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A study to determine the efficacy of controlled ovarian hyperstimulation regimen using a gonadotropin releasing hormone agonist versus antagonist in women of advanced reproductive age with varying degrees of oocyte reserve on outcome following in vitro fertilization-embryo transfer

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Summary

Purpose: To determine if the use of gonadotropin releasing hormone (GnRH) agonists (a) or antagonists (ant) allow better pregnancy rates when used in controlled ovarian hyperstimulation protocols in women of advanced reproductive age. Furthermore the study aimed to determine if the status of ovarian oocyte reserve has a confounding effect. Materials and Methods: A 12-year retrospective review was performed on all in vitro fertilization-embryo transfer (IVF-ET) cycles in women aged 40-44. Pregnancy rates were determined according to whether a GnRH-a or GnRH-ant was used. The data were also stratified according to normal or low oocyte reserve. Results: There was no significant difference in pregnancy rates according to whether a GnRH-a or GnRH-ant was used in women with normal oocyte reserve. Though a large majority of the women used a GnRH-ant, there was a 9% live pregnancy rate vs 0% in the women using a GnRH-a. Conclusion: Since it is unlikely that a larger study will ever be conducted, it is probably wise to use a GnRH-ant for the controlled ovarian hyperstimulation regimen in women aged 40-44 with diminished oocyte reserve.

Key words: Gonadotropin releasing hormone (GnRH) agonist; GnRH antagonist; Advanced reproductive age; Diminished oocyte reserve.

Introduction

There is a general consensus that after a learning curve, at least in women of a younger reproductive age, pregnancy rates are similar following in vitro fertilization-embryo transfer (IVF-ET) whether one uses gonadotropin releasing hormone (GnRH) agonists (a) or antagonists (ant) in the controlled ovarian hyperstimulation (COH) protocol [1]. The objective of the present study was to compare the effect of COH protocols using GnRH-a vs GnRH-ant in women of advanced reproductive age (age 40-44). Furthermore the study compared these two protocols according to whether there was diminished oocyte reserve or not.

Materials and Methods

A 12-year retrospective review was performed on IVF-ET cycles in women age 40-44. All cycles were used so that a couple could be utilized more than one time. Pregnancy rates and implantation rates were determined according to whether a GnRH-a or GnRH-ant were used.

Furthermore the data were stratified according to whether day 3 serum FSH was < 10 mIU/ml or > 10 mIU/ml (normal oocyte reserve vs diminished oocyte reserve). For inclusion the women had to be 40-44 years old. Women whose serum FSH was < 10 mIU/ml but whose day 3 serum estradiol was > 50 pg/ml were placed in the diminished oocyte reserve group.

Results

A comparison of clinical and live delivered pregnancy rates per transfer in women aged 40-44, with adequate oocyte reserve as determined by a day 3 serum FSH < 10 mIU/ml is shown in Table 1. Though the clinical and live delivered rates were 25-35% higher with the use of GnRH agonists vs antagonists, the differences were not significant.

A comparison of clinical and live delivered pregnancy rates per transfer in women with diminished oocyte reserve as determined by a day 3 serum FSH > 10 mIU/ml is shown in Table 2. For the diminished oocyte group there were no live pregnancies with GnRH-a but the group was small (n = 9) vs a 9% live delivered pregnancy rate with GnRH-ant.
Discussion

Though the study included 294 IVF-ET cycles in women aged 40-44, there was still insufficient power to determine if the 25-35% reduction in pregnancy rates in women with normal oocyte reserve using GnRH-ant compared to GnRH-a was merely by chance alone or whether a larger study eventually show statistical differences?

Similarly the same question applies for women with diminished oocyte reserve. There was a 9% live delivered rate with GnRH-ant but zero with GnRH-a. Since it is not likely that a study with more power will be forthcoming in the near future, it would probably be prudent, at least in the older reproductive group with diminished oocyte reserve, to use a GnRH-ant protocol.

