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Letter to the Editor

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In Memoriam:
Antonio Onnis

Antonio Onnis, an outstanding surgeon and researcher in gynaecological oncology, passed away on August 17, 2009. He was born in Cagliari on September 24, 1927. After graduating from the University of Padua in 1951 he worked in the fields of pathology, pharmaceutical chemistry, obstetrics and gynaecology at the Universities of Padua and Sassari from 1953 to 1968. He became Head Professor and Director of the Second Department of Obstetrics and Gynaecology of Padua University (1967 to 1969), then he moved to Verona University (1969 to 1974), and afterwards he returned to Padua University from 1974 to 1998. He was the author of more than 450 publications, monographs and textbooks on topics of pathology, pharmacology, endocrinology, obstetrics and gynaecology, surgery, gynaecological oncology, isotope and chemotherapy, and cancer prevention and early detection. His scientific publications covered many fields: gynaecology, obstetrics and especially gynaecological oncology. He was awarded the Musaia Medal from the University of Padua, the Gold Medal from the Italian Society of Gynaecology and Obstetrics, the Gold Medal from the Dexeus Institute of the University of Barcelona,
and the Gold Medal from the University of Sassari for scientific activity. He was a member of the Subcommittee for Cancer Prevention and Mass Screening of Cervical Cancer in the European Community’s programme “Europe Against Cancer”, of the Italian National Health Ministry for Women’s Health Care, and of the Italian National Health Ministry for Assisted Fertilization. He founded the Italian Society of Gynaecological Oncology in 1976 and the European Society of Gynaecological Oncology (ESGO) in 1983. For ESGO he organized and was president of many international meetings of Gynaecological Oncology in Venice in 1979, 1982, 1983, 1985 and 1991, and was honorary president of the meetings in Paris (1987), Barcelona (1993), Knokke (1995), Coimbra (1997) and Budapest (1999). He was an active and honorary member of many Italian and International Societies in every area of obstetrics, gynaecology, gynaecological urology, gynaecological oncology, and pelvic surgery. One of his greatest virtues was to open the door to scientific communication with colleagues from Eastern European countries involving them in an exchange and comparison of ideas and experiences. In gynaecological oncology he was a real pioneer with his experience in chemotherapy, trophoblastic disease, local-regional chemotherapy, and in the lymphographic technique for gynaecological cancers. His contribution to radical surgery avoiding useless demolition or mutilation was fundamental, especially for the “nonmutilant radical vulvectomy”. His humanity was always present in his approach to oncology patients. His surgical activity extended to every field of gynaecology but gynaecologic-oncological surgery was his principle involvement. In this activity he created a school for his pupils and for many gynaecologists from every part of the world who came to the Padua Clinic to learn the surgical techniques. Antonio Onnis retired from his activity in 1999 but was always involved in scientific research and meetings. He received the Laurea Honoris Causa from the Alma Mater University of Warsaw (2002) and Krakow University (2003) for his contributions, experiences, and high scientific value over his entire career. Above all his activities, Antonio Onnis was always a teacher and example of scientific honesty and humanity for his pupils and in his approach with colleagues and patients. The memory of him will be unforgettable to all those who knew him even if his character was not always the easiest. His intelligence, generosity and humanity – which at times were masked but never really abandoned – have always prevailed. The man, the scientist, and the teacher that we have lost will forever leave a void in all those who knew him.

M. Marchetti
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A practical approach to the prevention of miscarriage: Part 1 - progesterone therapy

J.H. Check, M.D., Ph.D.
The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To show evidence that progesterone therapy is useful in preventing miscarriages in women who are more prone to having them. Methods: Vaginal progesterone therapy was evaluated in women with a previous history of miscarriage or in women with infertility related to luteal phase defects. Results: The results favor the benefit of using progesterone to diminish the risk of miscarriage. Other methods of stimulating progesterone production, e.g., human chorionic injections, are also effective. Conclusions: Progesterone therapy, especially when given vaginally, is effective with few side-effects and is safe. Thus the evidence suggests that one should err on the side of over-treatment rather than under-treatment in certain circumstances, e.g., advanced woman’s age, previous history of miscarriage, or the use of follicle maturing drugs.

Key words: Miscarriage; Luteal phase defects; Progesterone therapy; Human chorionic gonadotropin; Follicle maturing drugs.

Progesterone is essential for maintaining a normal pregnancy

Surgical removal of the ovary with the corpus luteum of pregnancy prior to eight weeks when there may not as yet be adequate placental progesterone production generally leads to a miscarriage [1]. The use of a progesterone receptor antagonist during the first trimester, e.g., mifepristone, leads to a high rate of spontaneous miscarriages [2-4]. In contrast high pregnancy rates can be achieved in anovulatory donor egg recipients without a corpus luteum by giving exogenous progesterone [5].

The luteal phase defect and endometrial biopsy

Over 55 years ago Noyes et al., stated and provided histologic dating of endometrial biopsies and provided daily characteristic changes of the endometrium throughout the luteal phase [6]. The out of phase endometrial biopsy (defined as endometrium taken from the mid to late luteal phase appearing two or more days earlier than expected) was first studied by Jones and Delfa and the conclusion they made over 55 years ago was that the luteal phase defect was estimated to be the apparent etiologic factor in 35% of first trimester miscarriages [7]. However, an infertility or recurrent miscarriage pathological state related to persistent defective corpus luteum function has become a debated issue over the years including the present time. It is not clear how the policy was established, maybe by an ad hoc committee, but the decision was made that to diagnose a luteal phase defect the biopsy must be either two or more than two (another debate) days out of phase in two consecutive cycles [8]. Thus by this definition only a woman with two consecutive out of phase endometrial biopsies should be treated with progesterone if they have had a problem with recurrent miscarriages.

Nonetheless consider the paradigm of a woman who has a luteal phase deficiency in 50% of her menstrual cycles. If progesterone therapy would reduce her risk of another miscarriage by 50% and if sticking to the requirement of only treating if the biopsy is abnormal in two consecutive cycles, then only 25% of the women who could benefit from progesterone therapy in this paradigm would be offered progesterone treatment.

The other problem with establishing whether a woman has a luteal phase defect is whether the biopsy is more accurate when performed in the late luteal phase to gain more accumulative effect of progesterone [9-11] or mid-luteal phase when implantation occurs [12-14]. Other controversies involve whether abnormal should be considered if the biopsy is greater or equal to two days out of phase [15, 16] or greater than two days out of phase [17, 18]. Other concerns are that the same slide is frequently dated differently by different pathologists. These and other problems have led the majority of infertility specialists to abandon this diagnostic tool for infertility purposes. As I will discuss below, though I am a strong advocate that the use of exogenous progesterone can reduce the risk of miscarriage, I no longer use the endometrial biopsy to determine who should receive this therapy.

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Candidates for progesterone therapy to prevent miscarriage

The frequency of miscarriages in the normal population has been estimated to be approximately 14% [19]. One study suggested that approximately a third of women who previously had a miscarriage will have another loss in their next pregnancy [20].

Some clinicians argue that since accidental meiosis errors are responsible for the majority of any given miscarriage, many will advise a woman who had a miscarriage but no chromosomal evaluation of the fetus that probably based on statistics this was a fetus with an aneuploidy and that she has no greater chance of this happening again than a woman who did not previously miscarry.

If progesterone therapy can prevent miscarriage (and evidence of its efficacy will be presented subsequently) my argument is what if it was not a chromosome issue but a progesterone deficiency? I raise the question – which is worse, treating a woman with progesterone who did not need it, although she has a subsequent successful delivery that could have been achieved without progesterone, or a woman who has a need for progesterone but does not take it and subsequently has a miscarriage because of not taking it.

As women reach advanced reproductive age (≥ age 40) there is a higher rate of miscarriages related both to an increased rate of aneuploidy and also to an increased need for progesterone. A miscarriage at any age is psychologically devastating so if a trisomy abnormality is documented in a 41-year-old woman’s first pregnancy and miscarriage, I would still recommend the use of supplemental progesterone from the early luteal phase throughout the first trimester because irrespective of the cause of the first loss she is more prone to another loss from either a chromosome abnormality, which cannot be helped, or a relative progesterone deficiency, which can be helped.

Since many women who are merely more prone to miscarriage from a progesterone deficiency may still have a normal outcome if untreated it would take a very large study to show a significant improvement by progesterone therapy for women with only one or two previous losses, especially with some live deliveries thrown into the mix. This group is referred to as secondary aborters.

A study using supplemental vaginal progesterone suppositories started at a low dose of only 25 mg twice daily in the luteal phase then doubled with a positive pregnancy test and with further increases in dosage for bleeding or cramping resulted in a miscarriage rate of only 10% (10 of 100) in women who had at least one previous miscarriage [21]. Breaking them down according to how many previous miscarriages they had, there was a 5% miscarriage rate (1 of 20) in those with one previous loss, 4.8% with two miscarriages (3 of 62) and 33% (6 of 18) with three or more losses. Interestingly all six women with three or more losses who miscarried despite progesterone therapy were successful in the second treatment cycle of progesterone therapy [21]. This was not a controlled study but the 10% miscarriage rate showed a trend to be lower than the normal expected miscarriage rate of 14% [19] and certainly less than the 33.3% rate found in women with previous losses [20].

A study by Yeko et al. found that when a pregnant woman had a serum progesterone level less than 15 ng/ml a miscarriage is inevitable [22]. However another study found that with aggressive progesterone therapy given at the point of a serum progesterone < 15 ng/ml that 70% of the women will proceed to have a successful live delivery [23]. Another study even found a 60% live delivery rate with a serum progesterone less than 8 ng/ml with the use of aggressive progesterone supplementation [24]. Thus other candidates for progesterone therapy to try to prevent miscarriage are women who present with low serum progesterone levels during their pregnancy [23, 24].

It is not clear what the proper level of progesterone is during pregnancy but most normal pregnancies in my experience have levels over 30 ng/ml three weeks after conception. Thus I would start a pregnant woman on progesterone supplementation if she presents with a level < 30 ng/ml and I would suggest raising the dosage if she was already on it.

Though a low serum progesterone level during pregnancy would prompt the decision to raise or initiate progesterone therapy, a normal serum progesterone level does not preclude the use of progesterone to prevent miscarriage. The main mechanism by which progesterone prevents miscarriage may be through the stimulation of immunomodulatory proteins that in turn inhibit natural killer cells from attacking the fetal semi-allograft [25-27]. The relative role of progesterone and lymphocyte immunotherapy will be discussed more fully in part II of this editorial. Suffice it to say now that this 34 kDa protein has been synthesized by recombinant DNA technology. The development of ELISA assays that allow rapid results will soon be available.

Obviously if I think that progesterone therapy benefits women even with a history of one previous miscarriage even when live births have occurred I would think it should benefit women with recurrent miscarriages defined as at least three consecutive miscarriages. Studies have shown that the risk of subsequent miscarriage in women with three to five consecutive miscarriages ranged between 42-86%, 41-72% and 23-51%, respectively [28-31]. A group of women with recurrent miscarriages with four previous losses experienced a 51% miscarriage rate in their next pregnancy supplemented with progesterone [32]. As will be discussed in part II this may be the group who will show a more impressive response with lymphocyte immunotherapy [32]. It should be noted that many of these women in the aforementioned study had progesterone therapy previously and still had a miscarriage [32]. Perhaps the miscarriage rate would be lower in women with ≥ 4 recurrent miscarriages who never previously had progesterone therapy who are now treated with the progesterone hormone for the first time.
Methods of administering progesterone

One way of administering progesterone is by intramuscular (IM) injection. It is rapidly absorbed and produces measurable serum levels within two to eight hours. It has a slow clearance when administered in an oil vehicle. However IM progesterone in oil can be associated with a lot of side-effects. It is not unusual for women to develop an allergy to the peanut oil vehicle. Sometimes the progesterone is then suspended in olive oil and sometimes in ethyl oleate. However other complications including sterile abscesses, bleeding into the muscle, and pain at the injection site have occurred. Furthermore the use of IM progesterone requires the aid of another person for administration.

Parenteral IM progesterone has been used to treat infertility and miscarriages for over 45 years [7]. Compounded progesterone vaginal suppositories have been used for over 20 years [18, 33-36]. One of the disadvantages of vaginal progesterone suppositories compounded by pharmacies is that there is no control on batch-to-batch variations with no governing agency watching for quality control. Furthermore the suppositories result in a significant vaginal build up causing vaginal irritation [37]. They leak at room temperature and thus are messy and may lead to yeast infections [37].

One can reduce the irritation from these vaginal suppositories by adding vitamin E to the suppository.

To improve the efficacy and reduce side-effects of vaginal progesterone there have been attempts at commercial development of vaginal progesterone. These FDA approved preparations will be discussed subsequently.

There has been commercial development of progesterone which can be administered orally. Oral progesterone in 100 and 200 mg tablets has been marketed under the brand name Prometrium®. However it is rendered mostly ineffective by the rapid metabolism that occurs by the rapid first pass effect in the liver [38]. Thus though the drug produces good serum levels of progesterone the concentration is not very high in the endometrium where it counts [38]. Therefore oral progesterone is considered much less effective than IM or vaginal progesterone [39]. Furthermore the metabolites of oral progesterone can cause significant side-effects such as lightheadedness, vertigo, drowsiness, and gastric discomfort.

Vaginal progesterone preparation approved by the Food and Drug Administration

Progestrone gels - Crinone® and Prochieve®

Vaginal progesterone achieves lower serum levels but higher progesterone levels in the endometrial tissue than IM progesterone [40]. Crinone vaginal gel was the first progesterone preparation including oral or IM preparations approved for IVF-ET. It adheres very effectively to the vagina. Thus a 90 mg one-time daily insertion may be equal to a 400-600 mg compounded vaginal suppository. This adhesiveness leads to one of the main side-effects of Crinone vaginal gel and that is an accumulation of a significant buildup of the vaginal gel leading sometimes to irritation.

FDA approved vaginal progesterone tablets

Endometrin vaginal tablets (100 mg) are the newest vaginal natural progesterone approved by the FDA. The theoretical advantage of Endometrin compared to the vaginal suppository is that the tablets are made to absorb vaginal secretions and disintegrate into an adhesive powder that adheres to the vaginal epithelium thus facilitating sustained absorption [41]. Theoretically the formulation would cause less perineal irritation [41].

A study was performed comparing absorption and the side-effects of perineal irritation from Endometrin vs a commercially available vaginal progesterone suppository available in Europe known as Cyclogest [42]. The study found that 200 mg of Endometrin was able to produce the same serum levels after six days compared to 800 mg Cyclogest [42]. Though there was no significant difference in vaginal irritation between the two preparations there was a trend for less irritation from Endometrin [42].

Safety of progesterone treatment during pregnancy

The Food and Drug Administration (FDA) issued a warning stating that the use of synthetic progesterone or natural progesterone may be associated with various congenital abnormalities including VACTERL syndrome neural tube defects and heart abnormalities [8]. However, subsequent studies did not corroborate the FDA’s warning finding no risk of birth defects with the use of supplemental progesterone during the first trimester [43, 44]. Despite the widespread use of supplemental progesterone with assisted reproductive technology the FDA has never rescinded the warning even though their own Obstetrics and Gynecology Advisory Board recommended not to put this label on the progesterone [8]. In fact the use of progesterone may prevent neonatal consequences by preventing preterm deliveries [45]. Nevertheless most manufactured items still are using the FDA’s warning about progesterone.

Other therapies for luteal phase defects

As mentioned it is not my belief that only women with luteal phase defects may require progesterone therapy to prevent a miscarriage. Indeed in some instances the corpus luteum of pregnancy, which may have produced adequate hormones during the luteal phase, fails before the placenta is adequately secreting progesterone.

However, it is my contention that all women who have a luteal phase defect should be treated with extra proges-
terone. In a study of 100 consecutive women with a minimum of one year of infertility attributed to luteal phase defects as determined by endometrial biopsy the patients were divided into two groups according to whether they attained a mature follicle or not (as defined as reaching an average diameter of 18 mm and a serum estradiol ≥ 200 pg/ml) [34]. The group with mature follicles (n = 58) were either treated with follicle maturing drugs (n = 27) or supplemental vaginal progesterone (n = 31) [34]. Not only did only three of the 27 treated with follicle maturing drugs conceive within six months but two of the three had miscarriages. In contrast there were 24 of 31 conceiving in the 6-month period with supplemental progesterone with only one of the 24 having a miscarriage [34]. There were 25 women failing to conceive in the first six months with follicle maturing drugs treated with exclusive progesterone during the next six months; 16 conceived with only one miscarriage [34].

For the women with luteal phase defects and immature follicles seven of ten conceived but four of seven had a miscarriage. However in 20 women treated with the combination of follicle maturing drugs in the follicular phase and progesterone in the luteal phase 14 conceived with only one miscarriage [34]. Though only three of 12 with immature follicles conceived with just progesterone therapy, none of them miscarried [34]. Thus this study supported previous conclusions that supplemental progesterone therapy should be given even when follicle maturing drugs are not merely used in anovulatory women but in those with a luteal phase defects related to releasing the egg from an immature follicle [35].

There is evidence that anovulatory women with ovulation induction by follicle maturing drugs may still have persistent luteal phase defects in 30-50% of the cases [46, 47]. A matched controlled study found a 28% miscarriage rate in women treated with follicle maturing drugs without progesterone in the luteal phase vs only 6% of 50 women treated with both [21]. Thus in my opinion women with infertility related to anovulation or luteal phase defects related to releasing the egg before the follicle is mature should take progesterone in the luteal phase to decrease the risk of miscarriage [21, 34, 48].

The duration and amount of estradiol exposure during the follicular phase helps to develop endometrial progesterone receptors. Thus it is logical to not only treat women with a history of miscarriage with supplemental progesterone in the luteal phase but also the follicular phase should be corrected if an adequate serum estradiol is not attained.

It is the human chorionic gonadotropin made by the early pregnancy that is responsible for keeping the corpus luteum functioning during pregnancy. There is evidence that supplemental hCG during the luteal phase and first trimester can also reduce miscarriage rates [49-53]. A meta-analysis concluded that the use of hCG is beneficial in preventing miscarriage [54]. However it does not seem more beneficial than progesterone [55]. Disadvantages of hCG other than the injections is the risk of ovarian hyperstimulation in those women using higher dosages of follicle maturing drugs and the possibility of not being able to rescue a failing corpus luteum.

The corpus luteum not only makes progesterone but it also makes estradiol. Several studies have found low serum E2 levels in women who are aborting [56-59]. However, it is not clear if the low serum E2 is merely a marker for progesterone deficiency or if a low level plays a role in miscarriage. A study was performed evaluating E2 levels in women treated with follicle maturing drugs without progesterone in the luteal phase vs only 6% of 50 women treated with both [21]. Thus in my opinion women with infertility related to anovulation or luteal phase defects related to releasing the egg before the follicle is mature should take progesterone in the luteal phase to decrease the risk of miscarriage [21, 34, 48].

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References

A practical approach to the prevention of miscarriage: Part 1 - progesterone therapy


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
The effect of blastomere number on embryo survival upon freezing/thawing


The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Introduction

Previous evidence indicates a 6-7-fold increase in pregnancy rates following transfer of embryos with six to eight blastomeres versus 4-5-cell embryos [1].

The current study was conducted to determine the survival rate and likelihood of cleaving to an embryo worthy of transfer based on number of blastomeres present at time of freezing.

Materials and Methods

A retrospective cohort analysis was performed. Embryos were frozen using a biocool freezer and a one-step removal of the cryoprotectant 1, 2 propanediol [2].

The percentage of surviving embryos and those able to be transferred or re-frozen was evaluated based on the number of blastomeres from 4-cell to blastocyst at the time of freezing. This study did not include embryos frozen at the 2 pronuclear state.

Results

The effect of blastomere number at freezing time on survival and normal cleavage following thawing is given in Table 1. Although there seems to be a trend for a lower percentage of embryos transferred or refrozen at six cells, this seems fortuitous since the highest percentage was four cells.

Though there were only 48 blastocysts thawed, the simplified freezing protocol with a one-step removal of the cryoprotectant described also seems to be effective for blastocysts [2].

Discussion

The transferability rate was the same for both 4-cell and 7-9 cell embryos. Blastomere number of the embryo seems to be related to its implantation potential (based on previous data) rather than its ability to survive freeze-thawing [1].

Key words: Cryopreservation; Blastomeres; Survival rate.

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

Summary

Purpose: To determine if the blastomere number of embryos at the time of freezing is related to its quality post-thaw.

Methods: A retrospective cohort analysis of frozen/thawed embryos. Only multi-cell embryos were used for this study. If an embryo was of good quality it would either be transferred or re-frozen.

Results: There did not appear to be any trend for a lower percentage of good quality embryos with fewer numbers of blastomeres.

Conclusions: Though 4-cell embryos have a markedly lower implantation potential upon fresh embryo transfer compared to 6-8-cell embryos, this is not reflected in their ability to survive freeze-thawing.

Key words: Cryopreservation; Blastomeres; Survival rate.

Table 1. — Effect of the number of blastomeres at the time of freezing on the percentage of good quality embryos deemed transferable upon thawing.

<table>
<thead>
<tr>
<th>Cell Stage</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Morula</th>
<th>Day 5</th>
<th>Blast</th>
</tr>
</thead>
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<tr>
<td># embryos thawed</td>
<td>1403</td>
<td>599</td>
<td>555</td>
<td>441</td>
<td>430</td>
<td>151</td>
<td>17</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td># embryos survived</td>
<td>1218</td>
<td>461</td>
<td>443</td>
<td>372</td>
<td>376</td>
<td>132</td>
<td>14</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>% survived</td>
<td>86.8</td>
<td>77.0</td>
<td>79.8</td>
<td>84.4</td>
<td>87.4</td>
<td>87.4</td>
<td>82.4</td>
<td>70.8</td>
<td></td>
</tr>
<tr>
<td># transferred/refrozen</td>
<td>912</td>
<td>359</td>
<td>223</td>
<td>281</td>
<td>274</td>
<td>97</td>
<td>11</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>% transferred/refrozen</td>
<td>65.0</td>
<td>59.9</td>
<td>40.2</td>
<td>63.7</td>
<td>63.7</td>
<td>64.2</td>
<td>64.7</td>
<td>56.3</td>
<td></td>
</tr>
</tbody>
</table>

Revised manuscript accepted for publication September 22, 2008
A comparison of efficacy of freezing embryos at the 2 pronuclear (2PN) stage vs multi-cell when using a simplified freezing protocol with one-step removal of cryoprotectant


The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To compare the efficacy of freezing embryos at the 2 pronuclear stage vs multi-cell stage using a simplified freezing protocol with a one-step removal of the cryoprotectant. Methods: A retrospective analysis was performed. Survival, delivered pregnancy and implantation rates were compared in transfers of all embryos frozen at 2 pronuclear stage (2PN) or all embryos frozen at multi-cell stage. The results were further stratified and compared according to the number of high quality embryos transferred. Results: In all categories despite comparing similar numbers and quality of embryos transferred there was a significantly higher survival rate of 2PN embryos. Significantly higher delivered pregnancy and implantation rates were seen with 2PN vs multi-cell embryos when there was only one or two embryos with ≥ 6 blastomeres and < 25% fragmentation, and a trend for higher delivered pregnancy rates when there were three top quality embryos transferred. Conclusions: When given the option it is preferable when using this simplified freezing and thawing protocol to freeze at the 2PN stage.

Key words: Simplified freezing protocol; 2 pronuclear stage; Multi-cell embryos.

Introduction

An embryo cryopreservation technique is described using a single-step addition of the cryoprotectant 1,2 propanediol in freezing straws preloaded with sucrose, and then plunged into an alcohol bath, then placed in a control rate freezer (Biocool) and then finally plunged into liquid nitrogen [1]. This technique was found to be very effective for freezing at the 2 pronuclear (2PN) stage [1]. The purpose of this study was to determine the relative effectiveness of using this freezing protocol for day 3 multi-cell embryos as compared to the 2PN stage.

Materials and Methods

Embryos were cryopreserved using a simplified protocol with a single step removal of the cryoprotectant 1,2 propanediol upon thawing [1]. All transfers were on day 3 and were preceded by assisted embryo hatching [2].

Frozen embryos were derived as follows: 1) Fresh embryo transfer because of risk of ovarian hyperstimulation or endometrial factor, e.g., inadequate endometrial thickness or homogeneous hyperechogenic pattern on the day of hCG [3, 4]. Thus all the embryos were frozen at the 2PN stage. 2) Generally twice as many embryos as intended for transfer are allowed to cleave to day 3; the least quality embryos based on blastomere number and fragmentation are frozen at the multi-cell stage and the best ones transferred fresh. 3) Twice as many frozen embryos intended for transfer are thawed on day 3 and the lesser quality ones are re-frozen at the multi-cell stage [5]. 4) Germinal vesicle stage or metaphase I eggs are fertilized after another day in culture and then frozen at the 2PN stage [6].

Pregnancy outcome was compared for embryo transfer where all embryos thawed were at the 2PN stage vs all embryos thawed at the multi-cell stage. Pregnancy outcome was compared according to three groups: 1) At least one embryo in each group had an embryo with six blastomeres and < 25% fragmentation. 2) At least two embryos had six blastomeres and ≤ 25% fragmentation, and 3) At least three embryos had six blastomeres and ≤ 25% fragmentation.

