eastern Turkish intensive care unit - Z. Bedir, A. Ahiskalioglu, Ü. Esenkaya, E.O. Ahiskalioglu, A. Dostbil, M. Aksoy, N. Dogan, H. Kursad - Erzurum, Turkey .................................................. 795


The influence of sperm parameters on the outcome of intracytoplasmic sperm injection-embryo transfer cycle in poor responder women under 35 years of age - A. Usta, M. Karacan, Z. Cebi, A. Arvas, M. Ulug, C.S. Usta, T. Camlibel - Istanbul, Turkey .................................................. 808


Serum carcinogenic antigen (CA)-125 and CA 19-9 combining pain score in the diagnosis of pelvic endometriosis in fertile women - H. Zhu, H. Lei, Q. Wang, J. Fu, Y. Song, L. Shen, W. Huang - Chengdu, China .................................................. 826

Impacts of maternal anxiety on non-stress test parameters - S. Nergiz Avcioğlu, S.O. Altinkaya, İ. Kurt Ömürlü, M. Küçük, S. Demircan-Sezer, H. Yüksel - Aydın, Turkey .................................................. 830

Management of bladder endometriosis with combined transurethral and laparoscopic approach. Follow-up of pain control, quality of life, and sexual function at 12 months after surgery - A. Pontis, L. Nappi, F. Sedda, F. Multini, P. Litta, S. Angioni - Cagliari, Italy .................................................. 836

Is maternal Vitamin D associated with gestational diabetes mellitus in pregnant women in Cyprus? - I. Soytec Inancli, E. Yayci, T. Atacag, M. Uncu - Lefkoşa, Cyprus .................................................. 840


Immunohistochemical expression of hormonal receptors, collagen, elastin, and proteoglycans in genuine urinary incontinence - E. Pantatosakis, D. Karandrea, E. Liapis, A. Kondi-Pafiti, A. Liapis - Athens, Greece .................................................. 849

The assessment of maternal and umbilical cord homocysteine levels in obese pregnant women - B. Kaya, S. Kaya, H.L. Keskin, S. Aydoğmuş, G. Akçay Yegin, A. Ekin, A.F. Avşar - Ankara, Turkey .................................................. 853

Three-dimensional transperineal ultrasound: is there a correlation among age, weight, delivery mode, and a change in the pelvic floor architecture in Korean premenopausal women? - S.J. Kim, M.J. Kim, T.H. Kim - Seoul, Republic of Korea .................................................. 857

The role of hysteroscopy in unexplained infertility - M. Di Muzio, A.M.L. Gambaro, V. Colagiovanni, L. Valentini, E. Di Simone, M. Monti - Rome, Italy .................................................. 862

Monitoring and treatment results of 88 HBsAg-positive pregnant women - S. Kolgelier, S. Sumer, N.A. Demir, Z. Ascı, L.S. Demir, S. Ozçimen, O. Ural - Konya, Turkey .................................................. 866

Clinical analysis of 95 cases with ovarian pregnancy - A.W. Le, Z.H. Wang, L. Shan, X.Y. Dai, T.H. Xiao, X.R. Li - Shenzhen, China .................................................. 871
Effects of estradiol injection on outcome of in-vitro fertilization: a randomized, double-blind, placebo controlled trial - A. Samsami, A. Zarei, S. Shahrivar - Shiraz, IRAN ................................................................. 875

Using the LigaSure vessel sealing device in the large uterus at laparoscopic hysterectomy - M. Biçer, Z. Güner, C. Karas, A. Güclü, Mert Göl - Izmir, TURKEY ................................................................. 880

Analysis of the reason of abnormal uterine bleeding induced by copper corrosion of IUD Cu - L. Li, J. Li, N. Li, Y. Zhang, X. Feng - Shijiazhuang, CHINA ................................................................. 883

CASE REPORTS

Bulky fibroid and pregnancy: myomectomy is possible during pregnancy - J. Lepage, B. Merlot, J.P. Lucot, D. Subtil - Lille, FRANCE ................................................................. 887

A case of a 42-year-old patient with anomalous origin of the left main coronary artery from the pulmonary artery who delivered three times with no complications: presentation, diagnosis, and review - M.S. Arnaout, M. Serhan, C. Saade - Beirut, LEBANON ................................................................. 889


Successful transvaginal aspiration of interstitial pregnancy after failed methotrexate treatment - I.F. Yang, J.L. Hwang, H.J. Chen, L.W. Huang - Taipei City, TAIWAN ................................................................. 899