References


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Defective oocytes are not a common cause of unexplained infertility as determined by evaluation of sharing oocytes between infertile donors and recipients

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Summary
Purpose: To determine if defective oocytes or sperm may be a common etiologic factor in unexplained infertility. Materials and Methods: A retrospective comparison of fertilization rates and pregnancy rates from infertile donors with unexplained infertility trying to conceive with in vitro fertilization-embryo transfer (IVF-ET) and their respective recipients, who shared the other half of the oocytes with the recipient’s male partner for financial compensation was performed. Pregnancy rates from donors and recipients were also compared to other donor recipient pairs sharing oocytes from infertile donors with tubal or male factor or financially-compensated donors providing oocytes to two recipients. Results: Pregnancy rates from infertile donors with unexplained infertility were comparable not only to their respective recipients but to other donor/recipient pairs that received oocytes from donors with tubal or male factor or financially-compensated donors. Fertilization rates were somewhat reduced in the infertile donors. Conclusions: Abnormal embryos resulting from an oocyte or sperm defect do not appear to be a common cause of unexplained infertility. The possibility does exist that sperm may be an etiologic factor in reduced fertilization potential, which not only could be obviated by conventional oocyte insemination, but could be further improved by intracytoplasmic sperm injection (ICSI).

Key words: Oocyte sharing; Infertile donors; Oocyte recipients; Fertilization rates; Pregnancy rates.

Introduction
Sometimes a definitive cause for infertility is not identified or an infertility factor seems to be corrected but a successful pregnancy does not ensue. This group is considered to have unexplained or cryptic infertility [1]. Theoretically the problem could be defective oocytes despite the appearance of achieving follicular maturation and oocyte release, defective sperm despite normal semen parameters, defective tubal function despite the appearance of normal fallopian tubes, or some endometrial factor inhibiting implantation.

Defective oocytes or sperm may manifest in a few ways: either failing to fertilize the oocyte, or fertilizing the oocyte but failure to develop into an embryo or failure for a normal-appearing embryo to implant. Theoretically in vitro fertilization-embryo transfer (IVF-ET) by either exposing the oocyte to many more sperm or performing intracytoplasmic sperm injection (ICSI) may overcome the problem of defective fertilization of sperm or oocyte in some circumstances. However, it would not be expected to overcome problems of the creation of an abnormal embryo if there was a defective oocyte or sperm causing that problem.

Of course IVF-ET would be expected to be successful in cases where defective tubal function is the cause of the problems. IVF-ET may not be successful for cases of endometrial factor.

IVF-ET has been successful in cases of unexplained infertility. Nevertheless, a priori, based on the theoretical circumstances of defective oocytes or sperm leading to embryos of low implantation potential and the possibility of an endometrial factor, IVF-ET would not seem to be as likely to produce a live baby in cases of unexplained infertility, as compared to tubal or male factor problems unless the infertility factors requiring the need for more sperm and oocyte contact or the need to circumvent the fallopian tubes are the main factors involved in unexplained infertility.

It has been demonstrated that when infertile donors are used to provide oocytes for recipients requesting donor oocytes, these oocytes are equally as effective in establishing normal pregnancies as oocytes from financially-compensated donors [2]. However, many couples are reluctant to choose infertile oocyte donors with unexplained infertility for fear of poor quality oocytes preferring donors whose infertility was related to tubal or male factor problems.

There were two objectives of this retrospective comparison of pregnancy rates in women who are infertile vs their respective recipients: 1) Determine how likely are defective oocytes or defective sperm in creating embryos that do not implant as etiologic factors in unexplained infertility? 2) How effective are oocytes from infertile donors in establishing pregnancies in recipients when originating from donors with unexplained infertility vs oocytes from infertile donors with tubal disease or male factor vs financially-compensated donors?
incidence of multiple

According to whether ICSI was performed or not. The data was also stratified of the infertility in the infertile donors or whether they used a made between the various recipients according to the etiology type of infertility.

Comparisons of pregnancy and implantation rates were also made amongst infertile donors according to their type of infertility.

Table 1. — Pregnancy rates of oocyte donors based on their infertility types in shared IVF cycles.