Results

Table 1 summarizes the survival, implantation and delivered pregnancy rates. All three of these parameters were significantly higher for thawed 2PN vs multi-cell embryos when there was at least one or two good grade embryos transferred. Survival rates after thawing were also significantly higher for 2PN vs multi-cell embryos when there were three top quality embryos.

However, there was no significant difference in either implantation or viable pregnancy rates when three top quality embryos were transferred (but a trend for lower delivered pregnancy rates was seen even when three good quality embryos were frozen at the multi-cell stage).

Discussion

If the data had shown equal chances of achieving a pregnancy with embryos thawed at the multi-cell stage compared to the 2PN stage then we would have to ques-
A comparison of efficacy of freezing embryos at the 2 pronuclear (2PN) stage vs multi-cell when using a simplified freezing etc.

Table 1. — Survival, implantation and viable (ongoing past 16 weeks or delivered) pregnancy rates according to the stage of embryo freezing.

<table>
<thead>
<tr>
<th>Rate</th>
<th>All 2PN</th>
<th>All multi-cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least one 6-cell B embryo with ≤ 25% fragmentation Survival*</td>
<td>97.6% (5557/5691)</td>
<td>90.6% (722/797)</td>
</tr>
<tr>
<td>Implantation*</td>
<td>20.9% (665/3186)</td>
<td>12.3% (73/595)</td>
</tr>
<tr>
<td>Delivered pregnancy rate*</td>
<td>39.9% (386/967)</td>
<td>19.8% (41/207)</td>
</tr>
<tr>
<td>At least two 6-cell B embryos with ≤ 25% fragmentation Survival*</td>
<td>97.8% (4018/4107)</td>
<td>92.5% (481/520)</td>
</tr>
<tr>
<td>Implantation*</td>
<td>21.5% (489/2277)</td>
<td>14.9% (58/390)</td>
</tr>
<tr>
<td>Delivered pregnancy rate*</td>
<td>41.7% (283/679)</td>
<td>24.0% (31/129)</td>
</tr>
<tr>
<td>At least three 6-cell B embryos with ≤ 25% fragmentation Survival*</td>
<td>97.1% (2249/2316)</td>
<td>93.5% (243/260)</td>
</tr>
<tr>
<td>Implantation**</td>
<td>21.6% (272/1258)</td>
<td>19.4% (36/186)</td>
</tr>
<tr>
<td>Delivered pregnancy rate**</td>
<td>43.9% (155/353)</td>
<td>33.3% (18/54)</td>
</tr>
</tbody>
</table>

*p < 0.05 comparing all 2PN to all multi-cell stage. **p = NS comparing all 2PN to all multi-cell stage.

The pregnancy rate per oocyte harvest has been defined as the chance of a pregnancy from a given egg retrieval cycle without the need for performing another egg retrieval [7, 8]. Based on these data we will continue our policy of allowing only twice as many embryos as intended to transfer and then freeze the rest at the 2PN stage.

It is possible that a prospective study comparing the present policy described above vs allowing all embryos to cleave on day 3; choose the best ones, and freeze the remaining ones at the multi-cell stage could prove that a higher pregnancy rate on the fresh transfer could be achieved by having a larger number of embryos from which to select the best ones. However, the critical question from this prospective study is even if a higher fresh embryo pregnancy rate is found, if the fresh embryo transfer fails, will the less quality frozen multi-cell embryo lead to an overall lower pregnancy rate per oocyte harvest? Even if changing the policy to allow all embryos to cleave did not result in a lower pregnancy rate per harvest, would it lead to less chance of a second pregnancy for the first retrieval with subsequent frozen ETs?

One should note that possibly other freezing techniques might not show any advantage of freezing at the 2PN stage. Hopefully this study will encourage other IVF centers using a different technique for freezing to perform similar studies.

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
Pregnancy rates per embryo transfer (ET) may be improved by conventional oocyte insemination for male factor rather than intracytoplasmic sperm injection (ICSI)

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Introduction

The introduction of intracytoplasmic sperm injection (ICSI) has allowed successful conception by males with sperm of such poor quality that it would not have been likely with either intrauterine insemination (IUI) or conventional insemination of oocytes as part of in vitro fertilization (IVF) that a pregnancy would have been achieved [1, 2].

In vitro fertilization is expensive and labor intensive for both patient and physician. Thus many IVF centers (including our own) fearing failed fertilization may suggest ICSI to “play it safe” for milder male factor cases that in the past usually resulted in fertilization.

The possibility exists that the zona pellucida may be better able to select the best sperm to result in the best embryo as opposed to the embryologist selecting the best morphologic sperm. The present study probed the question as to whether performing ICSI for mild to moderate male factor problems may have a detrimental effect on pregnancy rates.

Materials and Methods

A retrospective review over a three-year period was performed in all IVF cycles whether ICSI or conventional insemination was used where the diagnosis included male factor. The general policy at the Cooper Center for IVF is to recommend ICSI for male factor for the reasons stated in the introduction. The general reason for a woman not choosing ICSI is to save money with conventional insemination.

Results

Pregnancy outcome according to method of oocyte insemination is shown in Table 1. As predicted only 29 of the 541 transfers (5.3%) were with conventional insemination. There were 118 ICSI cycles performed for low HOST or positive antisperm antibodies and all used ICSI. There were 51 clinical pregnancies (43.2%) and 48 live delivered pregnancies (40.6%) for this subgroup of low HOST scores and positive antisperm antibodies. Failed fertilization occurred in two of 31 retrievals (6.4%) using conventional insemination and in five of 517 (0.96%) using ICSI (p = NS).

There were 329 transfers in woman aged ≤ 35 and 183 in women aged 36-39 where ICSI was used. Clinical pregnancies occurred in 159 and 69 women, respectively (48.3% and 37.7%). The delivered pregnancy rates were 145/329 (44.1%) for women ≤ 35 and 58/183 (31.7%) for women 36-39.

For women having conventional oocyte insemination 16/21 (76.2%) aged ≤ 35 achieved a clinical pregnancy while 5/8 (62.5%) women aged 36-39 achieved one. The definition of male factor included: motile density < 8 x 10⁹/ml, motility < 30%, sperm morphology using standard WHO criteria or with strict criteria ≤ 4% normal, the presence of antisperm antibodies > 80% using direct immunobead assay, and a hypo-osmotic swelling test (HOST) score < 50%. Cases involving testicular and epididymal sperm aspiration were excluded.

Pregnancy outcome was then determined and compared for each group – ICSI vs conventional insemination. The data was also stratified according to two age groups: < 35 and 36-39.

Only cycles with at least two embryos transferred were evaluated. A clinical pregnancy was one where there was ultrasound evidence of pregnancy. A delivered pregnancy was one where a live baby was born.

Summary

Purpose: To determine if intracytoplasmic sperm injection (ICSI) for mild male factor may create embryos less likely to implant.

Method: A retrospective analysis of pregnancy outcome following oocyte fertilization with ICSI vs conventional egg insemination was performed. Results: Though there were many less cases using conventional oocyte insemination compared to ICSI so that a meaningful comparison of outcome could not be made, the data could suggest the fertilization by ICSI might result in embryos less likely to implant. Conclusions: This pilot study should encourage IVF centers to consider conventional oocyte insemination for mild male factor instead of ICSI. Only by evaluating a larger series can it be determined with certainty that fertilization by ICSI may lower the implantation potential of the embryo that is formed.

Key words: Intracytoplasmic sperm injection; Conventional oocyte insemination; Embryo implantation.
live delivered pregnancy rates were 66.7% (14/21) and 62.5% (5/8), respectively.

Overall for women ≤ 39 there were 253 clinical pregnancies in 512 transfers (44.5%) for oocytes fertilized with ICSI vs 21/29 (72.4%) with conventional insemination (p < 0.05, chi-square analysis). There were 224/512 (39.6%) delivered pregnancies for women ≤ 39 with ICSI vs 19/29 (65.5%) for conventional insemination (p < 0.05, chi-square analysis). The implantation rate was also significantly higher with conventional insemination vs ICSI (p < 0.01, chi-square analysis) (Table 1).

Table 1. — Pregnancy outcome following embryo transfer according to method of oocyte fertilization.

<table>
<thead>
<tr>
<th></th>
<th>Male factor with ICSI</th>
<th>Male factor without ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total ≤ 35 36-39</td>
<td>Total ≤ 35 36-39</td>
</tr>
<tr>
<td># transfers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% mature eggs retrieved</td>
<td>75.9 75.4 76.1</td>
<td>85.2 84.4 93.3</td>
</tr>
<tr>
<td>% mature eggs fertilized</td>
<td>64.5 64.6 66.4</td>
<td>62.7 61.5 70.1</td>
</tr>
<tr>
<td>Average # ET</td>
<td>2.2 3.0 3.3</td>
<td>3.2 3.1 3.4</td>
</tr>
<tr>
<td>% clinical pregnancy/transfer</td>
<td>44.5 48.3 37.7</td>
<td>72.4 76.2 62.5</td>
</tr>
<tr>
<td>% delivered pregnancy/transfer</td>
<td>39.6 44.1 31.7</td>
<td>65.5 66.7 62.5</td>
</tr>
<tr>
<td># embryos transferred</td>
<td>1831 991 607</td>
<td>107 65 27</td>
</tr>
<tr>
<td># embryos implanted</td>
<td>376 246 101</td>
<td>35 25 10</td>
</tr>
<tr>
<td>% implanted</td>
<td>20.5 24.8 16.6</td>
<td>32.7 38.5 37.0</td>
</tr>
</tbody>
</table>

Discussion

In our own experience the cut-off values for normal motile density or standard or strict morphology have not been very effective in predicting subfertile males either following intercourse, intrauterine insemination, or IVF with conventional insemination [3-7]. The long distance from cervical os to fallopian tube certainly favors the natural state of the sperm with the best motility.

The theoretical advantage of performing IVF with ICSI is the ability to ensure fertilization by one of the best morphologic sperms. However, one study comparing the effects of single sperm defects on pregnancy outcome following conventional oocyte insemination found a clinical pregnancy rate almost twice as high with sperm with strict morphology of ≤ 4% as compared to sperm which were considered without any abnormalities in motile density or morphology [7].

Thus, these new data are consistent with the hypothesis that certain properties of the zona pellucida allow the selection of sperm that have a better chance of resulting in a live pregnancy than selection of sperm with the best morphology by the andrologist/embryologist.

Though the results do show a significant difference between ICSI and conventional insemination in both clinical and ongoing/delivered pregnancy rates one should always use caution when interpreting a retrospective study especially when the sizes of the two study groups are so disproportional and one group very small in comparison.

It is hoped that this study will generate interest in other IVF centers to retrospectively evaluate their data in a similar manner and see if the same conclusions are reached. More importantly the present study may stimulate interest in a multicenter prospective cooperative study to better test this hypothesis.

The small numbers in the group having conventional oocyte insemination were too low to show a significant difference in failed fertilization with conventional insemination vs ICSI (6% vs 1%). However, if a large multicenter prospective study found the same results then one would have to determine if the improved pregnancy outcome with conventional oocyte insemination offsets the risk of failed fertilization.

Confirmation of this data could lead to a change in treatment philosophy, such as for alleged mild male factor, to inseminate at least half of the oocytes conventionally but do some percentage with ICSI as a back-up for failed fertilization. If the embryos formed have equal morphologic characteristics then the ones from conventional insemination should be transferred first, and the ones formed by ICSI frozen. Despite some initial data suggesting that embryos formed by ICSI do not freeze as well, subsequent studies found that not be the case [8-10].

These data will encourage our group to try more cases with mild male factor to attempt fertilization through conventional methods rather than ICSI allowing us to evaluate a larger series in the future.

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
Relationship of serum progesterone (P) level the day after human chorionic gonadotropin (hCG) injection on outcome following in vitro fertilization-embryo transfer (IVF-ET)

J.H. Check, J. Amui, J.K. Choe, D. Brasile
The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Introduction
There is evidence that a deficiency of progesterone (P) may be associated with infertility [1, 2]. Supplemental P after the use of follicle maturing drugs improves pregnancy outcome [3]. The majority of in vitro fertilization (IVF) programs used P supplementation after retrieval but before embryo transfer to supplement corpus luteum P production and improve IVF outcome [4-6].

The possibility exists that in some instances the corpora lutea do not secrete sufficient P, and even with the P supplementation, pregnancy does not ensue because of insufficient P. On the other hand, there is evidence that excessive P production would advance the implantation window so that if excessive P is generated prior to embryo transfer successful implantation may not occur [7, 8].

The present study evaluated the serum P level the day after the human chorionic gonadotropin (hCG) injection to see if either a relatively low response or an exaggerated response correlates with an inferior pregnancy outcome.

Materials and Methods
Serum P levels were drawn one day after the hCG injection in the a.m. This study evaluated all in vitro fertilization-embryo transfer (IVF-ET) cycles where the serum P was obtained the day after the hCG injection in women age <41 from 1997 to 2004. Both GnRH agonist and antagonist controlled ovarian hyperstimulation protocols were used.

The distribution of serum P levels post hCG was found and the cut-off for the deciles (10th, 20th, 30th, 40th) percentile was established. In vitro fertilization cycles were classified into three groups: P (ng/ml) levels post hCG < 1.9 (10th percentile), P levels post hCG > 1.9 and < 8.4 (40th percentile), P levels post hCG were > 8.4. These three groups were considered low, normal and high P levels, respectively.

The cut-off values for the deciles are presented in Table 1. Ovarian stimulation characteristics and embryo transfer outcomes were compared by P group.

Results
The stimulation characteristics analyzed included serum E2, P, FSH, and LH levels on the day of hCG and E2, FSH, and LH levels post hCG, number of embryos fertilized per retrieval, and number of embryos transferred. In each case there was a significant difference in mean value as the P levels increased as seen in Table 2 (p < .05, analysis of variance).

Table 1. — Cut-off used for deciles of serum P the day after hCG injection.

<table>
<thead>
<tr>
<th>Percentile</th>
<th>P (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.90</td>
</tr>
<tr>
<td>20</td>
<td>2.40</td>
</tr>
<tr>
<td>30</td>
<td>3.00</td>
</tr>
<tr>
<td>40</td>
<td>3.60</td>
</tr>
<tr>
<td>50</td>
<td>4.20</td>
</tr>
<tr>
<td>60</td>
<td>4.90</td>
</tr>
<tr>
<td>70</td>
<td>5.70</td>
</tr>
<tr>
<td>80</td>
<td>6.80</td>
</tr>
<tr>
<td>90</td>
<td>8.40</td>
</tr>
</tbody>
</table>

P: progesterone.
The clinical pregnancy rates in P groups 1 and 3 were significantly lower than group 2 as seen in Table 3 (p < .05, chi-square analysis). The outcome variables, ongoing/delivered pregnancy rates and implantation rates, however, did not differ by P groups as seen in Table 3 (p = NS, chi-square analysis).

### 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
Effect of the degree of fragmentation on embryo survival after freeze-thawing

J.H. Check, K. Swenson, W. Yuan, A. Nazari

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To determine if the degree of fragmentation of embryos prior to freezing correlate in a negative manner with survival after thawing. Methods: A retrospective review of frozen embryos thawed for purposes of embryo transfer was done. Survival and transferability rates were determined according to degree of fragmentation. Results: The chance that an embryo with < 25% fragmentation was deemed good enough for transfer upon thawing was 63.6% compared to 52.8% for embryos > 25% (p < .05). Conclusions: Though more fragmented embryos have a lower survival rate after freeze thawing, about 50% of embryos with > 25% fragmentation will still survive the thaw and be able to be transferred.

Key words: Fragmentation; Embryo freezing; Survival rate.

Introduction

Evaluation of the effect of embryo morphology on pregnancy outcome following single embryo transfers revealed that an increased blastomere number predicted an improved pregnancy outcome better than the fragmentation index [1].

The present study was conducted to determine if less fragmented embryos have a better chance of survival after freezing/thawing.

Materials and Methods

A retrospective cohort analysis was performed on frozen embryo transfers over a 10-year period. The embryos were all frozen using an alcohol bath rate controlled freezer and a one-step removal of the cryoprotectant 1,2 propanediol [2].

Based on the degree of fragmentation, the percentage of day 3 embryos that survived and were used for transfer or refrozen was evaluated.

Results

A progressive decrease in survival and normal cleavage is associated with increases in fragmentation as seen in Table 1. The chance that an embryo with ≤ 25% fragmentation will be deemed good enough for transfer was 63.6% (2,178/3,421) as compared to 52.8% (261/496) for embryos with ≥ 26% fragmentation (p < 0.05).

The effect of the degree of fragmentation at the time of embryo freezing on embryo survival and transferability is given in Table 1. Overall 85.4% (2,920/3,421) of embryos with ≤ 25% fragmentation survived the thaw vs 78.6% (390/496) of embryos with ≥ 26% fragmentation (p < 0.001). The transferability rates were 63.7% (2,128/3,421) for ≤ 25% fragmentation vs 52.6% (261/496) for ≥ 26% fragmentation (p < 0.001). Thus using the simplified freezing and thawing technique described only 12.6% (496/3,917) of the embryos had ≥ 26% fragmentation.

Discussion

Survival and transferability rates were significantly greater for those embryos with < 25% fragmentation versus those with > 25%.

Though these data suggest that more fragmented embryos are slightly less likely to result in embryos acceptable for transfer following subsequent freeze/thawing, the difference though statistically significant may not be as clinically important.

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

Table 1. — The effect of the degree of embryo fragmentation of day 3 embryos on embryo survival and transferability.

<table>
<thead>
<tr>
<th>Fragmentation</th>
<th>A 0%</th>
<th>B ≤ 25%</th>
<th>C 25-50%</th>
<th>D &gt; 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td># embryos thawed</td>
<td>305</td>
<td>3116</td>
<td>462</td>
<td>34</td>
</tr>
<tr>
<td># embryos survived</td>
<td>277</td>
<td>2643</td>
<td>365</td>
<td>25</td>
</tr>
<tr>
<td>% survived</td>
<td>90.8</td>
<td>84.8</td>
<td>79.0</td>
<td>73.5</td>
</tr>
<tr>
<td># transferred/refrozen</td>
<td>211</td>
<td>1967</td>
<td>246</td>
<td>15</td>
</tr>
<tr>
<td>% transferred/refrozen</td>
<td>69.2</td>
<td>63.1</td>
<td>53.2</td>
<td>44.1</td>
</tr>
</tbody>
</table>
A prospective comparison of in vitro fertilization (IVF) outcome following controlled ovarian hyperstimulation (COH) regimens using follitropin alpha exclusively or with the addition of low dose human chorionic gonadotropin (hCG) and ganirelix

J.H. Check, E. Davies, D. Brasile, J.K. Choe, J. Amui

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To determine if the addition of luteinizing hormone (LH) activity to a controlled ovarian hyperstimulation regimen for purposes of in vitro fertilization adds any additional benefit to the exclusive use of recombinant (r) FSH in antagonist protocols.

Methods: Women with normal endogenous gonadotropin levels were randomly assigned to receive either follitropin alpha exclusively or have the addition of 25 IU human chorionic gonadotropin (hCG) daily. Ganirelix was used when a 14 mm follicle was attained. The data would be analyzed after 70 women were selected for the study and divided into two groups.

Results: There were 22 women in each group who proceeded with embryo transfer (some purposely cryopreserved all embryos because of risk of ovarian hyperstimulation syndrome). There were no trends for differences in clinical or delivered pregnancy rates or implantation rates.

Conclusions: There does not appear to be any clinical advantage of adding exogenous LH activity to the drug regimen for stimulation of multiple follicles for purposes of in vitro fertilization when using follitropin alpha in an antagonist protocol.

Key words: Gonadotropin releasing hormone antagonist; In vitro fertilization; Luteinizing hormone; Follitropin alpha.

Introduction

Two separate meta-analyses comparing urinary follicle stimulating hormone (FSH) and human menopausal gonadotropin (hMG) found that uFSH yielded better results when used alone but observed similar outcomes in women pretreated with a gonadotropin releasing hormone (GnRH) agonist [1, 2]. However a carefully controlled randomized clinical trial comparing recombinant (r) FSH and hMG after down regulation with an agonist observed no difference in pregnancy rates [3]. Nevertheless a meta-analysis of five clinical trials found a higher clinical pregnancy rate in hMG-treated women when using an agonist protocol [3].

Most authors agree that there are so many opposite conclusions about the superiority of regimens that involve exclusive FSH or the addition of some hMG that they are probably equally effective [4, 5].

Most of the studies comparing FSH only to FSH with LH used GnRH agonist protocols. The present study compared the outcomes using a GnRH antagonist protocol.

Materials and Methods

Women were randomly assigned by random numbers table to receive either 300 IU daily of follitropin alpha only (group 1) or the same regimen with 25 IU hCG added (group 2). Ganirelix 250 IU day was added once a 14 mm follicle was obtained.

Oocyte retrieval was 35 hours from hCG injection. Embryo transfer was performed on day 3. Oocytes were inseminated by conventional means or by intracytoplasmic sperm injection. Only women having ≥ 2 embryos transferred were compared.

Women with hypogonadotropic amenorrhea or those with diminished egg reserve were excluded from the study. The plan was to analyze the data after there were 35 women in each group registered. If there appeared to be a trend for a difference in pregnancy rates between the groups in either direction we would perform a power analysis to determine how many more couples to recruit. If there did not appear to be a difference at this stage the study would be stopped.

Results

There were 35 women assigned to each treatment regimen. One woman assigned to group 1, and two in the group with hCG added (Group 2), conceived naturally and were eliminated from the study; one group 2 woman was stimulating poorly and dropped out of the study. Fresh embryo transfers were deferred and all embryos were frozen in 12 women taking FSH exclusively and in 12 adding low dose hCG to the COH regimen for risk of ovarian hyperstimulation. Thus fresh embryo transfers occurred in 22 group 1 women and 20 group 2 women.
The fertilization rate was 66.6% for the group receiving follitropin alpha only and 55.9% for group 2 receiving additional low dose hCG (p = NS). The clinical pregnancy rate per transfer (2 embryos or more) was 35% (7/20) in each group and the ongoing/delivered pregnancy rate was 30% (6/20) in each group. The respective implantation rates were 15% (9/60) for group 1 and 16.7% (10/60) for group 2. The average age for group 1 was 33.6 years vs 35.1 years for group 2. These results and other important data are provided in Table 1.

Conclusions

Only about 1% of LH receptors need to be occupied to support normal follicular maturation [6]. GnRH agonists usually result in low levels of LH by down regulation but the levels are usually sufficient to allow follicular maturation [7]. There are probably however a minority of women where the GnRH agonist suppressed the LH below the level necessary to allow normal follicular development [8]. There are data suggesting that fertilization, implantation and pregnancy rates may be adversely affected when LH levels are extremely low [9].

Theoretically if one used a GnRH antagonist protocol there should not be suppression of LH at all in menstruating women for most of the follicular phase. Besides using hMG, LH activity can be provided by the use of recombinant LH or low-dose hCG [10]. The present study prospectively evaluated whether the addition of LH activity by adding low-dose hCG provided any improvement in pregnancy rates over the exclusive use of rFSH alone in women using a GnRH antagonist protocol. The hypothesis was that without the marked LH suppression that could sometimes be seen with GnRH agonist protocols, the addition of LH activity would probably not improve the pregnancy rates.

The data did in fact support the aforementioned hypothesis. Thus the decision was made to conclude the study since no clinical differences were found in the data after 40 transfers of ≥ 2 embryos.

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

Table 1. — Pregnancy and implantation rates following IVF-ET in women using an antagonist controlled ovarian hyperstimulation regimen according to whether daily low dose hCG was added or not.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Follitropin and hCG (Group 2)</th>
<th>Follitropin Only (Group 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td># retrievals</td>
<td>66</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td># transfers</td>
<td>42</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td># transfers + 2 embryos</td>
<td>40</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Average age</td>
<td>34.3</td>
<td>35.1</td>
<td>33.6</td>
</tr>
<tr>
<td># follicles</td>
<td>1296</td>
<td>564</td>
<td>732</td>
</tr>
<tr>
<td>Avg. # follicles/retrieval</td>
<td>19.6</td>
<td>17.6</td>
<td>21.5</td>
</tr>
<tr>
<td># eggs retrieved</td>
<td>969</td>
<td>413</td>
<td>556</td>
</tr>
<tr>
<td>Avg. # eggs retrieved</td>
<td>14.7</td>
<td>12.9</td>
<td>16.4</td>
</tr>
<tr>
<td># metaphase II eggs retrieved</td>
<td>763</td>
<td>343</td>
<td>420</td>
</tr>
<tr>
<td>% metaphase II eggs retrieved</td>
<td>78.7</td>
<td>83.1</td>
<td>75.5</td>
</tr>
<tr>
<td># inseminated</td>
<td>819</td>
<td>376</td>
<td>443</td>
</tr>
<tr>
<td># fertilized</td>
<td>505</td>
<td>210</td>
<td>295</td>
</tr>
<tr>
<td>% fertilized</td>
<td>61.7</td>
<td>55.9</td>
<td>66.6</td>
</tr>
<tr>
<td># pregnancies</td>
<td>17</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>% pregnant/transfer</td>
<td>42.5</td>
<td>35.0</td>
<td>50.0</td>
</tr>
<tr>
<td>% clinical preg./transfer</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>% chemical</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>% ectopic</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td># delivered</td>
<td>12</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>% delivered/transfer</td>
<td>30.0</td>
<td>30.0</td>
<td>30.0</td>
</tr>
<tr>
<td>% miscarried</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>% miscarried</td>
<td>21.4</td>
<td>28.6</td>
<td>14.3</td>
</tr>
<tr>
<td># embryos transferred</td>
<td>120</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Average # embryos transferred</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td># cryopreserved</td>
<td>330</td>
<td>130</td>
<td>200</td>
</tr>
<tr>
<td># sac implanted</td>
<td>19</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>15.8</td>
<td>16.7</td>
<td>15.0</td>
</tr>
<tr>
<td># twins</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>% twins/clin. preg.</td>
<td>21.4</td>
<td>14.3</td>
<td>28.6</td>
</tr>
<tr>
<td># triplets</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>% triplets/clin. preg.</td>
<td>7.1</td>
<td>14.3</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Summary

Background: A novel immunoassay specific for the osteoclast-produced tartrate-resistant acid phosphatase TRAP isoform 5b was developed some years ago. By means of this assay, the usefulness of serum TRAP in monitoring the response to palliative treatment with clodronate in breast cancer patients with bone metastases was studied. Serum TRAP was examined for correlation with the activity of bone osteoclasts in these patients. Materials and Methods: Seventeen patients took part in this study taking 1600 mg clodronate daily as a tablet for five months. Eleven of these patients were evaluated. Results: TRAP activity correlated well with the grade of bone metastases and with the number of locations in the body. During the therapy with clodronate, TRAP activity in serum decreased. Conclusions: We conclude that the measurement of TRAP is useful in monitoring treatment with bisphosphonate clodronate in patients with bone metastatic breast cancer.