Analysis on two postmenopausal women with clinical symptoms resulting from completely encapsulated intrauterine device by fibrous tissue - D.Y. Wei, L. Yan, H.Q. Wang - Jinan, CHINA ................................................................. 911

Large pedunculated angiomyofibroblastoma of the vulva with concomitant anemia: a case report and mini review of the literature - O. Birge, A. Merdin, E.G. Ozbey, D. Arslan - Nyala, SUDAN ................................................................. 914
Index of Authors in alphabetical order

Abduljabbar A., 52
Abduljabbar A.H., 526
Abduljabbar H., 123
Abduljabbar H.S.O., 52, 526
Adamopoulos A., 406
Agacayak E., 345
Aguiar A., 812
Ahiskalıoğlu A., 795
Ahiskalıoğlu E.O., 795
Ahn H.S., 643
Ahn I.M., 643
Aka N., 560
Akçal B., 427
Akcay Yegin G., 853
Akkaya H., 25
Aksan Desteli G., 673
Aksoy A., 241
Aksoy M., 795
AlAAlwi, 457
Albers-Heinert P., 203
Alborzi S., 225
Alkashi M.A., 526
Alkhatieb M.T., 526
Althinkaıı S.O., 830
Aly J., 319, 787
Ambrosini G., 137, 268
Anagnostou E., 818
Anastasiadis P., 406
Ancer Rodriguez P., 555
Andrei C., 265
Anfınan N.M., 358
Anghelache Lupascu I., 374, 619
Angioni S., 304, 836
Angioi V., 161
Aminos P., 406
Anserini P., 467
Antonakopoulos N., 818
Antsaklis P., 209
Asoyama T., 192
Aptı, 723
Arıcı B., 388
Arısoy R., 844
Armijo L., 37
Arnauot M.S., 889
Arıçi S., 560
Arslan D., 140, 297, 914
Arvas A., 808
Aslanıı M., 274
Asci Z., 866
Ashkar L., 143
Asci Z., 866
Akkaya H., 25
Akbak M., 427
Aksan Desteli G., 673
Arıcı B., 388
Ayalı C., 673
Aydogan B., 388
Aydın S., 241
Ayhan I., 812
Aydogmuş S., 853
Babic U.M., 245
Babović I., 48, 896
Bae J.Y., 393
Bagci P., 463
Bagis T., 460
Bahir Gur E., 500
Bai W., 769
Bakacak M., 529
Bakar Y., 550
Bakas P., 446
Balot Ö., 414
Balık G., 463
Bao S.L., 742
Başaran T., 388
Basaranoglu S., 345
Batinenoori M., 723
Battmaz G., 241
Baykar S., 401
Bedır R., 463
Bedır Z., 795
Bekkers L., 203
Benedetti Panici P., 88
Berghmans B., 203
Beydag K.D., 691
Bi X., 486
Biacchi D., 431
Bian X.Y., 376
Biçer M., 880
Bigi N.F., 265
Bin Q., 727
Bigaglio S., 467
Bilgili Ş., 486
Birge Ö., 140, 297, 914
Bilel S., 818
Blidaru I., 374
Bodur H., 460
Boglisiolo S., 467
Bolantııı I., 774
Bollendorf A., 175, 186
Bonatos G., 384
Bonilla Jr. F., 16
Bonilla-Musoles F., 16
Bostancı M.S., 529
Bostancı S., 146
Bostancı M.S., 529
Bostancı S., 146
Bostancı M.S., 529
Bostancı S., 146
Bostancı M.S., 529
Bostancı S., 146
Boutou A., 230
Boutas I., 446
Boza A., 723
Bozdag G., 82, 597