<table>
<thead>
<tr>
<th>Donor Infertility Type</th>
<th>Type of Insemination</th>
<th>Unexplained</th>
<th>Conventional</th>
<th>Male factor</th>
<th>ICSI</th>
<th>Conventional</th>
<th>Total</th>
<th>Tubal</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. retrievals</td>
<td></td>
<td>21</td>
<td>5</td>
<td>16</td>
<td>96</td>
<td>86</td>
<td>10</td>
<td>212</td>
</tr>
<tr>
<td>No. transfers</td>
<td></td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>61</td>
<td>56</td>
<td>5</td>
<td>138</td>
</tr>
<tr>
<td>No. with 0% fertilization</td>
<td></td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No. with 1% but &lt; 50% fertilization</td>
<td></td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>% with low fertilization</td>
<td></td>
<td>33.3</td>
<td>0.0</td>
<td>43.8</td>
<td>14.6</td>
<td>15.1</td>
<td>10.0</td>
<td>17.9</td>
</tr>
<tr>
<td>% clinical pregnancies</td>
<td></td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>32</td>
<td>28</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>No. viable (12 weeks)</td>
<td></td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>29</td>
<td>25</td>
<td>4</td>
<td>62</td>
</tr>
<tr>
<td>% viable transfers</td>
<td></td>
<td>60.0</td>
<td>100.0</td>
<td>50.0</td>
<td>52.5</td>
<td>50.0</td>
<td>80.0</td>
<td>48.6</td>
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<tr>
<td>No. spontaneous abortion (SAB)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12.5</td>
<td>14.3</td>
<td>0.0</td>
<td>11.9</td>
</tr>
<tr>
<td>% SAB / clinical pregnancies</td>
<td></td>
<td>0.0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>No. deliveries</td>
<td></td>
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<td>2</td>
<td>4</td>
<td>28</td>
<td>24</td>
<td>4</td>
<td>59</td>
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<tr>
<td>% delivered</td>
<td></td>
<td>60.0</td>
<td>100.0</td>
<td>50.0</td>
<td>45.9</td>
<td>42.9</td>
<td>80.0</td>
<td>42.8</td>
</tr>
<tr>
<td>No. embryos transferred</td>
<td></td>
<td>24</td>
<td>7</td>
<td>17</td>
<td>165</td>
<td>152</td>
<td>13</td>
<td>377</td>
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<tr>
<td>Avg. no. embryos transferred</td>
<td></td>
<td>2.4</td>
<td>3.5</td>
<td>2.1</td>
<td>2.7</td>
<td>2.7</td>
<td>2.6</td>
<td>2.7</td>
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<tr>
<td>No. sacs implanted</td>
<td></td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>50</td>
<td>44</td>
<td>6</td>
<td>105</td>
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<tr>
<td>Implantation rate</td>
<td></td>
<td>41.7</td>
<td>57.1</td>
<td>35.3</td>
<td>30.3</td>
<td>28.9</td>
<td>46.2</td>
<td>27.9</td>
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</table>

Materials and Methods

A retrospective review of all donor oocyte cycles over a 12-year time period was made where oocytes were shared between two partners. The infertile donor shared oocytes in exchange for sharing financial obligations. There were two types of oocyte donors: ones that were infertile sharing half the oocytes with a recipient and using the other half to perform IVF-ET themselves and financially-compensated recipients providing oocytes for two recipients.

The infertile donors were divided into three groups according to the infertility etiology: unexplained infertility, male factor, and tubal factor. Clinical (ultrasound evidence of pregnancy at eight weeks), live-delivered pregnancy rates, and implantation rates were compared according to etiology of infertility in the infertile donor vs their respective donor oocyte recipients. Clinical and live-delivered pregnancy rates and implantation rates were also compared amongst infertile donors according to their type of infertility.

Comparisons of pregnancy and implantation rates were also made between the various recipients according to the etiology of the infertility in the infertile donors or whether they used a financially-compensated donor. The data was also stratified according to whether ICSI was performed or not.

Results

The pregnancy and implantation rates in infertile oocyte donors according to the etiology of their infertility are seen in Table 1.

The pregnancy and implantation rates in recipients according to the etiology of infertility in the infertile donors or if the source of oocytes was from financially-compensated donors are seen in Table 2.