Key words: Tartrate-resistant acid phosphatase (TRAP); Bone metastases; Clodronate.

Introduction

Bone metastases during metastatic breast cancer disease are common. Autopsy studies have shown that 70-85% of all women who died of metastatic breast cancer had skeletal metastasis [1]. One out of every four women who are diagnosed with breast cancer will suffer from bone metastases. In the United States where 180,000 women each year are diagnosed with a malignant breast tumour, 40,000-45,000 could benefit from bisphosphonate therapy each year.

Patients suffer an ongoing risk of skeletal complications that can have a significant impact on their quality of life. Bone metastases lead to typical complications like pain, fractures and nerve compressions after fractures in the spine. Characteristics of the terminal phase of the illness are hypercalcemia and bone marrow carcinoma.

Studies have shown that the development of bone metastases from breast cancer is facilitated by the release of substances from tumour cells that activate osteoclasts to lead to local osteolysis [2]. Use of agents such as bisphosphonates like clodronate inhibits tumour-induced osteolysis in vitro and prevents bone destruction and hypercalcemia in vivo [3].

Several studies pointed out that therapy with clodronate reduces the incidence of hypercalcemia and pathological bone fractures [4]. Kanis et al. showed that in breast cancer patients without obvious bone involvement, clodronate significantly reduced the risk of developing bone metastases [5]. In 2002, Powles et al. demonstrated that clodronate significantly reduced the occurrence of bone metastases with primary operable breast cancer during the medication period [6]. There was even a significant reduction in mortality for patients who were randomized to clodronate compared to a placebo group [6]. Dando and Wiseman describe in a review that clodronate was extensively used in patients with advanced breast cancer and was tolerated well. In patients with primary breast cancer clodronate was the only bisphosphonate which improved the survival rate and reduced the incidence of bone metastases in randomised controlled trials [7].

Tartrate-resistant phosphatase (TRAP) is an enzyme which could be used as a marker of the osteoclasts which are bone resorbant. There are two forms of this enzyme: isoform 5a and 5b; 5b is the active form and is specific for osteoclasts, and is detectable and measurable in serum.

TRAP belongs to the group of most proposed markers for assessing the state of skeletal turnover and in particular of bone resorption [8]. The study of Scanneccchia et al. demonstrated in 1991 that the measurement of serum TRAP activity may be useful in assessing bone turnover [9]. In their study, the TRAP concentration in healthy subjects was compared to patients with metabolic bone diseases, including seven patients with bone metastases secondary to breast cancer, and found to be significantly lower [9].

Therefore we hypothesised that the measurement of TRAP could be useful and helpful in monitoring bone resorption at any stage of disease by indicating if the bisphosphonate therapy with clodronate is working. TRAP
Figures 1-2. — Tumour stage at time of first diagnosis (n = number of patients).
Figure 3. — State of metastases at time of first diagnosis.
Figure 4. — Hormone receptors.

Fig. 1

Fig. 3

Fig. 2

Fig. 4

Fig. 5. — Forms of bone metastases.

Results

Patient characteristics

Figures 1-4 show tumor stage and metastases at the first diagnoses and hormone receptor status.

Bone metastases

Figures 5 and 6 show the forms of the bone metastases and the symptoms patients had when metastases were diagnosed. Out of 17 patients, there were four patients who only had bone metastases.

Patients and Methods

Seventeen patients admitted to the Department of Obstetrics and Gynaecology of the University Hospital of Saarland, Germany took part in this study. The median age of the patients was 62.5 years. The serum results of 11 patients were evaluable. All subjects had symptomatic or asymptomatic progressive bone metastases and had not received bisphosphonate therapy before. All patients were advised of the nature and purpose of the study before giving their informed written consent to participate. The study was approved by the ethics committee of the university. Patients were free of psychiatric problems and had sufficient liver and kidney function. They gave their consent to take one tablet of 1600 mg clodronate daily.

Before starting the therapy serum analysis of TRAP was taken; this was repeated every 14 days. At the end of the study period (five months of therapy) a conventional analysis of bone metastases was carried out (X-ray or bone scanning).
In Figures 7 and 8 the number of locations of bone metastases and the state of the illness before and after therapy of the patients (n = 11) who could be evaluated are shown; 3/11 patients had progressive disease, 8/11 showed stable disease.

The following figures indicate the activity of TRAP in serum before and during clodronate therapy. The reference value for serum TRAP activity is 3.43 IU/ml for premenopausal women and 4.63 IU/ml in postmenopausal women. Our study patients were all postmenopausal.

Figure 9 shows the baseline value of TRAP before the start of treatment. As the figure shows, baseline mean values increased without reaching statistical significance in our patients. The mean values of serum TRAP in patients with more than three locations of bone metastases were 9.95 IU/ml and significantly higher in comparison to normal values and to patients with less than three locations of bone metastases (mean values: 5.06 IU/ml).

The following five figures (Figures 11-15) give information about the development of TRAP during the time of therapy. Regarding all patients (as well as the two subgroups) there was a constant decrease in TRAP activity. Reduction of TRAP was the highest in patients with more than three locations of bone metastases.
Discussion

Our study showed that the evaluation of TRAP gives useful information about bone metastases in breast cancer in the primary diagnosis as well as a marker of monitoring therapy with clodronate. Continuous measurement of TRAP is necessary to evaluate the success of clodronate therapy. Like a tumor marker which is evaluated in a specific period of time, regression, progression or stable disease can be diagnosed. Capeller et al. demonstrated in

**TRAP at different stages of therapy (Figures 16-18)**

The analysis of TRAP at different stages of therapy (Figures 16-18) show a significant reduction of TRAP concentration in the serum at the third month of therapy in patients with more than three locations of bone metastases \( p < 0.05 \). In all patients and in the group of patients with less than three locations of bone metastases there was a reduction in TRAP activity (Figures 17 and 18) which was not significant.
TRAP in patients with breast cancer having bone metastases was much higher than in healthy donors and patients without skeletal injuries. We confirmed their results that TRAP concentration in serum of patients with multiple bone metastases due to breast cancer surpassed that in patients with single bone metastases. Diagnostic sensitivity and specificity of TRAP as a marker of skeletal metastases in patients with breast cancer were 82% and 87%, respectively. They concluded that the detection of TRAP in breast cancer patients with bone metastases held much promise for early diagnosis of skeletal metastases, estimation of severity, and monitoring the efficiency of bisphosphonate therapy [12].

Chao et al. determined TRAP in 30 early breast cancer patients without bone metastases, 30 aged-matched breast cancer patients with bone metastases, and 60 normal and 2003 that TRAP levels decline under bisphosphonate therapy when no progression is detectable. When progress of the bone metastases occurs, TRAP levels rise again [10]. We confirmed these data showing a reduction of TRAP during therapy with clodronate, independent of the number of locations of bone metastases.

Recently Chao et al. showed that TRAP is a useful serum marker for extensive bone metastases in breast cancer patients. In 168 breast cancer patients, including 81 who were newly diagnosed with early breast cancer, 20 with extraosseous metastasis, 24 with limited bone metastases and 43 patients with extensive bone metastases TRAP activity was measured. TRAP was significantly elevated in patients with extensive bone metastases compared to all other groups ($p < 0.0001$) [11].

Lyubimova et al. showed in 2004 that serum activity of
healthy volunteers as controls [13]. The mean TRAP activity in early breast cancer patients did not differ significantly from that of the normal group whereas it was significantly higher in patients with bone metastases ($p < 0.0001$) [13].

We found no studies regarding oral therapy with clodronate in measuring TRAP as a marker. However there is experimental evidence that therapy with pamidronate could be monitored by the determination of TRAP. Martinetti et al. treated 28 advanced breast cancer patients with bone metastases with the bisphosphonate of pamidronate using a total of 24 infusions over a treatment period of 61 weeks [14]. To evaluate the usefulness of TRAP in monitoring the treatment with pamidronate, patients were divided into two groups with respect to pain trend and analgetic intake. The results did not show any statistical significance in the baseline serum TRAP levels in the two groups. One week after the first pamidronate infusion TRAP serum levels decreased by 39% and 18% in group A and B. These levels persisted throughout the treatment period. They concluded that a decrease in TRAP serum levels may reflect the pharmacological activity of pamidronate [14].

Mose et al. monitored the effectiveness of local radiotherapy in 48 breast cancer patients with bone metastases by determination of the active isoforn 5b of tartrate-resistant acid phosphatase [15]. They found a significant decrease of TRAP in patients without progression in non-irradiated regions, whereas in progressive disease TRAP remained stable with a slightly increasing tendency ($p < 0.01$). Like our study, Mose et al. showed that in patients with more than three locations of bone metastases all TRAP values were significantly higher compared to patients with less than four locations ($p = 0.01$) [15].

Our study demonstrated that patients with progressive bone metastases can benefit from therapy with clodronate much more than patients with only one or two locations. As our data show a period of clodronate therapy of at least two-months is necessary to significantly reduce the activity of TRAP.

Conclusion

The possible significance of TRAP includes its use as a screening parameter in the first staging of diagnosed breast cancer. It may be able to indicate one day if change to another bisphosphonate is useful in the therapy of a woman with bone metastases. It would be of immense value in evaluating the risk of a breast cancer patient developing bone metastases in order to start prophylactic therapy at once.

References


Tartrate-resistant acid phosphatase (TRAP) as a serum marker for bone resorption in breast cancer patients with bone metastases


Address reprint requests to:
S. TAUCHERT, M.D.
Department of Gynaecology and Obstetrics
Caritas Hospital St. Theresia
Rheinstrasse 3
66113 Saarbrücken (Germany)
e-mail: sascha.tauchert@gmx.de

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Lesions of the subepithelial stromal zone of the lower female genital tract. An immunopathological study

A. Kondi-Pafiti¹, M. Frangou-Plemmenou¹, C. Bakalianou², M. Tsantopoulos¹, K. Papadias², A. Liapis²

¹Pathology Laboratory, ²2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital, Athens (Greece)

Summary

The stroma of the lower female genital tract is typically described as fibromuscular, consisting mainly of smooth muscle fibres and connective tissue. The study of Elliott and Elliott [1], however, has drawn attention to the presence of a 0.5-5 cm wide subepithelial stromal zone which runs from the endocervix to the vulva in mature females. There is evidence that this stromal zone may be the origin of fibroepithelial cervical and vaginal polyps, known as pseudo sarcomas because of the commonly reported presence of isolated large atypical cells [2, 3].

The purpose of this study was to investigate the morphology, histochemical and immunohistochemical properties of normal cervical stroma as well those of cervical and vaginal polyps and neoplastic processes of the cervix.

Materials and Methods

Cases for this study were obtained from the files of the Pathology Laboratory of Aretaieion University Hospital during the last decade. The following specimens were included in the study: ten fibroepithelial and ten adenomatous cervical polyps, 12 fibroepithelial vaginal polyps, 15 cone biopsies performed for cervical intraepithelial neoplasias, five cervices with squamous cell carcinoma and 20 cervices from total hysterectomies due to uterine leiomyomata. All tissues for light microscopy were formalin-fixed and paraffin-embedded and processed for hematoxylin and eosin stains and elastic van Gieson’s stain. Immunohistochemical procedures were performed by the Ventana Automatic Immunostaining System and the following antibodies were used: anti-vimentin (V9mab Eurodiagnostics) anti-desmin (D33mab Novosan) anti-smooth muscle actin (IA4 mabBiogenex, a-1-antitrypsin (pAb, Thermo), anti-collagen III (III-53mab, MediCorp), anti-CD34 (QBend mab, Novokasta,) ER (6F11Novokastra mab), PgR (IA6, Novocasta mab).

Internal controls, which included normal epithelium, fibrous tissue, smooth muscle and other specific tissues were assessed for immunostaining.

Results

Clinical data. The ages of the patients ranged from 30 to 70 years (mean 48 years) for the group of 25 total hysterectomies and from 24 to 47 years (mean 36 years) for the remaining patients with polyps, and dysplastic and neoplastic lesions of the cervix. Nine patients from the first group and one patient from the second were postmenopausal. None of the patients was pregnant, or had a history of hormonal therapy. All vaginal polyps and seven of the cervical polyps were discovered due to postcoital bleeding. The rest were discovered during routine examination. The size of the polyps varied from 6 to 35 mm.

Three of the 15 patients with dysplastic epithelial changes, in whom cone biopsies showed foci of microinvasive carcinomas, underwent more extensive surgical therapy. Three to five years follow-up was obtained in these patients and no recurrences were observed.

Hysterectomy specimens showed uterine leiomyomatia and the cervices were grossly normal. All cases with infiltrating squamous cell carcinomas were Stage 1 and appropriate therapeutic procedures were followed.

Microscopic features. A loose sub epithelial stromal layer, 2 to 6 mm in width, was detectable in both the endocervix and the ectocervix (Figure 1) in 17/20 normal cervical specimens, 16/20 cervical polyps, 10/12 vaginal polyps and in 6/15 cone biopsies, but not in neoplastic...
Lesions of the subepithelial stromal zone of the lower female genital tract. An immunopathological study

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specimens. This zone was more evident in the ectocervix and thinner in the endocervix. In three fibroepithelial cervical polyps, rare spindle-shaped cells with atypical nuclear forms were observed. Stromal mitoses were absent. A deeper zone consisting of fibromuscular tissue was also present in the cervical wall. In three normal cervixes the existing chronic inflammatory reaction covered the subepithelial stroma.

The same loose structure was focally observed in the stroma of 6/10 adenomatous cervical polyps and focally in the stroma of six cone biopsies where a co-existing inflammation covered the stroma. The specimens with microinvasive and the infiltrative carcinomas showed an inflammatory and/or focal fibrous stromal reaction which obscured the subepithelial stromal layer.

The vaginal polyps were well circumscribed or grossly papillary and covered by stratified squamous epithelium, with slight hyperplasia, sometimes with hyperkeratosis and parakeratosis, but no dysplasia. The core of these polyps was composed of a loose edematous matrix, quite similar to the subepithelial stromal layer previously described, with varying numbers of spindle and stellate-shaped cells and occasional giant and multinucleated-cells (Figure 2).

**Histochemical findings.** Histochemical staining revealed loose fibro-connective tissue and collagen fibers

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Figure 1. — Histological section of cervix showing loose subepithelial stroma over the fibromuscular wall (hematoxylin-eosin, x 25).

Figure 2. — Histological section of a fibroepithelial vaginal polyp showing atypical and giant cells in loose stroma (hematoxylin-eosin, x 40).

Figure 3. — Positive vimentin immunostain reaction in the subepithelial cervical stroma (immunostain, x 120).

Figure 4. — Positive desmin immunoreaction, focal in the subepithelial stroma and dense in the fibromuscular wall (immunostain x 120).

Figure 5. — Histological section of cervix showing positive immunostain reaction to ER in isolated stromal cells (immunostain x 250).
running parallel to the epithelial surface. Fine elastic fibers were also observed running perpendicular to the epithelial surface in the subepithelial zone, and parallel to the surface in the deeper layers.

**Immunohistochemical findings.** Vimentin staining was positive in normal cervicovaginal tissue and the stromal cells of all fibroepithelial polyps (Figure 3) and in 6/10 adenomatous polyps. In the vaginal polyps, in particular, the stromal spindle or stellate cells were strongly positive for vimentin and focal condensation of these cells under the surface epithelium was evident. Collagen III staining was detected only in scanty fibrils but a diffuse reaction of the stroma was observed in all cases.

Immunostaining for actin was negative but desmin was positive in the loose subepithelial zone, observed in scanty spindle-like cells. Both markers were markedly positive in the fibromuscular portion of the cervix (Figure 4). Alpha-1-antitrypsin was detected in a few isolated stromal cells and CD34 was positive in stromal cells around vessels. Estrogen receptors were observed only sparsely in a few stromal cells (Figure 5) and markedly in the fibromuscular wall. Similar results were focally observed in specimens with neoplastic changes, but the inflammatory and fibrous reaction obscured the characteristic distribution described above.

**Discussion**

It has been postulated that two different kinds of stroma exist in the lower female genital tract – a fibromuscular stroma, which is the main substrate of the cervical and vaginal wall, and a loose subepithelial myxoid stroma, first described by Elliott and Elliott [1]. The latter runs from the endocervix to the vulva in mature females, and according to our findings was usually thicker in the ectocervix. It is composed of loose fibroconnective tissue with fibroblasts, elastic fibers, type III collagen, and reticulin fibers.

The physiologic significance of the subepithelial zone is not very clear. Mucitelli et al. [4] suggest that the function of the zone may be to help the immense expansion of the vulvovaginal area during labor and its rapid reconstitution during puerperium. Ultrastructurally, large stromal cells have the distinctive features of an active contractile apparatus and may provide the necessary shrinkage of cervicovaginal tissue during puerperium [4].

The loose subepithelial zone, often populated by cells with bizarre nuclear features, may be the origin of the so-called fibroepithelial polyps of the vagina [1-4] and possibly of the cervix [5].

A striking similarity, both histological and immunohistochemical, of the stroma of vaginal polyps to that of the subepithelial zone found in normal vaginas has been reported [6].

Our study confirms the expression of vimentin, type III collagen and desmin in both vaginal polyps and the loose subepithelial zone.

The belief that polyps are the expression of a hyperplastic process arising in this zone is in agreement with our results.

The possible role of pregnancy and steroid hormones in this process has been argued by some investigators because 33% of fibroepithelial polyps of the vagina reported in the literature occurred in pregnant women and also because of steroid receptors detected in the atypical stromal cells.

None of our patients was pregnant or receiving any hormone therapy. It is interesting that we detected a loose subepithelial stromal zone not only in younger but also in elderly patients, and that the microscopic and immunohistochemical features of the zone were similar independently of age.

The histogenesis of the atypical and multinucleated giant cells in fibroepithelial polyps remains unclear. Some investigators have proposed an origin either from smooth muscle cells or from fibroblasts-histiocytes. A possible differentiation of multifunctional mesenchymal elements along two cell lines (divergent histogenesis) has also been proposed [4].

Based on immunohistochemical studies, Ostor et al. [3] concluded that the stromal cells were of fibrohistiocytic origin. On the contrary, Mucitelli et al. [4] supported the mesenchymal nature of those cells.

Rollason et al. [8], using electron microscopic examination, showed that fibroepithelial polyps had the ultrastructural features usually associated with myofibroblasts and concluded that these tumors would be better designated polypoid myofibroblastomas. Other investigators [9] proposed that adenomatous polyps may develop as a result of a granulation tissue reaction after some local injury of the vaginal mucosa. According to the same authors, delayed differentiation of myofibroblastic cells may explain why granulation tissue sometimes does not shrink but becomes a polyp.

In conclusion, the results of our study indicate that in the lower female genital tract there exists a loose subepithelial layer rich in fibroblasts, elastic and reticulin fibers characteristically arranged, which may be the origin of fibroepithelial vaginal polyps and possibly of commonplace cervical polyps.

**References**


Lesions of the subepithelial stromal zone of the lower female genital tract. An immunopathological study


Address reprint requests to:
A. KONDI-PAFITI, M.D.
Department of Pathology
Areteion Hospital
76, Vas. Sofias Ave.
Athens 115 28 (Greece)
e-mail: agathakondis@hotmail.com

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Regional anaesthesia for primary caesarean section in patients with preterm HELLP syndrome: a review of 102 cases

Ş. Palit¹, M.D.; G. Palit¹, M.D.; M. Vercauteren¹, M.D., Ph.D.; Y. Jacquemyn¹, M.D., Ph.D.
¹Department of Anaesthesiology, ²Obstetrics and Gynaecology, Antwerp University Hospital, UZA, Edegem (Belgium)

Summary

Objective: To determine the feasibility and the safety of combined spinal/epidural and spinal anaesthetic techniques for primary caesarean section in cases of preterm HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome. Methods: A retrospective study was carried out in a tertiary centre including all patients who underwent primary caesarean section for HELLP syndrome. The immediate preoperative and the lowest thrombocyte count, the method of anaesthesia and eventual complications were recorded. Patients were categorised as having antepartum or postpartum HELLP syndrome. Results: A total number of 102 charts was reviewed. Mean gestational age was 30.6 weeks (SD 2.7, range 23-36 weeks). There were seven (6.9%) patients with postpartum HELLP. In case of antepartum HELLP in 37 (36.3%) general anaesthesia was selected, in 53 (52.0%) combined spinal epidural anaesthesia and in 12 (11.8%) single dose spinal anaesthesia. Preoperative thrombocyte count was significantly higher (p < 0.01) in the combined spinal epidural group (113,000/mm³) while there was no difference between general (88,000/mm³) and spinal epidural anaesthesia (95,000/mm³). There were no cases of epidural haematoma. Two patients received a combined spinal epidural although their immediate preoperative thrombocyte count was < 50,000/mm³. Conclusions: Our data demonstrate that combined spinal/epidural anaesthesia is feasible and safe in selected cases of HELLP syndrome.

Key words: HELLP; Preeclampsia; Pregnancy; Epidural; Spinal; Combined spinal-epidural; Thrombocytes.

Introduction

HELLP syndrome is considered a severe form of preeclampsia. The acronym HELLP was first suggested by Weinstein in 1982 and describes hemolysis (H), elevated liver enzymes (EL) and low platelets (LP) [1].

Both regional and general anaesthesia are potentially associated with complications in HELLP syndrome. As low platelet count and liver dysfunction are risk factors for the development of epidural haematoma in case of neuraxial anaesthesia, spinal and epidural anaesthesia have been considered for a long time as a contraindication [2-5]. Although in the early nineties general anaesthesia was recommended as the technique of choice, impaired liver function and an altered metabolism of anaesthetic agents can result in unexpected reactions with general anaesthesia besides the risk of enhanced hepatotoxicity following the use of volatile substances, difficult intubation and hypertensive crisis during induction [2, 5, 6].

In our hospital combined spinal-epidural anaesthesia is the technique of preference for primary caesarean section. Epidural anaesthesia is only selected in case a catheter was already placed during (trial of) labour. Rarely patients with preterm severe preeclampsia or HELLP syndrome are considered for vaginal delivery. With respect to platelet count the following local guidelines have been recommended: any technique is possible with counts above 90,000/mm³, general anaesthesia with counts inferior to 60,000/mm³ while with intermediate values the technique used is at the discretion of the managing anaesthesiologist with some preference for a less traumatising single-dose spinal.

Studies on spinal and epidural anaesthesia in severe preeclampsia have almost always considered coagulopathy, including HELLP syndrome, a reason for exclusion [7-9]. To our knowledge only three small series have specifically documented regional anaesthesia for HELLP syndrome [10-12] and no report exists on the use of combined spinal epidural anaesthesia (CSE) with a double puncture either at a single or double interspace. We performed this retrospective study to further explore the feasibility and the safety of different regional anaesthetic techniques for primary caesarean section in cases of HELLP syndrome.

Material and Methods

After institutional approval by the local ethics committee we performed a retrospective chart analysis in a tertiary referral centre including all women who underwent primary caesarean section for HELLP syndrome at a gestational age before 37 weeks. The inclusion period was from January 1, 2002 to December 31, 2007. A difference was made between antepartum and postpartum manifestation of HELLP as in case of a first manifestation of HELLP syndrome during the postpartum period this would have had no influence on the choice of anaesthetic technique.

In our centre HELLP syndrome is classified according to the Mississippi three class system [13], based on the lowest measured maternal platelet count, either before or after delivery. In all cases haemolysis and hepatic dysfunction had to be present, demonstrated by an increase of lactate dehydrogenase (LDH) level ≥ 600 IU/L and aspartate transaminase (AST) and or...
Regional anaesthesia for primary caesarean section in patients with preterm HELLP syndrome: a review of 102 cases

Alanine transaminase (ALT) ≥ 40 IU/l. Women with class 1 HELLP syndrome have a maternal platelet nadir of ≥ 50,000/mm³; patients with class 2 HELLP syndrome a platelet nadir of > 50,000 but ≤ 100,000/mm³ and those with class 3 disease a platelet count of > 100,000 but < 150,000/mm³. Daily laboratory evaluation included liver function tests, complete blood count, fibrinogen and fibrin degradation products and renal function tests. Maternal hypertension was treated if systolic blood pressure was ≥ 160 mmHg and/or the diastolic value ≥ 100 mmHg. Treatment consisted of oral felodipine (5 to 20 mg daily) followed by urapidyl intravenously if blood pressure rose to ≥ 180 mmHg systolic or ≥ 110 mmHg diastolic values. If gestational age was < 35 weeks, betamethasone (2 x 12 mg) was given intramuscularly (if maternal thrombocyte count was > 50,000/mm³) or intravenously in case of a lower count.