Bozkurt M., 737
Brunel I., 443
Bülbül G.A., 516
Burada F., 287
Bustamante C., 534
Butora D., 774
Büyük B., 737
Caballero O., 16
Cadete C., 16
Cai J., 300
Cakir D.U., 82
Cakirolgu Y., 708
Çakır T., 569
Çakır D.U., 233, 650
Calház-Jorge C., 812
Calik E., 258
Caliskan E., 708
Camlibel T., 808
Can I., 737
Can S., 737
Canbolat M., 427
Candelieri M., 88
Candido-dos-Reis F.J., 512
Cao Y.X., 678
Capar M., 569
Capobianco G., 137, 268
Carati D., 198
Carauarena A., 374, 619
Garza G., 718
Caserta D., 354, 578
Cassibba S., 431
Catana P., 718
Cebi Z., 808
Cekmez Y., 509
Celik E., 500
Cernea D., 287
Cerovic-Popovic R., 291
Cetinkaya Demir B., 98
Cetkovic A., 63, 274
Ceviroglu A.S., 691
Chafouz AE., 7
Chaleshitori M.H., 713
Chang E., 787
Chang S.D., 766
Chao A., 766
Chao A.S., 766
Chatzipapas I., 209
Chatzis P., 614
Check D.L., 14
Check J.H., 14, 109, 112, 119, 173,
175, 178, 181, 184, 186, 189,
319, 328, 330, 332, 479, 484, 787
Chen H., 300
Chen H.J., 899
Chen H.Q., 134
Chen J.M., 397
Chen S.-H., 422
Chen W.Q., 103
Chen X.L., 448
Cheng J.X., 592
Cheng M., 777
Cheng X.Y., 417
Cherchi C., 268
Cherchi P.L., 137
Cho Y.K., 643
Choe J.K., 173, 330, 332
Choi C.H., 621
Cianci A., 638
Ciardi A., 431
Cignini P., 733
Cinar Özdemin Ö., 550
Cinar Tanrıverdi E., 612
Cingözde E., 588
Çok T., 673
Colagiovanni V., 862
Cooper L.J., 31
Corona Martinez M., 279
Cosmi E., 137
Creatas M., 446
Cuadra M., 905
Cui Y.Y., 103
D’Alfonso A., 718
D’Amelio R., 88
D’Arpe S., 88
Da Silva I.D.C.G., 685
Daher R.M.F., 512
Dai X.Y., 871
Dane B., 241
Dane C., 241, 759
Danava Markova A., 154
Dang Y-J., 631
Danilidis A., 265, 614
De Biasio P., 467
De Donno A., 198
De Moraes-Malinvernì A.C., 685
De Santiago J., 443
De Souza M.M., 685
De Stefano M.G., 88
Dekhordi G.B., 713
Delibas I.B., 612
Demir A., 82
Demir B., 584
Demir B.C., 401
Demir H., 146
Demir L.S., 866
Demir N.A., 866
Demircan-Sezer S., 830
Demirci O., 844
Derbent A.U., 516
Dessole M., 137
Dessole S., 137, 268
Dexeus D., 654
Dexeus S., 654
Di Muzio M., 862
Di Simone E., 862
Di Antonio A., 181, 189
Di Antonio A., 178, 181, 189, 328
Diestro M.D., 443
Dilibas B., 584
Dilibas S., 584
Dimitrijević D., 698
Dinas K., 265
Djakovič I., 774
Djamal ST., 52
Dimitrovic A., 245
Dogan A., 490
Dogan N., 795
Doger E., 708
Dong M.Y., 212
Dong Y., 742
Dostbii A., 795
Doster Y., 98
Dotlic J., 896
Drakakis P., 818
Drusko P., 565
Du Q., 703
Du X.-P., 422
Dukanac Stamjenović J., 63
Duran B., 550
Duroseau M., 189
Dursunsoy E.E., 98
EUROPEAN ACADEMY
OF GYNAECOLOGICAL CANCER, EAGC