The fact that the live-delivered pregnancy rates for infertile donors with unexplained infertility was 60.0% vs 53.3% for recipients receiving oocytes from infertile donors with unexplained infertility suggests that subtle oocyte or sperm defects leading to normal-appearing embryos that do not implant is not a common etiologic factor for unexplained infertility (Tables 1 and 2).

Discussion

The rarity of defective oocytes or sperm despite normal appearance in unexplained infertility is further substantiated by not finding lower pregnancy rates in the infertile donor with unexplained infertility compared to donors with male factor or tubal factor Table 1.

The live-delivered pregnancy rate of 43.7% (113/236) for recipients using oocytes from infertile donors was not significantly different from the 47.9% (356/743) found from those using financially-compensated donors confirming previous smaller studies [2].

These data found that two of 16 (12.5%) of the infertile donors with unexplained infertility had failed fertilization compared to only one of 85 (1.2%) with tubal factor (p = 0.06, Fisher’s exact test). None of the 12 recipients receiving oocytes from infertile donors failed to fertilize any oocytes (p = 0.52, Fisher’s exact test).

The group with the largest percentage of low fertilization rates with conventional insemination amongst infertile donors was the group with unexplained infertility (43.8%), compared to 10.0% for male factor, and 21.5% for tubal factor (Table 1). However, amongst recipients, there were similar rates of low percentage fertilization ranging from 17.6% to 22.2% in recipients according to type of infertility of infertile donors and recipients receiving oocytes from financially-compensated donors.

These data clearly show that defective oocytes or defective sperm leading to the formation of normal-appearing embryos that do not implant is not a very common cause of unexplained infertility based on the comparable pregnancy rates in donors and recipients receiving oocytes from infertile donors with unexplained infertility. This conclusion is supported by the fact that oocytes from infertile donors with unexplained infertility led to comparable pregnancy rates in both donors and recipients compared to other infertility etiologies in the other infertile donors and even compared to financially-compensated donors.
Table 2. — Pregnancy rates of oocyte recipients based on their infertility types of the oocyte donors in shared IVF cycles or whether they were financially compensated.

<table>
<thead>
<tr>
<th>Donor Infertility Type</th>
<th>Unexplained</th>
<th>Male Factor only</th>
<th>Tubal</th>
<th>Paid egg donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ICSI</td>
<td>96</td>
<td>54</td>
<td>212</td>
<td>945</td>
</tr>
<tr>
<td>Total Conv. Insem.</td>
<td>78</td>
<td>46</td>
<td>143</td>
<td>743</td>
</tr>
<tr>
<td>% with 0% fertilization</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>% with 1% but &lt; 50%</td>
<td>12</td>
<td>6</td>
<td>37</td>
<td>103</td>
</tr>
<tr>
<td>% with low fertilization</td>
<td>22</td>
<td>14</td>
<td>19.8</td>
<td>12.3</td>
</tr>
<tr>
<td>% clinical pregnancies</td>
<td>60.0</td>
<td>51.3</td>
<td>51.3</td>
<td>56.0</td>
</tr>
<tr>
<td>% viable transfers</td>
<td>53.3</td>
<td>39.7</td>
<td>39.1</td>
<td>50.7</td>
</tr>
<tr>
<td>No. transfers</td>
<td>15</td>
<td>7</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>No. retrievals</td>
<td>21</td>
<td>12</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>No. clinical pregnancies</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>No. viable (12 weeks)</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>No. spontaneous abortion (SAB)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% SAB / clinical pregnancies</td>
<td>11.1</td>
<td>11.0</td>
<td>16.7</td>
<td>11.3</td>
</tr>
<tr>
<td>No. deliveries</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>% delivered</td>
<td>53.3</td>
<td>37.0</td>
<td>37.0</td>
<td>49.7</td>
</tr>
<tr>
<td>No. embryos transferred</td>
<td>42</td>
<td>24</td>
<td>18</td>
<td>2010</td>
</tr>
<tr>
<td>No. embryos transferred</td>
<td>24</td>
<td>18</td>
<td>12</td>
<td>1171</td>
</tr>
<tr>
<td>Avg. no. embryos transferred</td>
<td>2.8</td>
<td>2.0</td>
<td>2.6</td>
<td>2.7</td>
</tr>
<tr>
<td>No. sacs implanted</td>
<td>14</td>
<td>4</td>
<td>10</td>
<td>648</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>33.3</td>
<td>16.7</td>
<td>55.6</td>
<td>32.0</td>
</tr>
</tbody>
</table>