All patients received intravenous MgSO4 (loading dose 5 g, followed by 1 g/hour IV) continued for 48 hours after the delivery. Thrombocyte transfusion was not systematically performed and the decision to give thrombocytes was left to the discretion of the obstetrician and anaesthesiologist.

When regional anaesthesia was selected patients were positioned either in the sitting or right lateral decubitus position. Before initiation of the spinal block, each patient received 500 ml of hetastarch 6% (Voluven®, Fresenius, France).

A skin wheal was raised with 1% lidocaine at the L3-4 or L4-5 intervertebral space. For the CSE technique an Adjustable Duraseaf® BD needle combination was used. The epidural space was identified with a 17-gauge Tuohy needle to which a glass syringe filled with 4 ml of air was connected. Subsequently if gestational age was < 35 weeks a lockable 27-gauge spinal Whitacre needle was introduced. After appearance of cerebrospinal fluid, 6.66 mg of hyperbaric bupivacaine and 3.33 μg of sufentanil were slowly injected with the orifice of the spinal needle directed cephalad. This combination is 2 ml out of a 3 ml mixture containing 10 mg hyperbaric bupivacaine 0.5% and 5 μg sufentanil.

In the case of insufficient initial cephalad spread, or when pain sensations reappeared intraoperatively, incremental epidural supplements consisting 0.75% of ropivacaine were injected, starting with 4 ml. When necessary, additional 2-ml boluses were given no earlier than 5 min after the preceding top-up.

For single-dose spinal anaesthesia a 27-gauge spinal Whitacre needle was introduced at the L3-4 or L4-L5 intervertebral space. Hyperbaric bupivacaine (8.3 mg) and 4.15 μg of sufentanil (i.e., 2.5 ml of above-mentioned mixture) were slowly injected.

Intraoperative haemodynamics were registered every two minutes. Ephedrine increments of 5 mg were given IV to treat hypotension, defined as a decrease in systolic blood pressure of 20% below baseline values or to less than 100 mm Hg.

General anaesthesia was induced after preoxygenation (100% oxygen for 5 min) with 3-5 mg/kg thiopental (Pentotal®) and 1.5 mg/kg succinylcholine (Lysthenon®) administered intravenously. Tracheal intubation was performed under cricoid pressure. Sevoflurane at 0.5 MAC with an oxygen:air mixture aimed at obtaining a FiO2 of 0.5 was given until delivery after which fentanyl and atracurium were given as required.

After delivery, all patients received a single dose of 2 g of cefazoline IV, 10 IE of oxytocine IV slowly over five minutes, and 10 IE IV over six hours. Starting on the first postoperative day all patients received low molecular weight heparin subcutaneously (nadroparine 2850 IE anti-Xa) daily for ten days. In case of combined spinal epidural anaesthesia the epidural catheter was left in place for at least 48 hours for patient-controlled analgesia delivering 4 ml levobupivacaine 0.1% with sufentanil 1 μg/ml per demand at a lock out time of 15 minutes.

On the second postoperative day the epidural catheter was removed if the maternal platelet count was ≥ 100,000/mm³, otherwise a repeat IV dose of 12 mg of betamethasone was given and the platelet count repeated one day later with the catheter staying in place.

For every patient the gestational age at the time of the caesarean section was documented as were the immediate preoperative thrombocyte counts, the lowest thrombocyte count reached and the method of anaesthesia. The preoperative thrombocyte counts in the different anaesthetic groups were compared using analysis of variance (one-way ANOVA on SPSS 15.0) with posthoc analysis using the Bonferroni test for eventual differences.

**Results**

During the period studied 102 patients fulfilling the criteria for HELLP syndrome had a primary caesarean section at less than 37 weeks gestational age. Mean gestational age at the time of caesarean section was 30.4 weeks (standard deviation 2.8; range 23-36 weeks). In seven (6.9%) patients HELLP syndrome first manifested after delivery – all of these had caesarean section for severe preeclampsia with a rapidly deteriorating maternal condition but without the laboratory changes necessary for diagnosing HELLP syndrome preoperatively; four received general anaesthesia and three had combined spinal epidural anaesthesia. Obviously these patients were excluded from further analysis as the postpartum diagnosis of HELLP syndrome can not influence the choice of anaesthetic method used.

Of the remaining 95 patients, there were 21 (20.6%) Mississippi class 1; 58 (56.9%) class 2 and 16 (15.7%) class 3 patients, based on the lowest thrombocyte count reached either pre- or postoperatively.

In 33 (36.3%) general anaesthesia was used, whereas in 50 (51.9%) combined spinal epidural anaesthesia was chosen and 12 (11.8%) received single-dose spinal anaesthesia. None of the patients received plain epidural anaesthesia alone. Table 1 gives the types of anaesthesia for the three classes in antepartum HELLP cases.

<table>
<thead>
<tr>
<th>Mississippi class and type of anaesthesia.</th>
<th>Class 1 HELLP</th>
<th>Class 2 HELLP</th>
<th>Class 3 HELLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined spinal epidural</td>
<td>11 (1)</td>
<td>29 (2)</td>
<td>10 (1)</td>
</tr>
<tr>
<td>Spinal single shot</td>
<td>1</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>General anaesthesia</td>
<td>9</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>Overall</td>
<td>21</td>
<td>58</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 2 describes preoperative thrombocyte count and the type of anaesthesia used. All patients had a preoperative count of < 150,000/mm³, but some had a preoperative count of > 100,000/mm³, which postoperatively went down to < 100,000 or < 50,000/ mm³. This explains the different numbers of patients in Tables 1 and 2.

We also looked for patients with a preoperative platelet count below 80,000/mm³. There were 27 women in this group, seven (25.9%) received CSE, three (11.1%) a single spinal shot and 17 (62.9%) general anaesthesia.
Despite local guidelines, two patients with a preoperative platelet count lower than 50,000/mm$^3$ received a combined spinal epidural. In one patient with a count of 46,000/mm$^3$, a thrombocyte transfusion was given and a combined spinal epidural was placed immediately thereafter without awaiting further results. The other patient had a combined spinal epidural placed based on blood results from four hours earlier (thrombocyte count 80,000/mm$^3$); a sample taken immediately before the patient went to the operating theatre later showed to be 46,000/mm$^3$.

Mean preoperative thrombocyte count was significantly different between groups, as demonstrated in Table 2 (F = 3.4; p < 0.05 in ANOVA). Posthoc analysis with the Bonferroni test demonstrated a significantly lower preoperative thrombocyte count for general anaesthesia versus combined spinal epidural anaesthesia (p < 0.05) but no significant differences between combined spinal epidural anaesthesia and spinal anaesthesia nor between spinal and general anaesthesia.

Thirteen patients received thrombocyte transfusions, 12 of these during the preoperative period (Table 2).

There were no cases of epidural haematoma. The only recorded anaesthetic complication was a bloody tap in a planned spinal anaesthesia, necessitating conversion to general anaesthesia. No major preoperative bleeding occurred, but one patient needed to be re-operated within 24 hours due to severe intraabdominal bleeding.

One patient scheduled for spinal anaesthesia developed an anaphylactic reaction upon the hetastarch with hypotension, dyspnoea and generalised oedema. She was transferred to the ICU where she received corticosteroids and supportive therapy until the next morning when platelet counts and liver tests had much improved upon which she underwent uneventful surgery under spinal anaesthesia (this patient has been reported on before) [14].

### Discussion

Our study describes a well-defined group of high-risk obstetric patients undergoing primary caesarean section in case of HELLP syndrome. No direct anaesthesia-related complications were found. The number of platelets was indeed higher for patients receiving a catheter technique whereas no difference in platelet count was found between general and single-dose spinal.

Based on case reports and assumptions HELLP syndrome is often considered a contra-indication for regional anaesthesia [4, 5, 15]. This is mainly due to fear of the development of an epidural haematoma, but other complications have been described such as intracranial subdural haematoma [16]. On the other hand, it is well known that general anaesthesia is a risk factor for maternal mortality, mainly due to problems of airway management or haemodynamic perturbations [17, 18]. Slow metabolic degradation of choline-ester drugs can occur in HELLP syndrome, probably due to decreased pseudocholinesterase activity [14]. The minimum platelet count above which it is safe to perform spinal or epidural anaesthesia is still unknown, but several studies suggest that this may be safely done at thrombocyte counts less than 100,000/mm$^3$ [17, 19-24]. None of these studies contains data specific for patients with HELLP syndrome nor is the use of combined spinal epidural mentioned.

Not many studies have reported on anaesthetic techniques in patients with HELLP syndrome. In the majority of reports the number of patients varies from 20 to 40 collected during four to six-year periods [2-5, 24] while some have mainly described and recommended intensive care treatment for these patients while strongly dissuading regional anaesthesia without strong supportive evidence [4, 5].

In a landmark study by Sibai et al. [11] 16 of 112 patients with HELLP syndrome received epidural anaesthesia. The mean platelet count in this group was 83 ± 8.10³/mm$^3$. There was one maternal bleeding in the epidural space in a patient with a platelet count of 93,000/mm$^3$. The catheter was kept in place for 24 hours and the bleeding stopped spontaneously. In their discussion they wrote that “the use of epidural anaesthesia in such patients is potentially dangerous”.

Crosby et al. [2] reported on 33 patients with HELLP syndrome of whom 32 received general anaesthesia and eight patients had uneventful epidural anaesthesia, but at the time of catheter insertion the diagnosis of HELLP syndrome had not been made in six of these eight patients. They had much evidence of abnormal haemostasis while 36% of patients received blood transfusions.

Miyamoto et al. [10] reported on 11 caesarean sections for HELLP syndrome, six under general anaesthesia, one epidural and four spinal blocks. No complications were noted.

In a report by Osmanagaoglu et al. [25] 27 caesarean sections in a group of 37 HELLP syndrome patients were performed. General anaesthesia was used in 12 and single-dose spinal anaesthesia in 25 patients. In this study no distinction could be made for the anaesthetic used in the vaginal delivery or the caesarean section group. There were no complications of regional anaesthesia but these were also not explicitly looked for. In this study maternal mortality was as high as 30% but it is unclear whether the risk was higher for vaginal than for abdominal delivery. In our hospital vaginal delivery was performed in only one non-included case during the study period evaluated.

The largest published series to date reported on 85 cae-
sarean sections [12], of whom 14 had a post caesarean and 71 a preoperative diagnosis of HELLP syndrome. In this series 58 (81.7%) had epidural anaesthesia, nine (12.7%) had general anaesthesia and four (5.6%) had spinal anaesthesia. Neurological complications or epidural haematoma were not diagnosed.

To our knowledge there is a lack of reports using combined spinal-epidural anaesthesia for caesarean section in HELLP syndrome. Although simply a combination of spinal and epidural anaesthesia it signifies a double risk of perispinal bleeding as two needles are introduced in two vascularised spaces. Theoretically this risk may be even greater when a double-interspace technique is used. Contrary to earlier publications on plain epidural we chose to use the catheter for patient controlled anaesthesia and leave it in place until the platelet count had risen over 100,000/mm³, also because meanwhile LMWH had been started.

Our results, as those from Vigil-De Gracia et al. [12], question the often cited advice that a platelet count below an arbitrary limit (be it 100,000 or 80,000/mm³) precludes the placement of an epidural catheter in cases of HELLP syndrome. In patients with a platelet count below 80,000/mm³ the anaesthesiologists in our centre prefer general or spinal anaesthesia three times more frequently than CSE anaesthesia.

From the point of view of maternal safety no data on anaesthesia-related maternal death in HELLP syndrome are available, but control of blood pressure is more difficult with general anaesthesia.

In a survey of severe neurological complications after a central neuraxial blockade [26] it was found that 33 spinal haematomas out of a total of 127 complications were present, two of which were in patients with HELLP syndrome, one with a spinal block and one with an epidural catheter being removed with apparent signs of coagulopathy. In this study the highest risk for developing spinal haematoma was in female orthopaedic patients subject to knee arthroplasty, and clearly not in obstetric patients.

Other studies on thrombocytopenic parturients have failed to demonstrate the feared complication of spinal haematoma [19, 23, 27, 28].

In a recent letter to the editor Frenk et al. [10, 29] reported their experience with regional anaesthesia in parturients with thrombocytopenia. No neurological complications nor spinal haematomas were reported, but this series did not include patients with HELLP syndrome, and neither did the series reported by Bernstein et al. [27].

It is evident that there is more than just the platelet count alone as altered platelet function has long since and repeatedly been suggested but has not yet been clearly demonstrated in HELLP syndrome [30].

Thrombocyte function is difficult to evaluate. Studies on the use of thromboelastography in HELLP syndrome found that in cases with preeclampsia women with a platelet count less than 100,000/mm³ are significantly hypocoagulable when compared to preeclamptic women with platelet counts ≥ 100,000/mm³, but the level of thromboelastographic parameters that would allow safe epidural anaesthesia to be performed in these women is not known [31].

Thromboelastography has been reported in a case report to reveal accompanying fibrinolysis in cases of HELLP syndrome [32]. No reports are available on the use of platelet function analysis (PFA-100) in HELLP syndrome, but in patients with preeclampsia false positives (suggesting disturbed clothing in normal patients) have been described [33]. On the other hand it was demonstrated that in patients with pregnancy induced thrombocytopenia, platelet function is not disturbed with counts as low as 60,000/µl. Conflicting results have been obtained demonstrating impairment of haemostatic function in severe preeclampsia with PFA-100 even with a normal thrombocyte count, but not when testing with thromboelastography [34].

Conclusion

Based on a small series, our study demonstrates that regional anaesthesia, including combined spinal/epidural anaesthesia for primary caesarean section in HELLP syndrome probably is feasible and safe, but until now no larger studies are available. Despite local guidelines regional techniques have been used more frequently, which may indicate that there is more than platelet count alone in the decision making of an anaesthetic technique such as the speed of platelet loss, technical aspects, emergency, anticipated intubation difficulty and personal preferences [35].

References


Prevalence of severe pelvic inflammatory disease and endometriotic ovarian cysts: a 7-year retrospective study

I. Grammatikakis¹, N. Evangelinakis¹, G. Salamalekis¹, V. Tziortzioti², C. Samaras³, C. Chrelias¹, D. Kassanos¹

¹3rd Department of Obstetrics and Gynecology, Medical School of Athens, General University hospital “Attikon”
²“Lito” Maternity Hospital, Athens (Greece)

Summary

Introduction: The purpose of this study was to delineate the association between endometriosis and pelvic inflammatory disease (PID) and the prevalence of this coexistence. Materials & Methods: The records of all patients with endometriotic ovarian cysts treated at the 3rd Department of Obstetrics and Gynecology of the University of Athens and in “Lito” Maternity Hospital of Athens from 2000 through 2007 were reviewed. Results: During this 7-year period 720 women underwent surgery due to endometriotic ovarian cysts. The average age was 40.9 years (range: 17-70). Median diameter of the cysts was 4.495 cm and 59% were located in the right ovary. PID was identified in 21 (2.9%) cases. The average age of these women was 31 years (range: 21-39). Half of the women presented with fever (10/21; 47.6%). Ultrasound examination was performed in all women, followed by laparoscopy. In 47.6% (10/21) the PID abscess was located in the right ovary and the rest (52.38%) in the left. The mean diameter of the endometriotic cysts in these women was 3.52 cm. Laparoscopy was the treatment of choice in all the women with the exception of five cases, where due to technical difficulties during laparoscopy, a laparotomy was performed. In all the cases with PID, abscesses were evacuated laparoscopically. No operative complications were observed. Conclusions: Endometriosis and PID are two conditions that can easily confuse the physician in setting the diagnosis, especially in the situation where they co-exist. In our study we report that the prevalence of PID in women with endometriosis is sufficiently higher than the prevalence in the general population.

Key words: Pelvic inflammatory disease; Endometriosis; Ovarian cysts.

Introduction

One of the most common conditions that a gynecologist has to deal with in women of reproductive age is endometriosis. Endometriosis is present in the 3-10% of the general population and in 25-35% in women with infertility problems [1]. Characteristic but not occlusive symptoms of endometriosis include dysmenorrhea, dyspareunia and chronic pelvic pain. It is obvious that a diagnosis of endometriosis is usually based clearly on symptomatology and a definite diagnosis can only be made during surgery. The above-mentioned symptoms are present in many women, especially in developed Western countries and the differential diagnosis includes irritable bowel syndrome (IBS) and pelvic inflammatory disease (PID). It is quite characteristic that more than one quarter of the population of Great Britain suffers from symptoms as the ones mentioned above and most of the time IRS is implicated [2]. There are a few articles published in the medical literature trying to distinguish these three entities (IRS, PID and endometriosis) that present with similar, common and torturing symptomatology [3], and many of the women visit successively gastroenterologists and gynecologists trying to obtain relief from these annoying symptoms. The purpose of this study was to delineate the association between two gynecologic conditions – PID and endometriosis – and the prevalence of the coexistence.

Materials & Methods

The records of all patients with endometriotic ovarian cysts treated at the 3rd Department of Obstetrics and Gynecology of the University of Athens and in “Lito” Maternity Hospital of Athens from 2000 through 2007 were reviewed. In all cases endometriotic ovarian cysts were diagnosed based on ultrasound (US) findings and clinical examination. Seven hundred and twenty women were included in the study after reviewing the age, main symptoms at admission, histologic examination, location of the cysts, bilaterality of the cysts and the diameter of the cyst.

Results

During this 7-year period 720 women underwent surgery due to endometriotic ovarian cysts. The average age was 40.9 years (range: 17-70). Median diameter of the cysts was 4.495 cm and 59% were located in the right ovary. PID was identified in 21 (2.9%) cases. The average age of these women was 31 years (range: 21-39). All the women with PID reported premenstrual exacerbation of pain, fornical tenderness, while physical examination was painful. Half of the women presented with fever (10/21; 47.6%). The majority of women without PID presented with abdominal pain and distension worsening at the onset of menses, nausea and/or vomiting and hemorrhagic fluid in the pelvis. US examination was performed in all women, followed by laparoscopy. In 47.6% (10/21) the PID abscess was located in the right ovary and the rest (52.38%) in the left. Mean diameter of the
endometriotic cysts in these women was 3.52 cm (Table 1). Laparoscopy was the treatment of choice in all the women with the exception of five cases, where due to technical difficulties during laparoscopy, a laparotomy was performed. In all the cases with PID, abscesses were evacuated laparoscopically. No operative complications were observed.

Discussion

In women aged less than 25 years 60-80% of PID is caused by sexually transmitted infections (STI), such as gonorhea and/or Chlamydia plus other commensals and anaerobic genital flora [4]. PID can also occur by ascending spread of genital commensals, often following surgical trauma, termination of pregnancy, delivery, oocyte retrieval, and intrauterine device insertion or removal. The minimal criteria for the diagnosis of PID are lower abdominal pain and tenderness, pain elicited on moving the cervix and adnexal tenderness/mass, while fever, dyspareunia, vaginal discharge and white cells on vaginal wet prep are considered as additional but not necessary criteria [3]. Consequently, endometriosis and PID are two conditions that can easily confuse the physician in making the diagnosis, especially in the situation where they co-exist.

In our study we report that the prevalence of PID in women with endometriosis is sufficiently higher than the prevalence in the general population. There are two main theories to explain this higher incidence of PID. The first one is that old blood that accumulates in the peritoneal cavity is an excellent isolated culture media for the bacteria that somehow are found there. Furthermore, the pseudocapsule of the endometriotic cyst along with the chocolate-like material inside it may prevent antibiotic prophylaxis from overcoming the transvaginal bacterial inoculation [5].

In contradiction, some researchers support the idea that the most important factor for the common emergence of PID in women with endometriosis is the altered immunological responses in these women. There are many articles in the medical literature proving that the immune system of women with endometriosis is dysregulated, stating that this is the main reason that endometriotic cells survive in other tissues [6]. Consequently, it is reasonable to suppose that the altered immune system of women with endometriosis is the main cause for not overcoming the inoculated bacteria.

A subpopulation that appears of interest is women in which severe endometriosis is the major cause of infertility. These women often undergo oocyte retrieval, a procedure that provides a path to the flora of the vagina in the pelvis. There are a number of cases described where after oocyte retrieval in women with severe endometriosis a tuboovarian abscess was formed [5]. Thorough vaginal preparation and avoidance of repeat penetration of the vaginal wall are specifically important in these patients. Close follow-up for signs of late pelvic infection is of utmost importance, while prior treatment of endometriosis before superovulation by aspiration or by operative laparoscopy should be considered. Finally, transabdominal oocyte retrieval under US guidance should be taken into account when planning for IVF in patients with multiple ovarian endometriotic ovarian cysts.

Table 1. — Characteristics of women with endometriotic cysts in comparison to women with PID.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Endometriotic ovarian cysts</th>
<th>Endometriotic ovarian cysts and PID</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>720</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Age (17-70)</td>
<td>40.9</td>
<td>(21-39)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Ovary implicated R:</td>
<td>59%</td>
<td>R: 47.6%</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Mean diameter of cysts</td>
<td>4.495 cm</td>
<td>3.52 cm</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

R: right; PID: Pelvic inflammatory disease.

References


Address reprint requests to:
N. EVANGELINAKIS, M.D.
“ATTIKON” General University Hospital
3rd Department of Obstetrics and Gynecology
Rimini 1, Chaidari
Athens (Greece) 12464
e-mail: evangelinakisnikos@yahoo.gr
Mid-trimester maternal serum markers in predicting adverse pregnancy outcome

G. Androutsopoulos¹, P. Gkogkos¹, V. Papadopoulos¹, G. Adonakis¹, V. Tsapanos¹, P. Vassilakos², G. Panayiotakis¹, G. Decavalas¹

¹Department of Obstetrics and Gynaecology, ²Department of Nuclear Medicine, ³Department of Medical Physics
University of Patras, Medical School, Rion (Greece)

Summary

Objective: In a prospective study, we investigated the association between mid-trimester maternal serum AFP (ms-AFP), maternal serum hCG (ms-hCG) levels and adverse pregnancy outcome in a South-Western Greek population. Materials and Methods: 126 healthy Greek women with spontaneous pregnancies were investigated for ms-AFP and ms-hCG levels between the 13th and 24th weeks of gestation and followed for adverse pregnancy outcome. Abnormal outcomes were considered as ms-AFP levels or ms-hCG levels > 2.0 multiples of the median value for gestation (MoM). Statistical analysis was performed by Pearson’s chi-square test. Results: Elevated ms-AFP levels were detected in a total of 25 out of the 126 women studied (19.84%). Elevated ms-hCG levels were detected in a total of four of the 126 women studied (3.17%). Conclusion: Multiparameter testing of placental function in the mid-trimester (uterine artery Doppler, placental morphology, ms-AFP and ms-hCG screening) may allow us to identify women with increased risk of developing severe placental insufficiency and pregnancy complications.

Key words: Maternal serum markers; AFP levels; hCG levels; Adverse pregnancy outcome.

Introduction

Maternal serum Alpha Fetal Protein (AFP) and human chorionic gonadotropin (hCG) were originally introduced for the detection of neural tube defects and trisomy 21 [1, 2]. However, increased quality of ultrasound equipment and sonographer expertise have greatly reduced the need for maternal serum AFP (ms-AFP) and maternal serum hCG (ms-hCG) screening in mid-trimester [2, 3].

Pregnancies with unexplained mid-trimester elevation in ms-AFP and/or ms-hCG, are at increased risk of pregnancy complications [intrauterine growth restriction (IUGR), intrauterine fetal death (IUFD), preeclampsia (PE)] resulting from placental insufficiency [4-8].

In our prospective study, we investigated the association between mid-trimester ms-AFP levels, ms-hCG levels and adverse pregnancy outcome in a South-Western Greek population.

Material and Methods

Between February 2005 and February 2008, 126 women with spontaneous pregnancies were referred to the Outpatient Clinic of the Obstetrics and Gynaecology Department of the University of Patras Medical School. All women were investigated for ms-AFP and ms-hCG between the 13th-24th weeks of gestation and followed for adverse pregnancy outcome.

Gestational age was estimated from the last menstrual period for women with regular (21-35 days) menstrual cycles or confirmed from ultrasonographic scan in the first trimester for women with irregular menstrual cycles. Women with multiple pregnancies, diabetes mellitus, pregnancy with chromosomal or structural abnormality, hypertension diagnosed before the 20th week of gestation, and history of PE in a previous pregnancy were excluded from the study.

All women had a dating ultrasound (US) examination at their first visit, followed by a detailed examination at the 18th-22nd week of gestation. The study was approved by the Ethical Committee of the hospital. Informed consent was obtained from each woman.