Chairman: Péter Bősze (Hungary)

Executive Board:
PIERLUIGI BENEDETTI PANICI (Italy)
CARLOS F. DE OLIVEIRA (Portugal)
GIUSEPPE DE PALO (Italy)
SANTIAGO DEXEUS (Spain)
WILLIAM DUNLOP (UK)
STELIOS FOTIOU (Greece)
GERALD GITSCH (Austria)
A. PETER M. HEINTZ (Netherlands)
MICHAEL HOECKEL (Germany)
JAN JACOBS (UK)
JACQUES LANSAC (France)
TIZIANO MAGGINO (Italy)
HARALD MEDEN (Germany)
JOSEPH MONSONEGO (France)
LASZLÓ PÁLFALVI (Hungary)
SERGIO PECORELLI (Italy)
DENIS QUELLEU (France)
STELIO RAKAR (Slovenia)
PIERO SISMONDI (Italy)
CLAES TROPÉ (Norway)
LÁSZLÓ UNGÁR (Hungary)
ANDRÉ VAN ASSCHE (Belgium)
RAIMUND WINTER (Austria)

International Advisory Board
Chairman: Antonio Onnis (Italy)

HUGH ALLEN (Canada)
CURT W. BURGER (Netherlands)
ALBERTO COSTA (Italy)
ANDRÉ GORINS (France)
NEVILLE F. HACKER (Australia)
MARIA MARCHETTI (Italy)
STELIOS P. MICHALAS (Greece)
MARIA TERESA OSORIO (Portugal)
ULF ULMSTEN (Sweden)
JAN B. VERMORKEN (Belgium)
GEORGE D. WILBANKS (USA)
JAN ZIELINSKI (Poland)

All questions concerning the Academy may be sent to:
PETER BOSZE, M.D.- P.O. Box 46 - Budapest 1301 (Hungary)
Phone: +36 1 4290317 - Fax: +36 1 2752172 - E-mail: eagc@cme.hu

www.cme.hu

Administrative Office:
1301 Budapest, P.O. Box 46 - Hungary
Fax (36 1) 4290318 - E-mail: eagc@cme.hu
CLINICAL AND EXPERIMENTAL OBSTETRICS & GYNECOLOGY
Subscription Order Card 2017

Founded in 1974 (ISSN 0390-6663) - Vol. XLIV. Issued bimonthly. All subscriptions are entered on a calendar-year basis. Individual rate is not applicable if payment is made through an Institution.

Subscriptions are entered with prepayment only and are accepted per calendar year only but can be backdated depending on availability. If not cancelled by the end of October, they will be tacitly considered as renewed; cancellations will not be refunded.

Discounts: 10% to book sellers and subscription agencies.

Please enter my subscription at the rate I have checked:

<table>
<thead>
<tr>
<th>PAPER ISSUE</th>
<th>ONLINE ISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institutional: 700 USD</td>
<td>Institutional: 450 USD</td>
</tr>
<tr>
<td>Individual: 500 USD</td>
<td>Individual: 270 USD</td>
</tr>
<tr>
<td>Single copy: 150 USD</td>
<td>Single issue: 100 USD</td>
</tr>
<tr>
<td>Single article: 30 USD</td>
<td></td>
</tr>
</tbody>
</table>

Payment: (USD ONLY)

☐ for PDF file: online through PayPal (all credit cards)
☐ for hard copy

Credit Card:
☐ Mastercard ☐ Visa ☐ Diners

Bank transfer: Beneficiary: 7847050 Canada Inc. - 4900 Côte St-Luc, #212 - Montréal, Québec, H3W 2H3 Canada - Account number 00001 003402-402245 SWIFT ROYCCAT2

N° ____________________ Exp. Date ____________

Signature ____________________ Date ____________

An invoice is issued only after payment is processed; no proforma receipts will be issued. The subscription order form is available through the Montréal office (Fax +1-514-485-4513) or Padua office (Fax +39-049-8752018) or through our website www.irog.net

EUROPEAN JOURNAL OF GYNAECOLOGICAL ONCOLOGY
Subscription Order Card 2017

Founded in 1980 (ISSN 0392-2936) - Vol. XXXVIII. Issued bimonthly. All subscriptions are entered on a calendar-year basis. Individual rate is not applicable if payment is made through an Institution.

Subscriptions are entered with prepayment only and are accepted per calendar year only but can be backdated depending on availability. If not cancelled by the end of October, they will be tacitly considered as renewed; cancellations will not be refunded.

Discounts: 10% to book sellers and subscription agencies.

Please enter my subscription at the rate I have checked:

<table>
<thead>
<tr>
<th>PAPER ISSUE</th>
<th>ONLINE ISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institutional: 700 USD</td>
<td>Institutional: 450 USD</td>
</tr>
<tr>
<td>Individual: 500 USD</td>
<td>Individual: 270 USD</td>
</tr>
<tr>
<td>Single copy: 150 USD</td>
<td>Single issue: 100 USD</td>
</tr>
<tr>
<td>Single article: 30 USD</td>
<td></td>
</tr>
</tbody>
</table>

Payment: (USD ONLY)

☐ for PDF file: online through PayPal (all credit cards)
☐ for hard copy

Credit Card:
☐ Mastercard ☐ Visa ☐ Diners

Bank transfer: Beneficiary: 7847050 Canada Inc. - 4900 Côte St-Luc, #212 - Montréal, Québec, H3W 2H3 Canada - Account number 00001 003402-402245 SWIFT ROYCCAT2

N° ____________________ Exp. Date ____________

Signature ____________________ Date ____________

An invoice is issued only after payment is processed; no proforma receipts will be issued. The subscription order form is available through the Montréal office (Fax +1-514-485-4513) or Padua office (Fax +39-049-8752018) or through our website www.irog.net