A poor pregnancy rate in donors with unexplained infertility following IVF-ET but a good success in their respective recipients would have suggested that sperm can create embryos that appear normal but do not implant. Alternatively, some occult endometrial factor could be hypothesized. Low pregnancy rates in both donors and recipients of oocytes from infertile donors would have suggested that oocytes can produce normal-appearing embryos that do not implant.

The relatively high rate of failed or low percentage fertilization rate in the donors with unexplained infertility using conventional insemination also suggests that fertilization failure may be a factor in unexplained infertility related to the sperm and not the oocyte. If the oocyte was the problem, one should have found that the recipients of oocytes from infertile donors with unexplained infertility would similarly be found to have the highest rate of low fertilization rates amongst the recipients and this was not the case. One can surmise that if exposing the oocyte to 50,000 sperm (as is done with conventional oocyte insemination) in women with unexplained infertility, results in a 12.5% failed fertilization rate and a 44% low fertilization rate, that in nature where after intercourse or intrauterine insemination, a far lower number of sperm reach the oocyte that fertilization failure may be one of the more common causes of unexplained infertility. Then based on donor-recipient comparisons, it would seem that it is the sperm, not the oocyte, that may be the main factor in failed fertilization. Therefore one reason why IVF-ET is successful despite unexplained infertility is by exposing the oocyte to a larger quantity of sperm that have a lower fertilization potential. What percentage of the cases of unexplained infertility is this mechanism operational vs other theoretical problem, e.g., sperm not reaching the oocyte, or abnormal fallopian tube formation, remains to be determined.

Though previous studies have found ICSI to provide higher fertilization rates than conventional oocyte insemination, the process of ICSI may lead to a lower pregnancy rate [3]. Considering extra costs to the patient and increased labor time for the embryologists, these data can help a given IVF center to develop certain strategies as to which infertility etiologies to perform conventional oocyte insemination in all the oocytes and which etiologies, e.g., unexplained infertility, where ICSI on all or half of the oocytes retrieved may be more advantageous to prevent failed or low fertilization rates and thus insufficient number of embryos generated [4, 5].

This study also shows that IVF centers using infertile donors as a source of oocytes for recipients should not eliminate those with unexplained infertility. Knowledge of these data may help patients to be more open-minded about choosing a donor with unexplained infertility as opposed to male or tubal factor as their source of donor oocytes.

References


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A comparison of clinical pregnancy rates and multiple gestation rates with 2 vs 3 embryos transferred with pairs matched for embryo quality

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Summary

Purpose: To determine the impact of embryo quality on multiple birth rates. Materials and Methods: A retrospective review of in vitro fertilization-embryo transfer (IVF-ET) cycles over ten years was performed. The data was stratified by number of embryos transferred (two vs three) and by percentage of embryos with < 6 vs ≥ 6 blastomeres. Results: Pregnancy rates (PRs) increase with the greater number of embryos with a higher blastomere count. However transferring more embryos with less blastomeres does not lower the risk of multiple births. Conclusions: Couples should consult the table, e.g. presented here, so they can make their best choice of how many embryos to transfer considering the importance of a higher pregnancy rate vs the risk of the complication of multiple births.

Key words: Blastomere number; Embryo quality; Multiple birth; In vitro fertilization-embryo transfer.

Introduction

One of the greatest challenges of the use of assisted reproductive technology is to avoid multiple pregnancies without compromising pregnancy rates. Multiple pregnancies are associated with increased risks for both the mother and fetus. Maternal risks include miscarriage, hemorrhage, preclampsia, diabetes, anemia, polyhydramnios, and Caesarean section. Fetal complications include preterm delivery, low birth weight, and various birth defects.