Serum samples were collected from all women between the 13th and 24th weeks of gestation. All serum samples were stored at -20°C. AFP levels were measured with immunoradiometric assay using two highly specific monoclonal antibodies for coating of the solid phase and the tracer. The tracer antibody and the coated antibody react simultaneously with the AFP present in patient samples or standards. Excess tracer is removed by a washing step and the radioactivity bound to the tube wall is measured in a gamma scintillation counter (IRMA-mat AFP, DiaSorin Inc). hCG levels are measured with immunoradiometric assay using two highly specific monoclonal antibodies for coating of the solid phase and the tracer. The tracer antibody and the coated antibody react simultaneously with the hCG present in patient samples or standards. Excess tracer is removed by a washing step and the radioactivity bound to the tube wall is measured in a gamma counter (h-CGR IRMA CT, Radim S.p.A.). Abnormal values were considered as ms-AFP levels or ms-hCG levels > 2.0 multiples of the median value for gestation (MoM).

All gestational complications with fetomaternal circulatory disturbances (placental abruption (PA), IUGR, IUFD, PE) were considered as adverse pregnancy outcomes.

Placental abruption (PA) was defined as the separation of the placenta from its site of implantation before delivery of the fetus [10]. Intrauterine growth retardation (IUGR) was defined as a birth weight below the 5th percentile for gestational age [11]. Intrauterine death (IUFD) was defined as fetal loss after 24 weeks’ gestation.
Preeclampsia (PE) was defined by a blood pressure above 140/90 mmHg after 20 weeks' gestation, proteinuria > 300 mg/24 hours or persistent 30 mg/dl (1+ dipstick) in random urine samples. The term severe preeclampsia is used when blood pressure above 160/110 mmHg is recorded at least six hours apart, and proteinuria of more than 5 g during 24 h occurs [12]. Statistical analyses were performed using the SPSS-13 for Windows. The chi-square test was used to assess the association between categoric variables.

Results

Serum samples were collected at a median gestation of 19 weeks (range 13-24). The median weight of the women at the time of serum sampling was 71 kg (range 50–105). The median age at the estimated delivery date was 31 years (range 17-50).

From the 126 women included in the study, 12 (9.52%) developed a gestational complication during the follow-up of their current pregnancy. The demographics of women with gestational complications compared to those without are shown in Table 1.

Table 1. — Women’s demographics (n = 126).

<table>
<thead>
<tr>
<th></th>
<th>Women with complications (n = 12)</th>
<th>Women without complications (n = 114)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pregnancies</td>
<td>1 pregnancy (100%)</td>
<td>96 (84.21%)</td>
</tr>
<tr>
<td></td>
<td>≥ 2 pregnancies (0%)</td>
<td>18 (15.79%)</td>
</tr>
<tr>
<td>Age of women &lt; 25</td>
<td>1 (8.33%)</td>
<td>20 (17.54%)</td>
</tr>
<tr>
<td></td>
<td>25-35</td>
<td>7 (58.33%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 35</td>
<td>4 (33.33%)</td>
</tr>
<tr>
<td>Complications in previous pregnancies</td>
<td>No (8.6%)</td>
<td>102 (89.47%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>No</td>
<td>10 (83.33%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4 (33.33%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 (10.53%)</td>
</tr>
<tr>
<td>NS: not significant.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abnormal ms-AFP levels were detected in a total of 25 of the 126 women studied (19.84%). Among them, only four women (16%) developed gestational complications in the current pregnancy (2 PA, 1 IUGR and 1 IUFD). Abnormal ms-hCG levels were detected in a total of ten of the 126 women studied (7.93%). None of them developed gestational complications in the current pregnancy. Abnormal ms-AFP and ms-hCG levels were detected in a total of four of the 126 women studied (3.17%). None of them developed gestational complications in the current pregnancy. These data are shown in Tables 2 and 3.

Discussion

AFP is initially synthesized by the yolk sac, followed shortly thereafter by the fetal liver. Because the human yolk sac involutes at the 9th week, the fetal liver is responsible for most of the AFP production during development [13, 14]. AFP synthesis by the proliferating fetal liver actually increases through the 20th week of gestation, after which it remains fairly constant until the 32nd week [13-15].

Elevated ms-AFP levels have been strongly associated with congenital abnormalities, placental dysfunction and preterm birth [15, 16]. When the fetus is structurally normal, mid-trimester high ms-AFP levels are thought to reflect a defect in placentaion and are associated with an increased risk for complications in later pregnancy, including severe PE, IUGR and IUFD [15-18]. In our study mid-trimester elevated ms-AFP levels were detected in a total of 25 of the 126 women studied (19.84%). Among them, only four women (16%) developed pregnancy complications (2 PA, 1 IUGR and 1 IUFD).

Serum hCG appears early during pregnancy [19]. Its concentration increases gradually by reaching a peak at the end of the first trimester, after which it progressively decreases until delivery [20].

During pregnancy hCG is produced almost exclusively in the placenta, but is also synthesized in the fetal kidney and fetal liver [21]. Most of the hCG in circulation is metabolized by the liver, whereas about 20% is excreted by the kidneys [22].

The etiology of the increased hCG production by the placenta is not clear. Experimental evidence from trophoblastic cells cultured in vitro showed that hypoxia
increases hCG production [23]. Many mechanisms leading to elevations of ms-hCG have been proposed.

Increased ms-hCG concentrations have been related to the presence of placental pathology, such as infarction, ischemic changes, villitis and intervillus thrombosis [9, 24]. Velamentous cord insertion has been described to be associated with elevated mid-trimester ms-hCG concentration [25]. The presence of chromosomally abnormal areas in the placenta known as confined placental mosaicism, has been found to be associated with high mid-trimester ms-hCG levels [26]. All these placental pathologies may be associated with overproduction of hCG [9, 24, 26-28].

Another possible explanation may be inadequate trophoblastic remodelling of the maternal uterine vasculature, with an absence of normal physiologic changes in the spiral arteries leading to placental hypoxia and hCG overproduction [9, 27, 28].

Pregnancies complicated by an unexplained mid-trimester elevation in ms-hCG are at increased risk of perinatal complications resulting from placental insufficiency, including combinations of IUGR, IUFD and PE [7-9, 29, 30]. In our study mid-trimester elevated ms-hCG levels were detected in a total of ten of the 126 women studied (7.93%). None of them developed pregnancy complications.

Combined elevations in ms-AFP and ms-hCG levels suggest a more complex type of placental pathology and they have a stronger association with complications in later pregnancy (PE, IUGR and IUFD) [31, 32, 33]. In our study mid-trimester elevated ms-AFP and ms-hCG levels were detected in a total of four of the 126 women studied (3.17%). None of them developed pregnancy complications.

In our study the main limitation was the small number of cases with gestational complications. According to the results shown in Tables 2 and 3, elevated mid-trimester ms-AFP and/or ms-hCG levels alone cannot detect all pregnant women with increased risk of developing pregnancy complications. However, uterine artery Doppler screening alone is superior to ms-AFP and ms-hCG screening for the identification of significant placental pathology leading to PE and IUGR [17, 34, 35].

In conclusion, multiparameter testing of placental function in the mid-trimester (uterine artery Doppler, placental morphology, ms-AFP and ms-hCG screening) may allow us to identify women with increased risk of developing severe placental insufficiency and pregnancy complications [34].

References


Address reprint requests to:
N. EVANGELINAKIS, M.D.
“ATTIKON” General University Hospital
3rd Department of Obstetrics and Gynecology
Rimini 1, Chaidari
Athens (Greece) 12464
e-mail: evangelinakissnikos@yahoo.gr
Evaluation of pain before and after vaginal delivery

B. Alves¹, M.D.; T. Zakka², M.D.; M.J. Teixeira³, M.D., Ph.D.; J.T.T. Siqueira⁴, Ph.D.;
S.R.D.T. Siqueira⁵, Ph.D.

¹School of Arts, Science and Humanities, University of São Paulo;
²Interdisciplinary Pain Center, Hospital das Clinicas, Medical School, University of São Paulo;
³Interdisciplinary Pain Center, Hospital das Clinicas, Medical School, University of São Paulo;
⁴Orofacial Pain Team, Hospital das Clinicas, Medical School, University of São Paulo, São Paulo (Brazil)

Summary

Purpose: The objective of this pilot study was to determine pain characteristics of pregnant women immediately before and after childbirth by vaginal delivery and to compare them with the pain intensity reported by physicians. Methods: We evaluated 20 Brazilian women between September and December 2007 with the WHOQOL-Bref instrument, VAS, McGill Pain Questionnaire, and Anxiety Adapted Scale. We interviewed the obstetrician with the VAS about the patient’s pain. Data were analyzed with the chi-square test. Results: Mean age was 22.35 years (SD = 6.24, range 15-39 years). It was necessary to use oxytocin in 15 (75%) patients, which had no correlation with anxiety degree. Higher intensity of pain (p < 0.05) and higher anxiety index (p < 0.05) were more common in women in the first pregnancy. Conclusions: Higher pain intensity was associated with higher anxiety levels (p < 0.05). Around half of the obstetricians’ VAS scores were lower than the VAS scores of women, and probably pain at labor was underestimated and not controlled. Higher indices of anxiety and pain were associated, and were more frequent in women in the first pregnancy.

Key words: Vaginal delivery; Pain; Quality of life; Childbirth; Anxiety.

Introduction

Pain is “an unpleasant sensory and emotional experience associated with real or potential tissue damage, or described in terms of such damage” (International Association for the Study of Pain). There are many pain behaviors due to the exposition and learning, previous experience and nociception. It is important to understand pain in a complex picture which includes individual and environmental influences [1]. Due to pain subjectivity, it is difficult to measure and variable signs, symptoms and behaviors are common. All perceived pain must be believed, evaluated, and treated with respect and knowledge in a broad way [2], and it is always associated with suffering [1].

Many studies have been performed aimed at alleviating pain of childbirth and labor. Pain can be modulated by affective factors, as fear and anxiety; cognitive factors, as pain anticipation; and organic causes, as sympathetic activation and increase in muscular tension [1]. The type of labor also influences the expected pain, and many women in Brazil prefer the cesarean technique because of cultural and social issues, and because of the hope of feeling less pain associated with labor [3]. Current anesthesia techniques permit higher female transoperative participation, and many alternative options can help (anesthesia techniques, pain therapy, and hydrotherapy) [3].

Before undergoing childbirth by natural labor, there is an imposition of routine and obstetric interferences which may inhibit the natural unchaining of physiological mechanisms, thus inducing pregnant women to feel incapa-
The interview was performed with the pregnant women and the obstetrician responsible for the case at both evaluations. Questions were asked in relation to childbirth pain (e.g., before delivery, women were interviewed regarding what they expected to feel and after delivery about what they experienced during childbirth).

**Interviews:**

1) Pregnant women: The following instruments were used at both evaluations.
   - Questionnaire WHOQOL-Bref (World Health Organization Quality of Life), validated in the Portuguese language [7, 8] to analyze quality of life;
   - Numeric visual analog scale (VAS) to analyze the maximum intensity of pain expected or felt;
   - McGill Pain Questionnaire validated in the Portuguese language [9] to analyze the quality of pain;
   - Anxiety scale, adapted from DAS (dental anxiety scale), validated in the Portuguese language [10] for anxiety aspects associated with surgical procedure expectations.

2) Obstetrician: The numeric VAS was implemented as an instrument to verify maximum pain intensity that was inferred about woman at both evaluations.

Data were statistically analyzed using the chi-square test, paired T-test and ANOVA with the significance level being 0.05.

**Results**

Twenty pregnant women were evaluated from September to December 2007, corresponding to all women in labor that were assessed for natural delivery at the University Foundation of Health of Taubate, Brazil. The mean age was 22.35 years old (SD = 6.24, range 15-39 years); eight women (40%) were in the first pregnancy, and the rest of had a mean of 2.25 children (SD = 1.5; range 1-6): It was necessary to use oxytocin in 15 (75%) and the rest of had a mean of 2.25 children (SD = 1.5; range 1-6): It was necessary to use oxytocin in 15 (75%) women. Mean dilatation of the uterine colon was 3.4 cm (SD = 1.9; range of 0-6 cm).

**Quality of Life (WHOQOL-Bref)**

There were no differences between either evaluation in the final scores of quality of life by the WHOQOL-Bref in this sample. However, for specific issues there were some differences, except for the psychological domain in negative feelings (Table 1). There was a slight but non significant increase on the dependence of medical aid, an improvement in mobility (<0.05) (Table 2), a reduction in sexual activity satisfaction (<0.05) (Table 3), an improvement in security sensation (<0.05) and a reduction in leisure activities (<0.05) (Table 4) after childbirth.

Work capacity was correlated with self-esteem (<0.05) and higher VAS scores (<0.05). Work capacity improved after childbirth (<0.05). The worst financial resources (<0.05) were correlated to higher VAS scores of women after childbirth. On the other hand, social support was correlated to lower VAS scores (<0.05).

**Quality of Pain (McGill Pain Questionnaire)**

The most common pain descriptors were hot (<0.001), heavy (<0.01) cold (<0.05), crushing (<0.05), and cruel (<0.05). We observed that the descriptor “cramping”, often associated with uterine pain, was maintained.

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**Table 1. — Psychological domain of quality of life (WHOQOL-Bref) (n = 20).**

<table>
<thead>
<tr>
<th></th>
<th>Before childbirth</th>
<th>After childbirth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>2 (10%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Sometimes</td>
<td>17 (85%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>Often</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Negative feelings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very often</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Always</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Did not answer</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*p* < 0.05: Reduction in leisure activities.

**Table 2. — Physical health domain of quality of life (WHOQOL-Bref) (n = 20).**

<table>
<thead>
<tr>
<th></th>
<th>Before childbirth</th>
<th>After childbirth</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5 (25%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Little</td>
<td>10 (50%)</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>High</td>
<td>4 (20%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Very high</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Dependence on medical aid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bad</td>
<td>3 (15%)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>Indifferent</td>
<td>7 (35%)</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Good</td>
<td>9 (45%)</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>Very good</td>
<td>1 (5%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Did not answer</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*p* < 0.05: Increased dependence on medical aid. **p < 0.05: Improvement in mobility.

**Table 3. — Social relationship domain of quality of life (WHOQOL-Bref) (n = 20).**

<table>
<thead>
<tr>
<th></th>
<th>Before childbirth</th>
<th>After childbirth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very unsatisfied</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Unsatisfied</td>
<td>2 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Indifferent</td>
<td>3 (15%)</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Satisfied</td>
<td>9 (45%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Very satisfied</td>
<td>6 (30%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Did not answer</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*p* < 0.05: Reduction in sexual activity satisfaction.

**Table 4. — Environmental domain of quality of life (WHOQOL-Bref) (n = 20).**

<table>
<thead>
<tr>
<th></th>
<th>Before childbirth</th>
<th>After childbirth</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Very little</td>
<td>3 (15%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Little</td>
<td>6 (30%)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>Secure</td>
<td>7 (35%)</td>
<td>14 (70%)</td>
</tr>
<tr>
<td>Very secure</td>
<td>4 (20%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Did not answer</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

**Table 5. — Intensity of pain (VAS) (n = 20).**

<table>
<thead>
<tr>
<th></th>
<th>Before childbirth</th>
<th>After childbirth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>7.7 ± 2.3</td>
<td>7.9 ± 2.5</td>
</tr>
<tr>
<td>Obstetricians</td>
<td>7.6 ± 2.1</td>
<td>7.0 ± 2.1</td>
</tr>
</tbody>
</table>

*p* < 0.05: Increased security perception. **p < 0.05: Improvement in security perception.
Intensity of Pain (VAS)

Although there were no differences in means of intensity of pain between the women and the obstetricians (Table 5), during labor only 23% of the data between the patient and obstetrician were the same, and after childbirth the similarity was 25%. Before delivery, 46% of the physicians expected lower pain than the expectation of the pregnant women, and after childbirth this percentage increased to 58.33%.

Among women, 50% had the same intensity of pain during labor and seven days after delivery, 30% had higher pain scores after childbirth and 20% reported lower perceived pain than what they had expected before.

The higher intensity of pain (VAS) \( p < 0.05 \) was more common in women during the first pregnancy and was correlated to higher pain intensity after childbirth \( p < 0.05 \). The higher levels of pain after childbirth were correlated with the following McGill descriptors: lacerating \( p < 0.05 \), cruel \( p < 0.05 \) and grueling \( p < 0.05 \).

Anxiety (DAS adapted)

Total score for mean of anxiety at labor was \( 14.7 \pm 3.2 \) and after childbirth it was \( 15.4 \pm 3.0 \). There was no correlation between the use of oxytocin and anxiety degree. The higher anxiety index \( p < 0.05 \) was more common in women in the first pregnancy. There was no correlation between number of children and anxiety.

The higher anxiety index of labor evaluation was associated with higher pain intensity after childbirth \( p < 0.05 \), and with the following McGill descriptors: heavy \( p < 0.01 \), splitting \( p < 0.05 \), grueling \( p < 0.001 \), beating \( p < 0.05 \), crushing (n.s.), piercing \( p < 0.05 \), cool \( p < 0.05 \) and lacerating \( p = 0.05 \); the descriptor “pounding” was associated with anxiety due to expectation of surgery \( p < 0.05 \). The descriptor “grueling” was correlated with all four questions of the DAS adapted questionnaire \( p < 0.001 \). After childbirth, the McGill pain descriptors correlated with higher levels of anxiety included pricking \( p < 0.05 \), hot \( p < 0.05 \), cruel \( p < 0.05 \), troublesome \( p < 0.05 \), tearing \( p < 0.01 \), cool \( p < 0.01 \) and piercing \( p < 0.05 \).

Discussion

Pain depends on biological, psychological and social factors, according to its own definition. In general, it is associated with negative feelings. Therefore, when associated with childbirth the motivation of pain (the fact of having a baby) complicates pain interpretation. Moreover, interpersonal relationships, living conditions and housing are involved in pregnancy, childbirth, and associated pain [8].

Many professionals do not consider parturient complaints, and when the behavior is exaggerated, they criticize and reprehend it. Pain is individual and depends on the previous experiences of the woman and on the psychosocial context in which she is involved [9]. In the absence of previous experience, painful behavior can vary, and in our study there was a significant correlation between higher pain intensity and women in the first pregnancy. Also anxiety was higher in these patients. The fear of the unknown, associated with the common belief of intense pain at childbirth is underlying. Western societies do not tolerate feeling any pain and search for relief with analgesic techniques or with less painful childbirth (as cesarean) which allows a higher degree of tranquility [8, 11, 12].

As pain is subjective, it cannot be measured only by the behavior that the individual has, nor can health professionals that are assessing the case always predict it. In the patients in this sample, we observed that before and after childbirth in only one quarter of the cases was there a correlation in the intensity of pain by the patient with the intensity predicted by the obstetricians (they reported the same value). It is noteworthy that in the majority of cases the intensity referred by the obstetrician was inferior to the intensity referred by the women, clearly suggesting that pain is underestimated by health professionals. This aspect has already been observed in other painful conditions, such as joint pain [13]. This attitude may result in lower analgesic strategies and more suffering by pregnant women. It is known that innumerable procedures, many non invasive, may collaborate to reduce the anxiety at the time of childbirth and aid in controlling pain.

In our data, we found differences between patient anxiety before and after childbirth but no differences in quality of life. It is known that anxiety contributes to pain and needs to be considered when attempting to control it. In fact, it was correlated with higher pain intensity predicted before childbirth. In this study we should also consider that shared rooms and often the impossibility to have a relative in the room during labor may be correlated with higher indices of anxiety in these patients. Pain involves cognitive factors, and the explanation and demystification of natural childbirth with clear information about labor and associated phenomena are necessary measures. It should also be remembered that pain during labor is visceral, and thus often does not have an exact localization. It is usually diffused and determines referral pain, often with high emotional connotations [14, 15]. In fact, descriptors of quality of life were correlated to the anxiety index and the reflexion of this great emotional impact can be observed by the descriptors: before childbirth, splitting, grueling, crushing (n.s.), piercing, lacerating, and after childbirth, cruel, tearing, piercing.

When we analyzed isolated questions about the quality of life, social support was significantly correlated to a lower intensity of pain and fewer financial resources with higher intensity of pain, which supports the role of socioeconomic factors in the general perception of pain [8, 16, 17]. Work capacity was significantly correlated to self-esteem and there was an increase in the sensation of security, which could reflect satisfaction with motherhood. Associated with the security that women feel while taking care of the baby, there is the responsibility and reduction in leisure activities, once that now she must occupy her attention and time for the child.

After childbirth, there was an increase in the need of
medical aids, which demonstrates that even in natural childbirth, the postoperative period needs special attention. Consequently it is interesting that the mobility increased, probably because of the reduction in weight and abdominal volume and the satisfaction with motherhood, which also increased the perception about the capacity of doing daily activities. The post-labor period up to seven days also reflected lower satisfaction with sexual activity than before delivery, which might be associated with taking care of the baby and depression that can occur in this period [8, 16].

In conclusion, we observed that there was no difference in general quality of life before and after childbirth, and higher pain intensity was associated with higher anxiety levels; higher indices of anxiety and pain were associated, and were more frequent in women in the first pregnancy. Intensity of pain between women and obstetricians corresponded only in one quarter of the cases, but in the majority the intensity of pain predicted by the obstetrician was lower than what was reported by the parturient.

Acknowledgement

We would like to thank CNPq (National Council of Scientific and Technological Development) of Brazil for the financial support for this research, and also The Institute of Mathematics and Statistics of the University of São Paulo for the statistical analysis.

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Address reprint requests to:
S.R.D.T. SIQUEIRA, Ph.D.
Rua Carlos Weber, 1319 apto P - 164
Vila Leopoldina, 05303-000
São Paulo - SP (Brazil)
e-mail: silviadowgan@hotmail.com
A comparison of low-dose and high-dose protocols of vaginal misoprostol for second trimester termination of pregnancy

K. Özerkan¹, G. Ocakoğlu², S. Rehimli¹, G. Uncu¹, O. Develioğlu¹

¹Department of Obstetrics and Gynaecology, Uludağ University, Faculty of Medicine, Bursa
²Department of Biostatistics, Uludağ University, Faculty of Medicine, Bursa (Turkey)

Summary

Sixty patients were randomized to low-dose and high-dose groups, receiving a maximum total dose 1400 g of misoprostol by the vaginal route to compare the efficacy of the protocols for second trimester termination of pregnancy. Outcome measures to be compared between the groups were success rates, time to termination, blood loss, complications and side-effects. Yet time to termination was significantly shorter in the high-dose than in the low-dose group (923 ± 571 vs 1307 ± 828 min; p < 0.05). The distance between the internal cervical os and the placenta was positively correlated with the duration of the termination process (r = 0.508, p < 0.001). Induction to the fetal expulsion period is shorter with the higher dose without any significant increase in morbidity. A shorter distance between the internal cervical os and the placenta may forecast a shorter termination process.

Key words: Pregnancy; Second trimester; Induced abortion; Misoprostol.

Materials and Methods

Study design

This was a randomized clinical trial designed to compare the efficacy of low-dose and high-dose protocols of vaginal misoprostol for second trimester termination of pregnancy between 13-24 weeks of gestation with post-abortion curettage of the uterine cavity. The study was approved by the Ethics Committee and Institutional Review Board of Uludag University Faculty of Medicine. All patients were informed about the trial and gave written consent.

Sixty women with various indications for pregnancy termination in the second trimester were randomized by computer-generated number lists to two groups of 30. Following collection of data about demographic and obstetrical parameters, all women underwent US by the practicing physicians to check for gestational ages and measurements of cervical length and distance between the internal cervical os and the placenta. Patients in either group received a maximum total dose of 1400 g of misoprostol by the vaginal route, starting with 400 g in the low-dose group with an additional 200 g tablets applied at two-hour intervals up to five doses. Patients in the high-dose group received a starting dose of 600 g, with an additional 400 g applications at four-hour intervals up to two doses. The next dose of the drug was skipped whenever there were effective uterine contractions. Patients were fully monitored throughout the procedure. If the procedure failed on the first day, it was undertaken the next day using the same protocol and starting at the same time as the previous day. Another method of pregnancy termination was called for in case the procedure failed on two consecutive days. The main outcome measures to be compared between the groups were success rates, time to termination, blood loss, complications, side-effects and cervical features defined ultrasonographically.

All statistical analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Categorical variables are presented as numbers and percentages, and continuous variables as means with standard deviation and range. Chi-square tests were used for comparison of categorical variables between the groups, and
Mann-Whitney U and Student’s t-tests for continuous variables, depending on the distribution of the data. Pearson’s correlations were used to define relationships between continuous variables $p < 0.05$ was accepted as statistically significant.

Results
This study started in July, 2006 and ended in December, 2007. Of the 60 patients included, there were no dropouts during the study. There were no differences between the groups regarding demographic characteristics or cervical features (Table 1).

Misoprostol alone was equally successful at low and high doses to induce termination of pregnancy (25/30; 83.3% vs 28/30; 93.3%). In addition to misoprostol, two women in each group needed augmentation of contractions by oxytocin to complete the termination process. Extraamnionic saline installation and cervical traction with a Foley catheter had to be implemented as additional methods in three others in the low-dose group. Termination was achieved in a significantly shorter period of time using the high-dose protocol $(923 \pm 571$ vs $1307 \pm 828$ min; $p < 0.05$), albeit at the cost of higher mean total dose of drug $(1153 \pm 50$ vs $886.67 \pm 532$ g; $p < 0.05$). While gestational age or gravidity were not found to be related to the duration of the termination procedure, a higher parity was shown to be correlated with a shorter induction-to-fetal expulsion period in the low-dose ($r = -0.400$; $p < 0.05$), but not the high-dose group.