Therefore the key question for every woman undergoing in vitro fertilization (IVF) is how many embryos to transfer? It has been shown that blastomere number is a better predictor of achieving pregnancy than fragmentation [1]. In a previous study of single embryo transfer (ET), the clinical pregnancy rate (CLPR) for six to eight cell embryos was 40.4% whereas the CLPR for four to five cell embryos was only 6.6% [1]. In contrast, in this same study, although embryos with < 25% fragmentation resulted in a 35% PR per transfer, those with > 50% fragmentation showed a 25% CLPR per transfer [1].

The objective of the present study was to evaluate PRs and multiple gestation rates (MGRs) according to the number of ETs and the number of embryos with ≥ 6 blastomeres. Because previous studies have looked at these two factors individually, this study also aims to look at these factors together by matched cohort comparison.

Materials And Methods

IVF cycles from January 1997 to May 2007 were reviewed according to whether two or three embryos were transferred in women aged < 38. CLPR (live fetus on ultrasound eight weeks from conception) and MGR were compared according to blastomere number. In addition, comparison was done for women receiving two vs three embryos but matched according to embryo quality based on blastomere number. Chi-square analysis was used for statistical analysis.

Results

For patients younger than 38 years transferring two of their own embryos, there is a statistically significant difference in the CLPRs by cell stage of ETs (p < 0.001). The highest PR of 46.1% was obtained in cycles where both embryos had six or more cells. In cycles where at least one of the two embryos had less than six cells, the CLPR were lower (21.6% for both < 6, 26.7% for 1 < 6 cell). These last two rates did not differ significantly for each other but combined they were significantly lower than the group with all embryos with > 6 blastomeres (p < 0.05).

In cycles where at least one of the two embryos had less than six cells, the CLPR were lower (21.6% for both < 6, 26.7% for 1 < 6 cell). These last two rates did not differ significantly for each other but combined they were significantly lower than the group with all embryos with > 6 blastomeres (p < 0.05).

In cycles where both embryos had six or more cells, 35.7% of the pregnancies had multiple sacs as compared to only 10.5% of pregnancies where both embryos had less than six cells (p ≤ 0.025). However there was not a significant difference in the multiple rates between cycles where one of the two embryos or both embryos were at least six cells (32% vs 35.7%, respectively).

For patients younger than 38 years transferring three of their own embryos, there is a significant difference in PRs
A comparison of clinical pregnancy rates and multiple gestation rates with 2 vs 3 embryos transferred and pairs matched for et c.

Table 1. — A comparison of pregnancy and multiple birth rates according to whether two or three embryos were transferred and percentage of embryos transferred with < 6 blastomeres.

<table>
<thead>
<tr>
<th>Blastomere</th>
<th>Two embryos transferred</th>
<th>Three embryos transferred</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All embryos &lt; 6</td>
<td>1 embryo &lt; 6</td>
</tr>
<tr>
<td>Clinical pregnancy rate/transfer</td>
<td>21.6% (19/88)</td>
<td>26.7% (50/187)</td>
</tr>
<tr>
<td>% with multiple births</td>
<td>10.5% (2/19)</td>
<td>32% (16/50)</td>
</tr>
</tbody>
</table>

Discussion

There is an improved chance of a clinical pregnancy with more embryos transferred especially when there is a greater percentage with six or more blastomeres which supports conclusions from previous studies [2-4]. However, there does not seem to be safety in preventing multiple gestations if there are less quality embryos being transferred.

This suggests that quantity rather than quality is more predictive of MGR. We suggest that each IVF center present their data in a similar fashion as in Table 1 to the couple seeking pregnancy by IVF-ET, so that after being properly counseled on the risk of multiple gestations and PRs they can decide on a two vs three ETs based on embryo quality as determined by blastomere number. For example by evaluating the Table, a couple would see that if none of the embryos have six blastomeres then transferring three embryos vs two raises the PR by 50% (30.2% vs 21.6%), but also more than triples the MGR (38.5% vs 10.5%). If a multiple birth rate of almost 40% is acceptable to the couple, they should have the right to have a greater chance of pregnancy from an expensive IVF-ET procedure.

References


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**General Section**

Placental and umbilical cord macroscopic changes associated with fetal and maternal events in the hypertensive disorders of pregnancy

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**Summary**

**Aim:** The purpose of this study was to identify placental and umbilical cord macroscopic changes and correlate them to maternal and fetal clinical events in hypertensive disorders of pregnancy (HDP).