Cervical features on pelvic examination or cervical length on US were not correlated with the duration of the termination process. A shorter distance between the internal cervical os and the placenta, on the other hand, heralded a shorter termination procedure ($r = 0.508$; $p < 0.001$).

The frequency of gastrointestinal side-effects, pain and fever, attributable to prostaglandins was not different between the groups (Table 2). Nor was the blood loss, as the mean hemoglobin levels either pre- or post-termination did not differ significantly between the groups (Table 3).

Discussion
Misoprostol is known to be an effective agent for cervical ripening and induction of labor, and its abortifacient properties in the second trimester of pregnancy have been well-defined by numerous studies. Since the earlier studies with the drug, initially used at lower doses in the range of 200 g b.i.d. [9], higher doses, more frequent application regimens, and alternate routes of administration have been studied extensively. Although direct comparison between studies is not realistic due to variations in indications for termination, differences in dosage and intervals of drug application, additional use of other abortifacients like oxytocin or mifepristone, and the definition of “success” with the procedure, previous work has in general shown that vaginal administration of the drug is more effective and better tolerated than oral [1], and that it is probably more efficacious at higher doses [4, 10]. On the other hand, research to define the minimum dose with the shortest induction-to-termination interval and the minimum side-effects is still on going since the first report by Jain et al. [11]. The present study was similarly designed to compare the efficacy of low-dose and high-dose protocols of vaginal misoprostol for termination of pregnancy in the second trimester. During the study, we also tried to collect data on cervical features and length, and placental location in order to analyze these parameters as predictors of the efficiency of the termination procedure.

To keep the total amount of drug used to a minimum, and hopefully the side-effects, in the present study the next dose of drug was skipped whenever there were effective uterine contractions. With this modification in protocol, the mean induction-to-termination intervals were $1307 \pm 828$ min $(21.7 \pm 13.7$ h) and $923 \pm 571$ min $(15.3 \pm 9.5$ h) in our low- and high-dose groups, respectively. The duration of the procedure in our high-dose group was similar to the mean of 14.5 h reported by Dickinson and Evans [2], and shorter than the means of 18.3 and 19.6 h reported by Akoury et al. [5] and Bebbington et al. [6], respectively. It is certainly possible, on the other hand, to...
complete the procedure in a shorter period of time as shown by the study of Carbonell et al., who reported a mean of 10.7 h with 600 mg of misoprostol every six hours up to four doses and a mean of 11.5 h 400 mg of drug every four hours up to five doses, with success rates of 98.1% and 94.3%, respectively [4]. Similarly, Bhattacharyya et al. reported success rates of 98.6% with a mean induction-to-termination interval of 13.0 h using 400 g of misoprostol every three hours, and 95.5% with a mean duration of 12.1 h using 600 g of drug followed by 200 μg every three hours [7]. The total amount of misoprostol used by Carbonell et al. [3] was 1320 ± 540 g, and 1701 ± 431 g by Bhattacharyya et al. [7]. Likewise, a success rate of 90.5% was reported by Wong et al., with a mean of 2021 ± 890 g of drug used [8].

In the face of success rates of 60-98% hitherto reported in the literature, and keeping in mind the relatively high total doses in the studies mentioned above, our success rates of 83.3% and 93.3% in the low- and high-dose groups, respectively, are fairly respectable. The lower total doses of misoprostol used in the present study appeared further to translate into a more favorable side-effect profile than in the other studies [4, 7, 8]. The frequency of nausea was 10% in our study, in comparison to the rate of 23% reported by Bhattacharyya et al. [7]. Likewise, the incidences of vomiting and diarrhea, observed in 23% and 38% of the cases by Carbonell et al. [4] were 7% in the present study. We observed fever in 10% of our cases, compared to the exact same rate of 32% reported by all three studies cited above [4,7,8]. Lastly, the occurrence of pain necessitating analgesia was observed in 10% of our cases, in comparison to 34% reported by Wong et al. [8].

Conclusion

Both protocols employed in the current study appear reasonable to use for termination of pregnancy in the second trimester with acceptable success rates and side-effect profiles. Another interesting finding of this study was that the distance between the internal cervical os and the placenta was positively correlated with the induction-to-termination interval. This finding needs of course to be further investigated in larger studies.

References


Address reprint requests to: K. ÖZERKAN, M.D.
Department of Obstetrics and Gynecology
Uludag University Faculty of Medicine
Görükle, Bursa 16059 (Turkey)
e-mail: doctorkemal@yahoo.com
Intramuscular fetal corticosteroid therapy
short-term effect on maternal-fetal Doppler velocimetry

I. Babović1, S. Plesinac1, J. Opalić1, I. Pilić1, Z. Radojičić2, M. Pervulov1, N. Radunović1, A. Ljubić1
1Institute for Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade
2Faculty of Organizational Sciences, University of Belgrade, Institute for Statistics, Belgrade (Serbia)

Summary

Aim: The aim of the study was to assess the short-term effects of intramuscular (IM) corticosteroid therapy (CST) on fetoplacental and fetal circulation in high-risk pregnancies of preterm labor. Method: We evaluated the effect of IM single-dose dexamethasone (4 mg/kg) on fetoplacental and fetal circulation two hours before and 0-4 hours after CST in 38 fetuses after the 32nd week of gestation. Result: Changes in the umbilical artery (UA) resistance index (RI) after fetal CST (AU RI1) were significantly correlated with gestational age after the 32nd week at recording r = 0.354; p < 0.05. There was a statistically significant difference of RI in the descending aorta (DAo) before and after therapy; p < 0.001 (-0.04-0.01), 95% confidence interval (CI) for differences. Conclusion: Short-time effects after fetal IM CST include an increased index resistance in DAo as well as decreased RI in UA after the 32nd week.

Key words: Fetal corticosteroid therapy; Fetoplastic; Fetal arterial velocimetry.

Introduction

Liggins [1] observed that fetal corticosteroid treatment trials resulted in early lung maturation in sheep. The pioneers of clinical trials, Liggins and Howie [2], documented that maternal antenatal corticosteroid treatment could decrease the incidence of respiratory distress syndrome in premature infants. Many studies have subsequently demonstrated that corticosteroids accelerate, in vitro and in vivo, the physiologic, biochemical and morphologic maturation of the fetal lung. Maternal administration of glucocorticoids has not been demonstrated specifically in intrauterine growth-restricted (IUGR) fetuses, whereas the use of antenatal glucocorticoid treatment has been widespread in this population, especially in cases of elective preterm delivery [3].

Knowledge of fetal hemodynamic effects of exogenous corticosteroids is limited. Szabo and Cosmi [4] reported an 8-fold increase in relation to the initial value of fetal lung perfusion (by three-dimensional color power Doppler) which was observed within 10 min after fetal betamethasone injection of 0.5 mg/kg of estimated fetal weight. Cohlen et al. found, following antenatal betamethasone therapy, no significant change in the pulsatility index of any of the fetal blood vessels [5]. A new technique for artificial lung maturation – direct fetal IM CST was included very successfully in clinical practice by Ljubić et al. [6], but there is no information about its effect on fetal behavior. Betamethasone causes profound but transient suppression of fetal heart rate parameters, which can mimic fetal distress. Evaluating fetal wellbeing with Doppler waveform studies after direct fetal intramuscular administration is therefore important.

The aim of our study was to evaluate short-term effects (0-4 h), of direct fetal IM CST on the resistance index of the umbilical artery (UA RI), the middle cerebral artery (MCA RI), aortic isthmus (AI RI) and fetal descending aorta (DAo RI).

Materials and Methods

Thirty-eight fetuses from singleton pregnancies after the 32nd week of menstrual age at risk of preterm delivery were prospectively included in the study at the Institute of Gynecology and Obstetrics, Clinical Center of Serbia, from June 2000 to June 2001. Gestational age was calculated according to the date of the last menstrual period and confirmed by first trimester ultrasound (US). If there was a discrepancy (more than five days), US was used to determine gestational age.

Preterm birth was anticipated on the basis of: 1. preterm contraction of the uterus (n = 20); 2. fetal intrauterine growth retardation (n = 11); 3. pregnancy-induced hypertension (n = 6); 4. preeclampsia (n = 4); and 5. premature rupture of membranes (n = 3).

We used a free-hand technique with Doppler mapping in order to avoid maternal and fetal blood vessels. An effective single dexamethasone dose of 4 mg (fetuses up to 1,750 g of estimated fetal weight were given 3 mg and those above that limit 4 mg) was administered directly to fetuses by transabdominal US-guided IM injection from 9 to 12 a.m. Fetal weight was estimated using Hadlock’s method based on measurements of the fetal head, body and femur. The site of application was the fetal gluteal musculature. A 22-gauge needle was used. The mothers were sedated during intervention (by diazepam). There were no incidents during or after corticosteroid applications. All women except one received additional medications: Partusisten (n = 20), antihypertensive drugs (n = 10), aspirin (n = 11), and antibiotics (n = 3). Tocolytic agents, used to stop preterm contraction of the uterus, were introduced before the first Doppler examination and discontinued after the last evaluation. Fenoterol (Partusisten Boehringer-Ingleheim) was administered via an infusion pump. The initial infusion was 0.5-1 μg/min, with the rate increasing when necessary every 30-60 min to a maximum of 3.0 μg/min. The Ethics Committee (no. 1262/99) approved the protocol. Patients were enrolled in the

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study after informed consent had been obtained. Doppler examination was performed two hours before an IM corticosteroid therapy test (CST) and 0-4 h after therapy. A combined real-time pulsed Doppler system fitted with a 3.75 MHz curvilinear probe (Siemens) was used. The spatial peak temporal average power did not exceed 87 mW/cm. The Doppler angle of insonation was less than 30, sweep speed was 2.5 cm/s and pulse repetition frequency from 3.5 KHz to 5.0 KHz. The women rested in a semi-recumbent position during Doppler examination. All measurements were performed by the same physician during fetal apnea. Blood velocity waveforms were obtained from both the umbilical artery and fetal middle cerebral arteries. The umbilical artery was insonated close to its placental insertions and the middle cerebral artery about 1 cm distal to its origin from the internal carotid artery. The descending aorta (DAo) was insonated just below the aortic valve from frozen real time images during systole. Doppler recordings from the aortic isthmus, down-stream of the left subclavian artery and just upstream of the ductus arteriosus connection, were obtained in a sagittal view simultaneously visualizing the aortic arch. We calculated the resistance index for each vessel by averaging the first two good quality resistance indexes obtained from two consecutive waveforms.

Multifetal pregnancies, intrauterine fetal demise, and suspected fetal congenital malformations were excluded from the study.

Continuous variables are presented as mean (standard deviation as 95% confidence intervals) assessed for normality or not, and comparisons were made using the paired sample t-test to estimated differences between the resistance index (RI) in the umbilical artery (UA RI), middle cerebral artery (CM RI), aortic isthmus (AI RI) and descending aorta (DAo) before and after fetal IM corticosteroid therapy. A probability value below 0.05 was considered statistically significant. Spearman’s correlation test was used to compare gestational week at recording and indexes of resistance in the umbilical artery, middle cerebral artery, descending aorta and aortic isthmus.

Results

The mean age of all women participating in the study was 29.2 years. Of the study women 26.3% (10/38) were older than 35 years. The majority were nulliparas (60.5%, 23/38). In 24 (63.2%) pregnancies, a cesarean section was performed and 14 neonates were born vaginally. Mean neonatal weight was 2,591.54 ± 639.33 g and there were the same number of male and female neonates (50.0%, 19/38).

Mean gestational age at dexamethasone administration was 35.13 ± 1.99 weeks (min = 27 weeks, max = 35 weeks). Most of the fetuses were above the 32nd week of gestation (92.1%, 35/38).

The mean RI in the umbilical artery was 0.66 ± 0.08 before the direct fetal IM steroid therapy (UA R1I0) and 0.64 ± 0.09 after the therapy (UA R1I1). Changes in the umbilical artery RI (UA R1I) was significantly correlated with gestational age after the 32nd week at recording ($r = 0.3540; p < 0.05$; Spearman’s correlation test). There was not a statistically significant difference between umbilical artery index resistance before or after direct fetal CST ($p = 0.625$; 95% CI).

The mean RI in the middle cerebral artery was 0.66 ± 0.08 before the direct fetal IM steroid therapy (UA RI0), and 0.64 ± 0.09 after the therapy (UA RI1). Changes in the umbilical artery RI (UA R1I) was significantly correlated with gestational age after the 32nd week at recording ($r = 0.3540; p < 0.05$) (Figure 1).

There was not a statistically significant difference between umbilical artery index resistance before or after direct fetal CST ($p = 0.625$; 95% CI).

The mean RI in the middle cerebral artery was 0.80 ± 0.08 before fetal dexamethasone therapy (CM RI0) and 0.77 ± 0.08 after the therapy (CM RI1). Changes in the middle cerebral artery RI (CM R1I) were not significantly correlated with gestational age after the 32nd week at recording ($r = 0.074$). The main RI in the aortic isthmus was 0.89 ± 0.03 before the fetal IM steroid therapy (AI RI0) and 0.90 ± 0.04 after the therapy (AI R1I). No significant changes were observed between the middle cerebral artery index before (MCA RI0) and after CST (MCA RI1) (95% CI), as well as between resistance indexes of the aortic isthmus before (AI RI0) and after therapy (AI R1I) (95% CI).
The mean RI in the descending artery was 0.83 ± 0.03 before the direct fetal IM steroid therapy (DAo RI0) and 0.85 ± 0.03 after the therapy (DAo RI1). There was a statistically significant difference of index resistance in the descending aorta before and after therapy (p < 0.001; 95% CI) (Figure 2).

Discussion

In the study 77.3% of women were younger than 35 years and the majority were nulliparas. The synthesis and secretion of surfactant are a complex sequence of biochemical events involving lipids. Specific enzymes included in metabolism of surfactant use glucose, phosphate, and fatty acids as substrates for phospholipids synthesis. These reactions are determined by fetal oxygenation [7]. Hypertensive disease is a frequent indication for preterm delivery. Almost one-third of all pregnancies in our study were complicated by maternal hypertensive disorders. Corticosteroids might initiate hypertension in normotensive women and aggravate it in those with already elevated blood pressure. The 48-hour postponement of delivery required to complete antenatal CST may be unacceptable because of the maternal risk of preeclampsia that is aggravated by CST. Only healthy fetuses without US signs of fetoplacental insufficiency at the time of administration IM fetal CST were considered. In over twice the pregnancies cesarean section was performed.

Lemons and associates [8] reported that of approximately 70% of neonates who received antenatal corticosteroids, 50% were delivered by cesarean section. After the 29th week birth weight appeared to be a better predictor of survival than gestational age [9]. Mean neonatal birth weight was 2,600 g.

We found that the changes in the umbilical artery resistance index (AU RII) were significantly correlated with gestational age after the 32nd week at recording. But IR in MCA was insignificantly correlated with gestational age after that. We were not able to define a gestational age after the 32nd week as an age window in which the effect of dexamethasone was significant.

There was no statistically significant difference between umbilical artery RI before or after direct fetal CST. As Urban et al. [10] reported, animal data have shown that dexamethasone in fetal sheep reduced fetal partial pressure of oxygen in arterial blood, suggestive of umbilical artery vasoconstriction.

We found a statistically significant difference between RI in the DAo before and after direct fetal CST. The pulsatility index of DAo was the only one where the peripheral vascular resistance index did not correlate with fetal cardiac output. The constant PI may indicate that descending aortic flow is integral for fetal development and may remain at a relatively low vascular resistance. Chang et al. [11] reported that changes of cardiac output did not influence the vascular resistance. However glucocorticoids increase blood pressure and vascular resistance in human neonates, and similar changes are likely to occur in human fetuses, although these may not be reflected by changes in the flow velocity waveforms of the UA and MCA [10]. In UA and DAo the acceleration time changed with fetal heart rate.

There was no control group since we were unable to recruit mothers.

Conclusion

Short-term effects of direct fetal CST include increased index resistance in the DAo and decreased resistance indexes in the UA after the 32nd gestational week. The short-time effects of IM fetal CST could be modified by intrauterine hypoxia and additional medication.

References


Address reprint requests to: I. BABOVIC, M.D.
63/27 Luke Vojvodica Street
Belgrade (Serbia)
e-mail: ivana.r.babovic@gmail.com
Chorionic villus sampling: analysis of the first 350 singleton pregnancies by a single operator

G.O. Ajayi

Prenatal Diagnosis & Therapy Centre, College of Medicine, University of Lagos, Surulere, Lagos (Nigeria)

Summary

Objective: To assess factors that might influence the success rate, safety and reliability of chorionic villus sampling (CVS). Design: Analysis of the outcome of 350 cases of CVS (215 transabdominal and 135 transvaginal). Setting: The outpatient prenatal diagnosis and therapy laboratory of a university tertiary care centre. Subjects: 350 pregnant women that underwent CVS for prenatal genetic diagnosis between nine and 32 gestational weeks. Results: Fetal genotype was the most common indication for CVS 45% (158/350). The overall sampling success rate was 98% (343/350). The majority of cases, 92% (322/350), required one or two aspirations. Out of 331 cases in which CVS was successful 305 continued with the pregnancy. Thirty-five had therapeutic termination of their pregnancies and ten resulted in spontaneous abortions. There was an overall fetal loss rate of 1.7% (6/350). Early bleeding complications occurred in 11.4% (40/350), PROM 0.86% (3/350), preterm 5.1% (18/350), and placenta disorders 1.1% (4/350) did not exceed the expected values. Conclusion: CVS is a relatively safe and reliable method of prenatal genetic diagnosis. It needs be done by experienced personnel.

Key words: Chorionic villous sampling; Fetal; Prenatal genetic diagnosis.

Introduction

Chorionic villus sampling (CVS) allows diagnosis of genetic diseases within the first trimester of pregnancy.

Based on earlier identification of affected fetuses as compared with amniocentesis and relative safety, this procedure spread worldwide very rapidly [1, 2].

It became widely popular owing to its advantage of first trimester diagnosis, which meant early reassurance or early termination [3].

Several methods of chorionic villus sampling under ultrasonic guidance have been evaluated. The most commonly used are aspiration through a cathether [4-8] or biopsy by forceps [9, 10] introduced through the cervix, and transabdominal sampling [11-14].

In this study, we assessed factors that might influence the success rate, safety and reliability of chorionic villus sampling using the transabdominal and transvaginal approach under ultrasound (US) guidance in 350 cases at risk of genetic problems.

Materials and Methods

Between February 1994 and February 2005 a total of 350 patients who had either transabdominal (215) or transvaginal (135) chorionic villus sampling for different genetic problems followed-up until delivery were analyzed.

CVS was carried out according to Holzgreve et al. [8] and Kaplan et al. [9].

No premedication or local anesthetic was administered. Betamethasone solution was used for cleansing of the external genitalia, vagina, cervix and abdominal wall depending on the method of approach.

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For the transabdominal procedure we used a freehand needle technique and for transvaginal we used a Holzgreve cathether (Angiomed/Germany). US was carried with a 7.5 mHz transducer (Combison 320-5, Kretz-Technik, Austria) and Siemens-Sonoline (Germany).

Sector and real-time scanners with transducers were used. Rhesus negative, unsensitized patients were given anti-D immunoglobulin in accordance with the guidelines of the German Society of Obstetrics and Gynecology and American College of Obstetrics and Gynecology [15, 18]. At the end of the procedure and at 12, 16 and 21 weeks, a US control study was performed to screen for trophoblasts, hematoma and fetal vitality. The weight of chorionic villi obtained was visually estimated by the author using the Holzgreve method [8]. After separation of maternal tissue from fetal tissue, DNA was extracted using a standard method [19]. Analysis for the sickle cell mutation was carried out on 200 ng of DNA by polymerase chain reaction (PCR) [20] on an Autogene thermocycler (Grant Instruments, England) to amplify a 770 bp segment of the $\beta$-globulin gene, followed by Dde 1 (GIBCO-BRL) restriction analysis of the PCR product. Where results seemed equivocal the analysis was repeated by the PCR - ARMS method [21].

The procedures were done after genetic counseling and STORCH antibody screening [22].

Results

The gestational age and time of sampling ranged between nine and 14 weeks of gestation, although about 55% of the patients were sampled within the tenth week.

The entire procedure was completed within 10-30 min. A sufficient amount of chorionic villi was obtained in all cases tested with a success rate of 98% (343/350). In 119 (34%) a sufficient amount of villi was obtained on the first attempt, in 203 (58%) the second attempt, and in six (21%) after the third or more attempts. In seven
cases sampling was repeated one week later because of either an insufficient amount of trophoblasts obtained (four cases) or incomplete digestion of the DNA (three cases). The estimated weight of the chorionic villi obtained ranged from 10-70 mg. Sampling was more difficult in patients with a posterior placenta or antverted uterus or uterine leiomyomata. Seventy percent of the samples were pure without any decidual contamination which were easily purified under the microscope. Minor bleeding without any complications was observed in 40 (11.4%) cases; subchorionic hematomas were noted in 38% (10.86%) of the patients at the US control study done immediately after sampling. No case of amniotic fluid leakage occurred. There were six (1.7%) fetal loses (Table 3). The interval between sampling and fetal death or abortion was 1-12 weeks. In two of these cases, abortion was probably unrelated to the procedure because the pregnancies occurred four to ten weeks after cytomegalovirus IgG/IgM and rubella IgG/IgM seropositivity test result were positive.

There was no relationship between time of sampling and incidence of fetal loss. Eighteen patients (51%) delivered before term and 305 (87.1%) at term; 35 (10%) had therapeutic termination of pregnancies.

### Table 1. — Indications for CVS in 350 patients.

<table>
<thead>
<tr>
<th>Type</th>
<th>No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal genotype</td>
<td>158</td>
<td>45.1%</td>
</tr>
<tr>
<td>Previous family history of hydrocephalus/</td>
<td>97</td>
<td>27.7%</td>
</tr>
<tr>
<td>spina bifida/Down’s syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Down’s syndrome and other defects</td>
<td>95</td>
<td>27.2%</td>
</tr>
</tbody>
</table>

### Table 2. — Number of insertion attempts for CVS (350 patients).

<table>
<thead>
<tr>
<th>Type</th>
<th>No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>First attempt</td>
<td>119</td>
<td>34%</td>
</tr>
<tr>
<td>Second attempt</td>
<td>203</td>
<td>58%</td>
</tr>
<tr>
<td>Third attempt</td>
<td>21</td>
<td>6%</td>
</tr>
<tr>
<td>Third or more attempts</td>
<td>7</td>
<td>2%</td>
</tr>
</tbody>
</table>

### Table 3. — Problems and outcome of diagnostic chorionic villus sampling.

<table>
<thead>
<tr>
<th>Problems</th>
<th>No. of cases (n = 350)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapeutic termination of affected fetuses</td>
<td>35</td>
<td>10 (%)</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>10</td>
<td>2.85%</td>
</tr>
<tr>
<td>Delivery at term</td>
<td>287</td>
<td>82%</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>18</td>
<td>5.1%</td>
</tr>
<tr>
<td>Fetal losses</td>
<td>6</td>
<td>1.7%</td>
</tr>
<tr>
<td>Early bleeding</td>
<td>40</td>
<td>11.4%</td>
</tr>
<tr>
<td>PROM</td>
<td>3</td>
<td>0.86%</td>
</tr>
<tr>
<td>Placenta disorders</td>
<td>4</td>
<td>1.1%</td>
</tr>
<tr>
<td>Subchorionic hematomas</td>
<td>38</td>
<td>10.86%</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>16</td>
<td>4.57%</td>
</tr>
</tbody>
</table>

### Discussion

This study has shown that chorionic villus sampling transabdominally and transvaginally under US guidance is an efficient procedure with a success rate of 98% confirming the results of others [8, 9, 23, 24].

The amount and quality of DNA obtained was suitable for diagnosis of sickle cell diseases and Down’s syndrome by oligonucleotide analysis in the vast number of cases.

In this report – which is the first since we introduced and established prenatal diagnosis in Lagos in 1994 – the fetal mortality rate was 1.7% which is relatively less than the figure of 5% reported in the international registry regarding 13,506 cases reported by Jackson [19]. This could be the result of the introduction of TORCH serological antibody screening before the procedure and our experience, although our sample size was small.

Other complications of chorionic villi in this series included abdominal cramps in 16 (4.57%), premature rupture of the membrane in three (0.86%), and vaginal bleeding in 40 (11.4%) which differs from the report of Lippman et al. [20] showing abdominal cramps in 2.7% and vaginal bleeding in 26.6%.

In conclusion, CVS is a relatively safe and reliable method of prenatal genetic diagnosis but it needs to be carried out by experienced personnel.

### Acknowledgement

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In memory of the late Prof. O. Coker, Prof. O. Akinrimisi, Prof. A. Akinkugbe and my late parents.

### References

Chorionic villus sampling: analysis of the first 350 singleton pregnancies by a single operator


Address reprint requests to:
G.O. AJAYI, M.D.
Department of Obstetrics & Gynecology
College of Medicine/University of Lagos
P. M. B. 12003 Surulere, Lagos (Nigeria)
e-mail: prenataldiagnosticcentre@yahoo.com

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mailto: IGCS_2010@mail.vresp.com
Prevalence of acute hemoperitoneum in patients with endometriotic ovarian cysts: a 7-year retrospective study

N. Evangelinakis¹, I. Grammatikakis¹, G. Salamalekis¹, V. Tziortziot², C. Samaras²; C. Chrelias¹, D. Kassanos¹

¹ 3rd Department of Obstetrics and Gynecology, Medical School of Athens, General University Hospital “Attikon”
² Lito” Maternity Hospital, Athens (Greece)

Summary

Introduction: Endometriosis is a quite common condition in women of reproductive age. The purpose of this study is to delineate the association between hemoperitoneum and endometriosis. Materials & Methods: The records of all patients with endometriotic ovarian cysts treated at the 3rd Department of Obstetrics and Gynecology of the University of Athens and at “Lito” Maternity Hospital of Athens from 2000 through 2007 were reviewed. Results: During this 7-year period 720 women underwent surgery due to endometriotic ovarian cysts. The average age was 40.9 years (range: 17-70). The median diameter of the cysts was 4.49 cm and 59% were located in the right ovary. Hemoperitoneum was identified in 16 (2.22%) of them. The average age of these women was 28.5 years (range: 22-44). Ten (62.5%) of these women presented with acute and strong abdominal pain and moderate signs of cardiovascular shock. The rest presented with abdominal pain and distension worsening at the onset of menses, nausea and/or vomiting and hemorrhagic fluid in the pelvis. Ultrasound examination was performed in all women and afterwards they underwent laparoscopy to identify the source of bleeding. In all cases a ruptured endometriotic cyst was found. In 68.8% (11/16) the ruptured cyst was located in the left ovary and the rest (31.2%) in the right. A thorough examination did not reveal any other sources of bleeding. No operative complications were observed. Discussion: The simultaneous occurrence of ascites and endometriosis is rare. A physician, though, must always take into consideration endometriosis in the differential diagnosis of ascites and acute abdominal pain or pelvic mass.

Key words: Hemoperitoneum; Endometriosis; Ovarian cysts.

Introduction

Endometriosis is a quite common condition in women of reproductive age. The prevalence is calculated as 3-10% in the general population and 25-35% in women with infertility problems. Characteristic but not occlusive symptoms of endometriosis include dysmenorrhea, dyspareunia, and chronic pelvic pain [1]. Ascites when emerging in a woman raises the suspicion of a malignancy rather than any other pathology. Despite that, there have been several reports of endometriosis causing massive hemoperitoneum [2, 3]. Purpose of this study is to delineate the association between hemoperitoneum and endometriosis and in particular how often these co-exist.

Materials & Methods

The records of all patients with endometriotic ovarian cysts treated at the 3rd Department of Obstetrics and Gynecology of the University of Athens and at “Lito” Maternity Hospital of Athens from 2000 through 2007 were reviewed. In all cases endometriotic ovarian cysts were diagnosed based on ultrasound (US) findings and clinical examination. Seven hundred and twenty women were included in the study after reviewing the age, main symptoms at admission, histologic examination, location of the cysts, bilaterality of the cysts and the diameter of the cyst.

Results

During this 7-year period 720 women underwent surgery due to endometriotic ovarian cysts. The average age was 40.9 years (range: 17-70). The median diameter of the cysts was 4.49 cm and 59% were located in the right ovary. Hemoperitoneum was identified in 16 (2.22%) of them. The average age of these women was 28.5 years (range: 22-44). Ten (62.5%) of these women presented with acute and strong abdominal pain and moderate signs of cardiovascular shock. The rest presented with abdominal pain and distension worsening at the onset of menses, nausea and/or vomiting and hemorrhagic fluid in the pelvis. US examination was performed in all women and afterwards they underwent laparoscopy to identify the source of bleeding. In all cases a ruptured endometriotic cyst was found. In 68.8% (11/16) the ruptured cyst was located in the left ovary and the rest (31.2%) in the right. A thorough examination did not reveal any other sources of bleeding. No operative complications were observed.

Discussion

The simultaneous occurrence of ascites and endometriosis is rare. The first case was reported in 1954 by Brews [4]. It has been suggested that rupture of endometrial cysts into the peritoneal cavity can lead to the formation of ascites and dense adhesions [5]. Blood and endometrial cells irritate serosal surfaces resulting in ascites. As far as the rare cases of endometriosis co-exist-
The importance of personal history should be emphasized. The recognition of adnexal masses in US examination pelvic malignancy may also be observed and in combination with identification of adnexal masses in US examination pelvic malignancy may be the initial diagnosis [13]. At this point the importance of personal history should be emphasized.

Table 1. — Characteristics of women with only endometriotic cysts in comparison to the cysts with hemoperitoneum as well.

<table>
<thead>
<tr>
<th></th>
<th>Endometriotic ovarian cysts</th>
<th>Endometriotic ovarian cysts and hemoperitoneum</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>720</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>40.9 (17-70)</td>
<td>28.5 (22-44)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Ovary implicated</td>
<td>R: 59%</td>
<td>R: 31.2%</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

R: right.

Most of these women describe progressive dysmenorrhea, and exacerbation of symptoms coincides with onset of menses and of course all of them were of reproductive age.

A physician must always take into consideration endometriosis in the differential diagnosis of ascites and acute abdominal pain or pelvic mass. Definitive therapy in any case is mostly surgical and is targeted to stop mass bleeding and dissect as many endometriotic lesions as possible. This approach may be at the cost of inflicting fertility on a nulliparous woman, illustrating the need to consider endometriosis preoperatively as a possible diagnosis in a woman of reproductive age. Ascites has been suppressed with hormonal therapy and this is a quite promising solution, especially in women wishing to preserve their fertility [14].

References


Address reprint requests to:
N. EVANGELINAKIS, M.D.
“AFTIKON” General University Hospital
3rd Department of Obstetrics and Gynecology
Rimini 1, Chaidari
Athens, 12464 (Greece)
e-mail: evangelinakisnikos@yahoo.gr
Two-year results of a new two-minute hot liquid balloon endometrial ablation system (Thermablate): a pilot study

D. Karamanidis, P. Nicolaou, A. Byros, Ger. Koutsougeras

Department of Obstetrics and Gynaecology, University General Hospital of Alexandroupolis (Greece)

Summary

Purpose: To evaluate the feasibility, safety and clinical outcomes of a new 2-minute hot liquid balloon endometrial ablation system (Thermablate). Material and Method: This prospective observational study included 72 premenopausal women with menorrhagia. All patients were treated from February 2005 through February 2008 under general anaesthesia in the Department of Obstetrics and Gynaecology at the University Hospital of Alexandroupolis, Greece. Thinning of the endometrium was achieved by sharp curettage immediately prior to the procedure. Pretreatment evaluation of menstrual blood flow, duration of menses and frequency of menstruation was performed on all patients. Post procedure pain and required medication, dysmenorrhea, satisfaction and menstrual bleeding patterns. Results: Follow-up at three months (n = 72), 6 months (n = 62), 12 months (n = 47) and 24 months (n = 17) showed a trend towards reduced monthly blood flow. Combined amenorrhea and hypomenorrhea rates at 3, 6, 12 and 24 months were 39%, 73%, 77% and 70%, respectively. The corresponding satisfaction rates were 86%, 93.5%, 93.5% and 82.4%, respectively. Dysmenorrhea rates increased from 37.5% prior to surgery, to 57% at three months and decreased to 23.5% at 24 months (p < 0.0001). Conclusion: Endometrial ablation with the Thermablate system is a safe and effective therapy for dysfunctional uterine bleeding when other therapies are contraindicated or have been tried and failed.

Key words: Menorrhagia; Dysfunctional uterine bleeding; Endometrial ablation; Thermablate.

Introduction

Abnormal uterine bleeding is a common presenting symptom in clinical practice. It affects as many as 20% of otherwise healthy, premenopausal women over age 35, and causes approximately 5% of women aged 30 to 49 years to seek medical care each year [1]. As a rule, in women of childbearing age, a detailed history, complete physical and pelvic examination, as well as appropriate laboratory tests enable the physician to rule out other causes of bleeding such as pregnancy and pregnancy-related disorders, iatrogenic causes, systemic conditions and obvious genital tract pathology. Dysfunctional uterine bleeding (anovulatory or ovulatory) is diagnosed by exclusion of these other causes [2, 3].

In premenopausal women with certain risk factors for endometrial neoplasia, initial evaluation should include endometrial biopsy, saline-infusion sonohysterography and/or diagnostic hysteroscopy in accordance with clinical practice guidelines. Women of childbearing age at low risk for endometrial neoplasia may be assessed initially by transvaginal ultrasonography [4].

Medical management of anovulatory dysfunctional uterine bleeding may include oral contraceptive pills or cyclic progestins [5]. Menorrhagia may also be managed effectively with nonsteroidal anti-inflammatory drugs (NSAIDs) or levonorgestrel intrauterine systems (LNG-IUS). When such therapy is ineffective, not tolerated or refused by patients for reasons of personal choice, many women seek surgical treatments, especially if fertility is not an issue [6]. Initially, hysterectomy was the only choice for patient and surgeon but in the 1980s targeted destruction or excision of the endometrium under hysteroscopic guidance was introduced. However, hysteroscopic endometrial ablation requires additional training and a significant degree of surgical skill. This requirement led to the introduction of a number of automated, usually non-hysteroscopic controlled devices, aimed at achieving endometrial destruction in a predictable fashion. These devices use different energy sources such as hot liquid systems, microwave, bipolar radiofrequency, laser or cryotherapy [7].

Device description

The Thermablate system consists of a disposable, pre-filled catheter-balloon cartridge and a reusable hand-held treatment control unit (TCU) that runs on direct current and is responsible for heating the liquid in the cartridge. Hot liquid is forced through the catheter into the uterine balloon by a pneumatic pump. Treatment time, pressure, and temperature, as well as safety checks are microprocessor controlled by the TCU. The cervix is dilated to 6-7 mm to allow introduction of the catheter. The liquid treatment temperature is much higher than other units at 173°C, pressure is similar at 200 mmHg, and treatment time is reduced to 128 seconds. Balloon ablation devices were designed for women with structurally normal endometrial cavities devoid of intracavitary pathology. For Thermablate, the range for the sounded uterine length should be between 7.5 and 12 cm.

In our department, we have used thermal balloon endometrial ablation for the treatment of dysfunctional uterine bleeding since 2001. In the present study, we evaluated the feasibility, safety and clinical outcomes of the Thermablate system for two years following treatment.
Material and Methods

A total of 72 patients were treated from February 2005 through February 2008 at the Obstetric and Gynaecological Department of University General Hospital of Alexandroupolis, Greece. All patients were premenopausal with a history of at least two heavy menstrual bleedings requiring dilation and curettage within the last year. The age ranged from 35 to 51 years, with a median of 41. Table 1 shows the patient demographics. Candidates for balloon ablation were those women with structurally normal endometrial cavities, sounding between 7.5 cm and 12 cm, and no desire for fertility. Women with intrauterine lesions (myomas or polyps), intramural leiomyomas, endometrial hyperplasia with cellural atypia, active genital track or urinary infection, and those with a history of classical caesarean section or transmural myomectomy were excluded. The procedure was performed under general anaesthesia. Thinning of the endometrium was achieved by sharp curettage immediately prior to the procedure. Curettage, in addition to the thinning of the endometrium, provided additional endometrial tissue for histologic examination. Antibiotics (second generation cephalosporin or doxycycline) were administered to all patients.

Pretreatment evaluation of blood flow, duration of menses and frequency of menstruation were determined for all patients, except 14 women (Table 2). Patient records were reviewed for adverse events, post-procedure pain and required medication, satisfaction and dysmenorrhea and bleeding status. Patients were followed at three, six, 12 and 24 months.

Results

None of the 72 patients experienced intraoperative or postoperative adverse events. Postoperative pain was experienced by 46 of 72 (63.9%), however only 11 of 72 (15.3%) requested analgesics.

Results at three months (n = 72), six months (n = 62), 12 months (n = 47) and 24 months (n = 17) are shown in Figure 1. Thirteen patients were lost to follow-up. Figure 1 shows an increasing trend towards reduced monthly flow at two years. The combined amenorrhea and hypomenorrhea rates increased from 39% at three months to 73%, 77% and 70% at six, 12 and 24 months, respectively (p < 0.0001).

Table 1. — Patient demographics, median and range.

<table>
<thead>
<tr>
<th>Description</th>
<th>Age (years)</th>
<th>Parity</th>
<th>BMI (kg/m²)</th>
<th>Comorbidities (hypertension, diabetes, cardiovascular disease, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3- to 5-year old</td>
<td>41</td>
<td>35-51</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>6- to 15-year old</td>
<td>2</td>
<td>1-5</td>
<td>27</td>
<td>38/72</td>
</tr>
<tr>
<td>16- to 24-year old</td>
<td>38</td>
<td>52.8%</td>
<td>38</td>
<td>52.8%</td>
</tr>
</tbody>
</table>

![Fig. 1](image1.png)  
Figure 1. — Clinical outcomes - menstrual blood flow.

![Fig. 2](image2.png)  
Figure 2. — Clinical outcomes - dysmenorrhea.

![Fig. 3](image3.png)  
Figure 3. — Post-treatment patient satisfaction.

![Table 2](image4.png)  
Table 2. — Pretreatment bleeding pattern of patients.

<table>
<thead>
<tr>
<th>Description of flow</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy</td>
<td>47</td>
</tr>
<tr>
<td>Very heavy with clots</td>
<td>11</td>
</tr>
<tr>
<td>Not recorded</td>
<td>14</td>
</tr>
<tr>
<td>Duration of menses (days)</td>
<td>No.</td>
</tr>
<tr>
<td>Median</td>
<td>9.2</td>
</tr>
<tr>
<td>Range</td>
<td>5-16</td>
</tr>
<tr>
<td>Frequency of menses (days)</td>
<td>No.</td>
</tr>
<tr>
<td>Median</td>
<td>25.3</td>
</tr>
<tr>
<td>Range</td>
<td>17-32</td>
</tr>
</tbody>
</table>
Figure 2 shows that dysmenorrhea increased from 37.5% before surgery to 57% at three months and decreased to 23.5% at 24 months follow-up. The significance of this remains elusive.

Figure 3 shows the satisfaction rate with a slight increase from 86% at three months to 93.5% at six and 12 months ($p < 0.00001$). A slight decrease of satisfaction to 82.4% was noticed at 24 months of follow-up ($p < 0.0001$).

Discussion

Hysteroscopic endometrial ablation has resulted in short-term success rates of 75-100% [8]. These methods are skill-dependent, require intensive training and expertise and are not free of complications such as perforation, haemorrhage, visceral injury and excessive fluid absorption. It is apparent that optimal outcomes require a level of skill and experience that may not be achieved by the average surgeon [9]. During the last two decades, however, there has been clear progress in the search for a less invasive yet still effective treatment with lower risk of complications. Recently, a number of devices have been developed to treat menorrhagia by non-hysteroscopic ablative methods. The advantage of these is that they require far less physician skill to perform successfully, and typically have reduced risk of complications. Today, there is an emerging trend toward simpler, quicker, safer and yet effective procedures that can take place in an outpatient or office setting under minimal analgesia. Indeed, two pilot studies have shown that Thermablate is feasible and safe in a clinic setting [10, 11].

Thermal balloon ablation requires minimal training and is easy to perform. Rates of 2-4% of minor adverse events such as postoperative infection or haematometra have been reported. Recently, serious complications such as bowel and other thermal injuries have been reported during the use of second generation endometrial ablation systems [12].

Appropriate patient selection is of utmost importance to ensure good clinical outcomes. A large uterus (> 12 cm cavity length), active pelvic infection, evidence of malignant or premalignant endometrial changes, the desire to maintain fertility and patient’s expectation of amenorrhea, are absolute contraindications. The presence of myomas or suspected adenomyosis, are likely to reduce success.

Rates of success with the Thermablate endometrial ablation system have paralleled other ablation techniques with 70-100% patient satisfaction [11-15]. This device is the smallest, simplest and fastest of the ones presently available. A short treatment time of two minutes along with the small diameter catheter (6 mm), allow the device to be used with ease and minimal anaesthesia.

Conclusion

The Thermablate endometrial ablation system is safe and effective in treating dysfunctional uterine bleeding when other therapies are contraindicated or ineffective. High rates of menstrual reduction and patient satisfaction make this device a very attractive option for the treatment of menorrhagia.

References


Address reprint requests to:
D. KARAMANIDIS, M.D.
28th October str. 40
Alexandroupolis 68100 (Greece)
e-mail: drdimk@gmail.com
Arthrogryposis multiplex congenita: Analysis of twelve cases

B. Dane¹, C. Dane¹, F. Aksoy¹, A. Cetin¹, M. Yayla¹

¹Department of Gynecology & Obstetrics Clinic, Division of Perinatology, Haseki Training & Research Hospital, ²Department of Pathology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul (Turkey)

Summary

Objective: The term arthrogryposis multiplex congenita (AMC) refers to multiple joint contractures present at birth. AMC is not a specific disorder but is the consequence of neurological, muscular, connective tissue, and skeletal abnormalities or intrauterine crowding, which may lead to limitation of fetal joint mobility and the development of contractures. Methods: Cases referred to our perinatology department for detailed examination were retrospectively analyzed. Results: Twelve cases with AMC were detected during the antenatal period. The ultrasound features related to the “lack of movement” included limb abnormalities (multiple contractures, clenched hands, and clubbed feet), short umbilical cord, polyhydramnios, pulmonary hypoplasia, camptodactyly, and micrognathia. Five of the early detected cases (71%) were found to have increased nuchal translucency or nuchal fold. All of the cases at the third trimester resulted in neonatal death. Conclusion: First trimester screening may be useful for early diagnosis of AMC. Sonographic findings in late pregnancy might be helpful in predicting the prognosis. Due to the high recurrence risk, a specific screening program should be performed for the following pregnancies by examination of the fetus several times for movement and position of the limbs.

Key words: Arthrogryposis multiplex congenita; Nuchal edema; Contractures; Polyhydramnios; Hydrops; Fetal akinesia.

Introduction

Arthrogryposis multiplex congenita (AMC) is defined as multiple joint contractures. The incidence of this condition is 1/3,000 births [1, 2]. Antenatally diagnosed arthrogryposis is often lethal. In a recent Finnish study the total incidence (selective terminations, stillbirths, and infant deaths included) of lethal arthrogryposis was reported to be 1/6,985 [3]. The joint contractures occur because of decreased fetal movement (fetal akinesia). Neurogenic processes affecting either the peripheral or the central nervous system are the most common causes. Other causes of fetal akinesia include muscle or connective tissue abnormalities, uterine anomalies or fibroids, intrauterine vascular compromise after bleeding, and maternal diseases such as diabetes mellitus, multiple sclerosis and myotonic dystrophy [2]. AMC may be associated with a number of different syndromes. Prenatal diagnosis of AMC is focused on diminished fetal movement and the presence of joint contractures or skeletal deformities. The classic sonographic findings include clenched hands, clubfeet and often polyhydramnios [4]. We describe the sonographic findings, clinical courses and postmortem examinations of 12 cases.

Materials and Methods

Cases referred to our perinatology department for detailed examination for different reasons were retrospectively analyzed. Data on obstetric history and ultrasound (US) findings were recorded at the time of sonographic examination. Arthrogryposis was defined as joint contractures present in at least two regions of the body as seen at US, postmortem examination or autopsy. The clinical, laboratory, autopsy, and US data were re-evaluated.

Result

During the 3-year period (2004-2007) we found 12 cases which had been diagnosed prenatally as having multiple contractures of different etiologies. Of these 12, five were in the third trimester and all of these cases suffered neonatal death. Eight cases between 16-30 weeks of gestation resulted in selective pregnancy termination. The second trimester screening was unremarkable in all cases in the third trimester. Six of the 12 cases (50%) had a history of a previous baby with various anomalies. All of the cases had severe bilateral talipes and fixed flexion deformities of the wrists and elbows (Figure 1). Either fixed flexion or extension of the knees was detected in 11 of the cases (Figures 2, 3); the case with muscle dystrophy had only distal arthrogryposis. Other findings and prognoses are presented in Table 1. Nuchal edema (2 had cystic hygroma) was detected in 71% (5 of the 7) of the cases in the first and second trimesters. In four cases, amniocentesis and in one case fetal umbilical cord blood sampling was performed uneventfully. In one case chorionic villus sampling was performed for specific immunostaining of collagen type VI due to the sonographic findings and a previous child with Ullrich type muscle dystrophy.

Postnatal examination of the fetal muscle revealed dystrophic changes. Chromosomal analysis was normal in six cases. Specific diagnosis could be made in seven (58%) of the cases based on the necropsy findings, previous history, sonographic findings, and immunostaining of the chorionic villi (Table 2). Five cases were autosomal recessive forms of AMC and one case had X linked recessive inheritance. Necropsy of case number one revealed occult spina bifida at the cervical region.
nostic neuroimaging of the craniocervical junction in patients with AMC has already been stressed by some authors [9]. In our first case the presence of the neural tube defect at the cervical region could not be detected by sonographic examinations. The necropsy of the fetus provided the specific diagnosis.

Early diagnoses of AMC have been based on an abnormal nuchal translucency in the late first trimester, rather than abnormalities in limb positioning and contractures [10-12]. However, our cases, which were detected by first trimester scanning, had contractures (case numbers 5 and 6), suggesting very early onset of the pathology.

Fetal hydrops and polyhydramnios are mentioned as poor prognostic signs [13]. It has been suggested that immobility causes accumulation of subcutaneous fluid and atony in the diaphragm, leading to a change in thoracic pressure and a disturbance in lymphatic circulation [14, 15]. It is still not clear why hydrops was not seen in all cases. Scalp edema and nuchal edema were detected in seven of our cases (58%). One of these cases in the third trimester also had ascites and hydrothorax. In this case, at 30 weeks of gestation the parents were counseled...
lethal arthrogryposis, since fetal muscle movement is required for the normal growth of craniofacial bones. The reported frequency is 41% in AMC cases [11]. Micrognathia was also detected in two of our cases in the third trimester (40%) as a possible consequence of neuromuscular dysfunction.

In AMC cases pulmonary hypoplasia was probably present due to absent or decreased fetal respiratory activity [17, 18]. Four of the cases above 26 weeks (80%) failed to inspire, while one case (case 4) had weak respiratory activity that lasted 24 hours.

Distal arthrogryposis (DA), which affects distal joints, can also result from various cerebral, neuromuscular and connective tissue lesions, and may occur as part of a number of congenital syndromes [2]. In one of the cases (case 9), after detection of DA by serial US examinations, chorionic villus sampling was performed. The diagnosis of Ullrich type muscle dystrophy was made based on the weak staining of collagen VI.

AMC is a component of a number of genetic syndromes. In one series of 350 AMC cases, 14% exhibited autosomal dominant inheritance, 7% autosomal recessive inheritance, and 2% X-linked recessive inheritance [1]. In our series, specific diagnoses could be made in seven cases: five cases had autosomal recessive (41.6%), and one case had X linked recessive (8.3%) inheritance. The recurrence form of the lethal anomaly in additional three cases (cases 2, 4, 12) pointed out also an autosomal recessive form of inheritance.

Four genetic loci associated with autosomal recessive AMC syndromes have been described [19-23]. In a recent study the findings suggested that there was genetic heterogeneity of congenital arthrogryposis in the same population: the same phenotype was caused by mutations in different genes and the authors concluded that AMC is of even greater genetic heterogeneity than previously known [24].

regarding the dismal prognosis and opted to terminate the pregnancy. The baby was born with an Apgar score of 0.

In the cases with cystic hygroma associated with multiple contractures, the diagnosis of lethal multiple pterygium syndrome was considered. This is an autosomal recessive condition characterized by soft tissue webbing between contracted joints (Figure 4). This disorder is uniformly lethal and pathological assessment of fetuses suggests delayed cannulation between the jugular lymphatic sacs and the internal jugular vein, which leads to an accumulation of lymphatic fluid around the fetal neck [16]. Our cases also had hydrothorax and the pregnancies were terminated after amniocentesis (cases 5, 6).

Polyhydramnios, due to impaired swallowing, was present in about 20% of cases in a series after 26 weeks of gestation [11]. Polyhydramnios was found in three of the cases in the third trimester (60%). Frequently, in severe cases of AMC, the umbilical cords are short because the infants have not been moving and stretching the cord as occurred in four of our cases (33%). This might also be a sign of early onset.

Micrognathia is also a common finding in fetuses with lethal arthrogryposis, since fetal muscle movement is required for the normal growth of craniofacial bones. The reported frequency is 41% in AMC cases [11]. Micrognathia was also detected in two of our cases in the third trimester (40%) as a possible consequence of neuromuscular dysfunction.

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<p>| Table 1. — Sonographic, macroscopic findings, history and prognosis of the cases. |</p>
<table>
<thead>
<tr>
<th>Number</th>
<th>Findings</th>
<th>Ankles</th>
<th>Knees</th>
<th>Elbows and wrists</th>
<th>History</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PHA, MG, Short umb. cord, CS, AOM</td>
<td>PE</td>
<td>Extended</td>
<td>Flexed</td>
<td>1 NND at term</td>
<td>NND at term</td>
</tr>
<tr>
<td>2</td>
<td>PHA, MG, camptodactyly, scalp edema, hypertelorism, AOM</td>
<td>PE</td>
<td>Extended</td>
<td>Flexed</td>
<td>1 NND at term</td>
<td>NND at 38 weeks</td>
</tr>
<tr>
<td>3</td>
<td>Microftalmy, hypertricosis, clenched hands</td>
<td>PE</td>
<td>Extended</td>
<td>Flexed</td>
<td>NND at 38 weeks</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>AOM</td>
<td>PE</td>
<td>Extended</td>
<td>Flexed</td>
<td>NND at 39 weeks</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cystic hygroma, webbing, hydrops, Short umb. cord</td>
<td>PE</td>
<td>Flexed</td>
<td>Flexed</td>
<td>TOP at 16 weeks</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Cystic hygroma, webbing, hydrops, Short umb. cord</td>
<td>PE</td>
<td>Flexed</td>
<td>Flexed</td>
<td>TOP at 17 weeks</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ventriculomegaly, adducted thumb, NE, short umb. Cord</td>
<td>PE</td>
<td>Flexed</td>
<td>Flexed</td>
<td>TOP at 18 weeks</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>MG, NE, diaphragmatic hernia</td>
<td>PE</td>
<td>Extended</td>
<td>Flexed</td>
<td>TOP at 22 weeks</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Scoliosis, MG, webbing, NE</td>
<td>PE</td>
<td>Flexed</td>
<td>Flexed</td>
<td>TOP at 20 weeks</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Clenched hands, AOM</td>
<td>PE</td>
<td>–</td>
<td>Flexed</td>
<td>TOP at 22 weeks</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Hydrops, PHA, Short umb. cord, clenched hands</td>
<td>PE</td>
<td>Extended</td>
<td>Flexed</td>
<td>TOP at 30 weeks</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>NE, clenched hands</td>
<td>PE</td>
<td>Extended</td>
<td>Flexed</td>
<td>TOP at 22 weeks</td>
<td></td>
</tr>
</tbody>
</table>


<p>| Table 2. — Chromosome analysis and specific diagnosis of the cases. |</p>
<table>
<thead>
<tr>
<th>Number</th>
<th>Chromosomal analysis</th>
<th>Specific diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal (CS)</td>
<td>Spina bifida at the cervical region</td>
</tr>
<tr>
<td>2</td>
<td>Normal (CS)</td>
<td>Cerebro-oculo-facio-skeletal-syndrome (AR)</td>
</tr>
<tr>
<td>3</td>
<td>Normal (AS)</td>
<td>Lethal multiple pterygium syndrome (AR)</td>
</tr>
<tr>
<td>5</td>
<td>Normal (AS)</td>
<td>Lethal multiple pterygium syndrome (AR)</td>
</tr>
<tr>
<td>6</td>
<td>Normal (AS)</td>
<td>X-linked hydrocephalus</td>
</tr>
<tr>
<td>8</td>
<td>Normal (AS)</td>
<td>Pena Shokeir (AR)</td>
</tr>
<tr>
<td>10</td>
<td>Normal (CVS)</td>
<td>Ullrich type muscle dystrophy (AR)</td>
</tr>
</tbody>
</table>

Conclusion

Despite new findings the availability of early prenatal diagnosis is critical. A case specific screening program should be planned for the following pregnancies of the affected families. Since increased nuchal translucency has been found in fetuses with lethal arthrogryposis before contractures become apparent, first trimester screening should be carried out. Scanning the fetus at 16, 20, 24, and 32 weeks to evaluate fetal movements and characteristic fetal positions could reveal congenital contractures. If the diagnosis is made after viability, US findings of poor prognosis may be helpful in obstetric management.

References


Address reprint requests to:
B. DANE, M.D.
Emlakbank Bloklari B: 1 D: 12
Vatan caddesi Fatih
34019 Istanbul (Turkey)
e-mail: cemdane@yahoo.com
Spontaneous umbilical endometriosis: a case report with one-year follow-up

E. Spaziani¹, M.D., Ph.D.; M. Picchio², M.D.; A. Di Filippo¹, M.D.; C. De Cristofano³, M.D., Ph.D.; F. Ceci⁴, M.D.; F. Stagnitti¹, M.D., Ph.D.

¹Department of Surgery, University of Rome “Sapienza”, Polo Pontino, Terracina, Latina
²Department of Surgery, Civil Hospital “P. Colombo”, Velletri, Rome
³Department of Experimental Medicine, University of Rome “Sapienza”, Polo Pontino, Latina
⁴Department of Surgery, Civil Hospital “A. Fiorini”, Terracina, Latina, Italy (Italy)

Introduction

Endometriosis is the abnormal growth of endometrial tissue outside the uterine cavity. It has been estimated that endometriosis affects 15% of women of reproductive age and up to 50% of infertile women [1]. It may be pelvic or extrapelvic. Extrapelvic endometriosis may occur in up to 12% of women with endometriosis [2]. Cutaneous endometriosis has also been described and generally occurs in a surgical scar from abdominal or pelvic procedures, including cesarean sections, hysterectomy, episiotomy, and laparoscopy [3].

We report a case of cutaneous endometriosis of the umbilical region in a young woman whose past medical history was unremarkable.

Case Report

A 36-year-old woman presented with a nine-month history of a reddish cutaneous nodule, located in the umbilical region. The lesion showed cyclic changes: increase in size and consistency with associated pain during the first two days of menses and secretion of a clear liquid on the 14th and 15th day of the menstrual cycle. Past medical history was unremarkable. Obstetric history showed two full-term spontaneous vaginal deliveries, two spontaneous abortions and two therapeutic abortions. On physical examination the umbilical mass was hard and irreducible and measured 1 cm in diameter. Pelvic examination and transvaginal ultrasonography (US) were normal, as well as thoracic and abdominal computed tomography (CT).

The umbilical mass was widely excised together with the umbilicus, fascia and peritoneum (Figure 1). The ensuing defect was primarily closed without using prosthetic mesh.

Postoperative recovery was uneventful. Histological examination of the specimen showed the presence of endometrial glands with a stromal component, compatible with the diagnosis of endometriosis. At one-year follow-up the results of surgery were satisfactory with no sign of endometriosis recurrence and or parietal defect occurrence.

Discussion

Umbilical endometriosis is rare with an estimated incidence of 0.5% to 1.0% of all patients with endometrial ectopia [4]. It may be associated with abdominal surgical scars or occur spontaneously. Several theories exist for the development of umbilical endometriosis. Postsurgical cutaneous endometriosis may be due to transplantation of viable endometrial cells into scars at the time of surgery, especially if the surgical procedure includes possible contact with endometrial tissue (i.e., episiotomy, hysterectomy, cesarean section, and ectopic pregnancy) [5]. Spontaneous umbilical endometriosis is supposed to arise from transport of endometrial cells from the pelvis via lymphatic and vascular channels, or develop through metaplasia of urachus remnants [5].

Diagnosis of umbilical endometriosis is sometimes difficult. An umbilical mass associated with cyclic pain, bleeding and discharge may suggest the correct diagnosis. Differential diagnoses include hernias, neoplasms, abscesses, various granulomas and embryological rests. US is useful in determining whether a mass is cystic or solid, but the appearance of endometrioma on US is non-
specific [6]. CT or NMR may help in finding the correct diagnosis and are useful for excluding intraabdominal extension of a lesion [7]. A recent study has suggested a distinctive dermatoscopic feature of cutaneous endometriosis, comprising small red globular structures [8].

Surgery is the treatment of choice. Extensive surgical excision, including the adjacent fascia and peritoneum is recommended to avoid local recurrence [9]. Surgeons should be aware that rare cases of endometrial carcinoma arising from endometriosis have been reported [10]. Because of the risk either of recurrence or neoplastic transformation of endometriosis, we chose the primary repair of the umbilical defect without using prosthetic materials. Moreover, there is no evidence in the literature that prosthetic materials can be safely used in patients with umbilical endometriosis [11].

Histopathological features of umbilical endometriosis are similar to those found within the uterine endometrium at each of the main phases of the menstrual cycle [12]. These findings allow a correct diagnosis to be defined in most cases. Immunohistochemical examination can help in differentiating the endometrial tissue in dubious cases [13].

In conclusion, umbilical spontaneous endometriosis is rare and should be suspected when a mass with cyclic pain, bleeding and discharge is present. Imaging techniques do not usually allow a correct diagnosis to be made with the exception of dermatoscopy. Surgical treatment involving wide local excision with clear margins is the treatment of choice. Use of prosthetic materials is not recommended because of the risk of recurrence and neoplastic transformation.

References

Primitive breast localisation of Buruli ulcer in an endemic zone: a rare case

K. N’Guessan1, P. Guié2, P. Iovenitti3, G. Carta4, V. Loue1, V. Angoi1

1Department of Gynaecology and Obstetrics, CHU Cocody, Abidjan
2Department of Gynaecology and Obstetrics, CHU Treichville, Abidjan
3Department of Gynaecology and Obstetrics, Medical Centre, Saint Louis Orione d’Anyama (Ivory Coast)
4Department of Gynaecology and Obstetrics, University of L’Aquila (Italy)

Summary
A case of Buruli ulcer with primitive breast localisation with evident epidemiological and clinical aspects is reported. This localisation is exceptional; the differential diagnosis with breast cancer is essential. If diagnosed early, it can be cured with surgery, broad-spectrum antibiotherapy and thermotherapy.

Key words: Breast; Buruli ulcer; Mycobacterium ulcerans.

Introduction
Buruli ulcer is an infectious skin disease caused by Mycobacterium ulcerans. In many cases Buruli ulcer is probably the third most common mycobacterial disease after tuberculosis and leprosy among immunocompetent patients. The first case was described by MacCallum et al. in Australia in 1948 [1]. The disease is absent in Europe, and affects mainly Africa, Oceania and South America. The Ivory Coast is one of the countries in the world most affected by this disease. Between 1988 and 1997 over 10,000 cases were registered [2, 3]. The upper and lower limbs have always been the preferred location of the disease. In 2005 an Ivorian team reported two cases of Buruli ulcer with genital localization in humans [4]. A case of primitive breast localization in a woman is presented together with the clinic characteristics, diagnostic and therapeutic procedures.

Case Report
A 37-year-old illiterate woman living in Adzope, a highly endemic area, discovered a painless nodule in the left breast by self breast examination in June 2005. She had four children and no previous history of breast pathology. She did not consult a health centre for two years given the absence of symptoms. The patient was consulted in August 2007 by the Breast Pathology Service of CHU Cocody (Abidjan, Ivory Coast). Her second child had suffered from a Buruli ulcer of the left knee; he was cured after treatment in a specialised centre in 1999. The general condition of the patient remained unchanged. Examination of the left breast showed a necrotising ulcerative lesion (6 cm in diameter in the periareolar area with a portion of the areola). The edges were raised and palpation did not reveal any nodule under the lesion. There were no palpable axillary lymph nodes and other areas were normal.

Mammography and CA 15-3 analyses were carried out. The biological samples revealed the presence of acid alcohol resistant bacillus. Mammography did not show suspicious lesions of malignancy. Polymerase chain reaction (PCR) was not carried out. Carcinoembryonic antigen and CA 15-3 were normal. Care of this patient required a multi-disciplinary team (Dermatology, Infectious diseases and Senology). Excision of the necrotic area under general anaesthesia was performed followed by local disinfectant solution for ten days, with dressings every other day. Broad-spectrum antibiotherapy and local heating to 40-50°C with serum helped heal the injury.

Discussion
According to the World Health Organization (WHO) at least 27 countries around the world have reported Buruli ulcers, mostly in tropical regions. In several of these countries, this disease is not considered as a public health threat [2]. The Ivory Coast is a country with a high endemicity of Buruli ulcers and the region of Adzope is one of five centers selected by the WHO for treatment-monitoring-evaluation of this ailment [2-4]. This region was where our patient resided. One of her children had Mycobacterium ulcerans. The disease often occurs in the vicinity of ground water and aquatic insects belonging to the genus Naucoris (Naucoridae family) and Diplonychus (Belostomatidae family) which are responsible for this ailment. Some authors argue that the mode of transmission of this disease is not fully understood. They believe the lifestyle of people who have permanent contact with water is not the main element in the transmission chain. For these authors, a prior traumatic injury is the gateway to the germ [5, 6]. In our patient previous trauma in the breast was not found, nor a primitive location. The breast lesion was the first site of an outbreak of the disease; data from the literature does not note primitive breast localisation in women. Two genital localisation cases reported by Sica et al. [4] in the same region were all secondary sites and primitive injuries were on the lower limbs. The predominance of this infection

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among children aged from 2-14 years and among women has been recognised, however no racial or socio-economic class is safe [7]. Female domestic chores, in an African context (especially in rural areas), and bathing children in rivers can explain this distribution in relation to the age and sex. Epidemiology has a key in establishing the diagnosis of Buruli ulcers. It should be considered with any nodule or ulcer in an endemic area until proven otherwise. Moreover, the clinical aspects outlined by Yamoussoukro [13] allow us to discuss the diagnosis of Buruli ulcers. The case definition used is as follows: infectious disease involving the skin caused by Mycobacterium ulcerans. It is characterised by a nodule, papule, plaque or painless swelling evolving into a painless ulcer, often leading to crippling after-effects [2]. In our case, although the patient did not present at the nodular stage of the disease there was slow ulceration, painless necrosis with a background and edges slightly off. In an epidemiological context symptoms were evocative enough to make the diagnosis. Bacteriological testing with the presence of acid alcohol resistant germs confirmed the diagnosis. In our health-developing countries, the germ culture has a long duration (10 weeks), and PCR is very expensive [8]. The differential diagnosis of a malignant breast lesion was easy. Mammography and the biopsy results did not allow diagnosis of a primitive malignant breast lesion. Currently, there is not any medicine for Mycobacterium ulcerans, and the use of conventional antibiotics (rifampicin and amikacin) alone or in combination have shown limitations. Surgical treatment remains the only recourse; blanket broad-spectrum antibiotics pre- and postoperatively for ten days are required [3-6]. In our case immediate suturing post excision was not performed because of the extensive necrosis. Cleaning and dressing with a daily solution of Dakin® and permanent thermal contacts provided some benefit in reducing the lesion. This therapeutic approach adopted by our team is confirmed by other authors [9, 10]. Some authors [11, 12] believe that rifampicin, amikacin and clarithromycin could promote healing of early pre-ulcerative or ulcerative lesions (< 2 cm), but they are ineffective for extended lesions (> 2 cm). Continuous local warming at 40°C promotes healing, even without excision, if applied consistently for four to six weeks. This therapy would promote blood circulation, penetration of antibiotics and phagocytosis. Hyperbaric oxygenation could also promote healing but it is expensive and not available in many endemic areas [11-13].

Conclusion

Localisation of a primitive breast Buruli ulcer is exceptional. The fear of breast cancer cannot exclude this possible diagnosis, especially in endemic areas. Early diagnosis and adequate treatment of this pathology can achieve healing.

References


Address reprint requests to:
P. IOVENITTI, M.D.
Via G. D’Annunzio, 8
67100 L’Aquila (Italy)
e-mail: piero.iove@yahoo.it

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Placental chorioangioma and chorioangiosis.  
Clinicopathological study of six unusual vascular lesions of the placenta - case reports

A. Kondi-Pafiti, K. Bakalianou, N. Salakos, C. Iavazzo, K. Papadias, A. Liapis

2nd Department of Obstetrics and Gynecology, Pathology Laboratory, Aretaieion Hospital, University of Athens (Greece)

Summary

The clinopathological features are presented of two cases of placental chorioangiomas and four cases of chorangiosis diagnosed in a total of 2,400 placental specimens examined in our laboratory. The differential diagnosis, clinical effects and therapeutic approach are discussed.

Key words: Placenta; Chorioangioma; Chorioangiosis;

Introduction

Placental chorioangioma is the most frequent benign nontrophoblastic tumor of the placenta, analogous to hemangiomas at other body sites. It has an incidence of approximately 1% or 1/3,500-5,000 depending on whether the lesions are examined microscopically or clinically, respectively [1, 2]. Chorioangiomas are composed of capillary vascular channels and rare stromal cells, and are surrounded by trophoblasts. The differential diagnosis of chorioangioma comprises placental chorangiosis and chorangiomatosis.

Chorangiosis or villus capillary hypervascularity is an important sign of placental malperfusion and long-standing fetal hypoxia. The diagnostic criteria of placental chorangiosis were established by Altshuler (1984) [3] and included the presence of a minimum of ten villi, each with ten or more vascular channels, in ten or more areas of three or more random, noninfracted placental areas when using ocular magnification (X10) [3]. Characteristic is the fact that the mesenchyme/capillary ratio is increased for gestational age. Chorioangiomatosis usually presents histologic features similar to chorangioma but extends between the normal placental structures, lacking the configuration of chorangioma.

There is also a distinct age distribution; chorioangiomas and chorangiomatosis are observed at the 32nd-36th weeks of pregnancy, while chorangiosis is usually observed at term [7].

We present two cases of placental chorioangiomas studied in our department during the last decade, among 2,400 placentas routinely examined.

We focused on the clinical presentation and the pathological findings of this entity.

Cases Reports

Case 1

A 37-year-old patient was admitted to the 2nd Clinic of Obstetrics and Gynecology of our hospital at term and in labor.

External fetal monitoring showed a heart rate ranging from 120-130 bpm. A healthy male fetus was delivered weighing 2,500 g with an Apgar score of 9 at 1 min. The placenta weighed 430 g and measured 15 x 12 x 2 cm. The umbilical cord was 20 cm long and 1.5 cm wide with two arteries and one vein. The placenta presented a brown circumscribed tumor located at the umbilicus and measuring 3 cm in the greatest diameter. Multiple old infarcts measuring 0.5-1.5 cm in diameter were also observed.

Case 2

The second pregnancy of a 35-year-old woman was terminated at the 33rd week because of maternal preeclampsia. A stillborn male fetus was delivered without any congenital malformations, showing meconium aspiration and signs of pneumonitis.

The placenta measured 12 x 10 x 2 cm and the umbilical cord was 21 cm long and 1.5 cm wide with two arteries and one vein.

The placenta presented a circumscribed tumor, brownish-hemorrhagic and of firm consistency measuring 4.5 cm in the greatest diameter, reaching the margin of the placenta. Severe chorionamnionitis was also observed.

Histological examination of both these placental tumors showed the features of chorioangioma (Figure 1).

Cases of chorangiosis

Four term placentas among 2,400 routinely examined during the last decade in the Pathology Laboratory presented lesions with histological features consistent with chorangiosis according to the criteria of Altchuler [3]. Women’s age ranged from 29-41 years. All embryos were female. Three were born alive and one was stillborn presenting pulmonary adenoid cystic malformations.

All placentas presented signs of chorionamnionitis as well.

Discussion

Chorioangiomas are vascular neoplasms of the placenta, usually situated mainly in the parenchyma and occasionally in the membranes of the placenta or the...
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umbilicus. They can be single or multiple [7] and red, brown, yellow or white in color. Most are small in size and found incidentally, however “giant” tumors have been described [7]. Tumors larger than 5 cm have been reported to be associated with maternal and fetal complications.

The studies of the pathogenesis of this tumor show an overexpression of angiogenic cytokines such as VEGF induced by hypoxia and some authors consider them as hamartomatous lesions [8].

Chorangiomas are classified as capillary, cavernous, endotheliomatous, fibrosing and fibromatous forms [9]. There are three microscopic types: angiomatous, cellular and degenerate [10].

Chorangiomas are reported to coexist with arteriovenous shunts leading to maternal and fetal coagulopathies, hemolytic anemia, preterm labor, hydranmio, intrauterine retardation, and fetal hydrops [11-13].

Chorangiomas may be detected during pregnancy by ultrasound scanning as arterial vessel-dependant tumors of the placenta with an elevated speed in the fetal blood flow. They are well circumscribed with an echogenicity different from the placental tissue [14]. Color Doppler is used in the differentiation between placental chorioangioma and other tumors such as hematoma, teratoma, partial mole, infarcts and intervillous thrombosis [15, 16]. The 3D power Doppler can show that the vascular channels of the tumor are in continuation with the fetal circulation and moreover they are characterized by an increase of the tumor volume parallel to the gestation age and reduction of VI (vessel density) [17].

The treatment is proposed to be conservative (with close follow-up) in tumors with a maximum diameter less than 5 cm. Symptomatic tumors have been treated with indomethacin [18] and digoxin [19]. Furthermore, amniodrainage, intrauterine blood transfusion, endoscopy-guided ligation and/or diode laser coagulation have also been used [20].

The newborn could face problems due to congestive heart failure which is the major implication caused by the arteriovenous shunt of the chorioangioma, and further-more due to the hypoalbuminemia and anemia caused by the main products of the tumor.

The differential diagnosis of chorioangioma includes chorioangiosis, a relative entity linked to a chronic hypoxic state as well. Many factors have been considered in the pathogenesis including maternal, fetal or placental conditions (Table 1) [21]. Assisted reproductive technology is not associated with such a condition [22]. Long-standing placental hypoperfusion or low-grade tissue hypoxemia is a common pathophysiologic pathway. A diffuse vascular malformation related to chorioangioma, a dysplasia secondary to chronic hypoxia or a proliferation of blood vessels due to infection such Bartonella sp. could also explain such a pathology [23-25]. Such hypoxia is usually expressed as microscopic chorionic pseudocysts in placental membranes which are common findings in chorangiosis [26]. The role of a chronic hypoxic state in the formation of chorangiosis is also shown in the study of Soma et al. who found chorangiosis due to hypervascularisation in Nepalese women that lived at high altitudes [27].

Chorangiosis is associated with acute problems of pregnancy such as preeclampsia, diabetes mellitus, placentalomegaly, delayed villous maturation, chronic villitis, nuchal cord and placental abruption [6, 28, 29]. Zhang et al. in their case series showed that chorangiosis is correlated with 10.9% of pregnancy-induced hypertension [30]. It is associated with increased perinatal morbidity and mortality [3]. For example, it is correlated with reversible cardiac failure, anemia and thrombocytopenia

<table>
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<th>Factor</th>
<th>Maternal</th>
<th>Fetal</th>
<th>Placental</th>
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<tr>
<td>Preeclampsia, eclampsia, diabetes mellitus, drug ingestion, urinary tract infection</td>
<td>Major congenital anomalies, trisomies 21, 18, 13</td>
<td>Umbilical cord anomalies, single umbilical artery, abruptio placentae, placenta previa, chorangiosis, amnion nodosum, villitis (rubella, CMV, syphilis)</td>
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of the newborn [31]. Moreover, elevated total serum hCG could be associated with chorangiosis when no other anomaly can be identified [32].

Chorioangiomatosis is a rare benign mesenchymatous placental malformation which usually leads to high-risk pregnancies as it is referred to be connected with severe anemia [33, 34], heart failure [33, 34], fetal cerebral embolism [35] and low birth-weight infants [36]. The pathophysiology also seems to be related to an overexpression of vascular growth factors [37].

References


Letter to the Editor

APPROPRIATE USE OF THE TERM “PSEUDOXANTHOMATOUS SALPINGIOSIS” IN GYNECOLOGICAL PRACTICE

In “pseudoxanthomatous salpingiosis” tubal plicae are expanded by accumulation in the lamina propria of pseudo-xanthomatous cells containing old blood pigment and brown lipofuscin (ceroid). Inflammatory cells are absent. The lesion is not of inflammatory nature. Therefore, the authors think that the use of the term “pseudoxanthomatous salpingitis” is inappropriate in the diagnostic report.

Recently we observed a pseudoxanthomatous salpingiosis in the right fallopian tube of a 48-year-old woman. The patient underwent bilateral salpingo-oophorectomy with a preoperative diagnosis of pelvic inflammatory disease. Endometriotic foci were found in the right tubaric wall. Tubal plicae were expanded by accumulation in the lamina propria of pseudo-xanthomatous cells containing old blood pigment and brown lipofuscin (ceroid). Inflammatory cells were absent. A diagnosis of “pseudoxanthomatous salpingiosis” was made. Because of the rarity of this lesion, we examined the reports published in the English literature. We read with interest Kostopoulou and colleagues’ report about “xanthogranulomatous salpingitis”: they reported three cases and compared them with a case of “pseudoxanthomatous salpingitis” [1]. These authors described the histopathologic features of the fourth lesion in the following terms: “the fallopian tube exhibited distension of the plicae, with numerous histiocytes within the lamina propria that contained light brown pigment. Lymphocytes and plasma cells were present in small numbers focally. These findings were consistent with a diagnosis of pseudoxanthomatous salpingitis”. In agreement with Seidman et al. [2] we think that use of the term “pseudoxanthomatous salpingitis” is inadequate. The lesion is not of inflammatory nature. Therefore, the term “salpingitis” is inappropriate and should not be used in the diagnostic report of “pseudoxanthomatous salpingiosis”.

References


M.G. ZORZI, M.D.; T. PUSIOL, M.D.; F. PISCIOLI, M.D.
Institute of Anatomic Pathology
S. Maria del Carmine Hospital Rovereto, Rovereto, Trento (Italy)

Corresponding Author:
M.G. ZORZI, M.D.
Institute of Anatomic Pathology
S. Maria del Carmine Hospital Rovereto
Rovereto, Trento (Italy)
e-mail: mgraziazorzi@interfree.it

The authors, E. Kostopoulou et al. were invited to respond to the letter by M.G. Zorzi et al. but no reply has been received.

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In Memoriam: Antonio Onnis

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Reproductive Biology Section


Pregnancy rates per embryo transfer (ET) may be improved by conventional oocyte insemination for male factor rather than intracytoplasmic sperm injection (ICSI) - J.H. Check, B. Katsoff, D. Summers-Chase, W. Yuan, D. Horwath, J.K. Choe

Relationship of serum progesterone (P) level the day after human chorionic gonadotropin (hCG) injection on outcome following in vitro fertilization-embryo transfer (IVF-ET) - J.H. Check, J. Amui, J.K. Choe, D. Brasile

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No. 4, October, November, December

In Memoriam: Antonio Onnis

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