**Materials and Methods:** The authors examined 150 placentas, 30 from each HDP group, totaling 120, and 30 from the control group. All placentas and umbilical cords were examined, recorded, and photographed.

**Results:** The mean placental weight in the control group (526.3 ± 95.6 g) was greater than in the HDP (435.5 ± 43.1 g). Calciphylaxis was the most common macroscopic change found in the control and HDP groups in 27 (90%) and 118 cases (98.3%), respectively.

**Discussion:** Pregnant women with HDP were relatively younger. In addition, due to low blood flow seen in HDP, the macroscopic changes found included lower placental weight, calciphylaxis in the maternal surface, and fibrin in the fetal surface. Because of all complications associated, most women with HDP had preterm infants who developed respiratory problems and had shorter umbilical cords.

**Key words:** Hypertensive; Newborn; Placenta; Pregnancy; Umbilical cord.

**Introduction**

A well-functioning placenta is crucial for fetal development and morphological changes found may indicate fetal and maternal clinical changes associated with intrauterine development [1].

Macroscopic examination of the placenta and umbilical cord can help determine fetal/neonatal prognosis. Placental weight, distance between the placental margins, and thickness and any macroscopic abnormalities are recorded. Further evaluation is required in the presence of some nonspecific abnormalities seen in diabetes, infections in the last trimester of gestation, and hypertensive disorders of pregnancy (HDP) [2].

Placental infarctions are common in the placenta of women with HDP. The maternal surface of the placenta is firm with a yellow-white appearance. Cone-shaped lesions measuring approximately 0.5 to one cm are seen in the decidua. They may be superficial or involve the entire thickness of the placenta [2].

The examination of the umbilical cord can provide valuable information on the progression of the pregnancy [3-5]. Abnormalities in any structure of the umbilical cord can indicate compromised fetal development, fetal anomalies, perinatal complications or even intrauterine fetal death [5].

Pathologic examination of the umbilical cord includes the measurement of its length and mean diameter. Umbilical cord site and type of insertion into the fetal surface of the placenta should be assessed, while taking note of the distance between the placental margin and insertion site, and color of the Wharton’s jelly surrounding the umbilical blood vessels and their tortuosity. When present, focal pathological features including torsion, true knot, rupture, hematoma and constriction are recorded [2].

Hypertensive complications during pregnancy are the leading cause of maternal and fetal morbidity and mortality worldwide, occurring in about ten percent of all pregnancies. They are more common in nulliparous women, multiple gestations, women suffering from hypertension for more than four years, history of hypertension in a previous pregnancy, history of renal disease, and women with family history of preeclampsia [6].

Some potentially serious conditions are often associated with HDP, such as placental abruption, disseminated intravascular coagulation, cerebral hemorrhage, pulmonary edema, liver failure, and acute renal failure. Perinatal complications include: increased prematurity, intrauterine growth restriction, fetal distress, perinatal death due to intrauterine hypoxia, and increased risk in...
neonatal deaths. Maternal changes involve diffuse endothelial disruption associated with the development of circulatory disorders, while fetal changes involve hypoxemia and nutritional deprivation [7].

The main risk factors are mainly on the maternal side and include obesity, chronic hypertension, and genetic factors. Hypertension and proteinuria during the second half of pregnancy are key diagnostic criteria but clinical presentation, laboratory tests, and associated events may reflect a variety of disease manifestations [6].

To minimize the complications of HDP and reduce the high rates of maternal and perinatal morbidity and mortality, it is key to improve scientific knowledge through the study of placental and umbilical cord macroscopic changes. Research together with obstetric care enable to devise health actions and evaluate care quality for the prevention, promotion, and rehabilitation of pregnant women and fetuses/newborns through strategies and specific protocols of obstetric, neonatal, and placental assessment [8]. In addition, further investigation of factors associated with placental changes of HDP can help elucidate the etiology and pathogenesis of gestational diseases and predict fetal/neonatal outcome.

The current study aimed to identify placental and umbilical cord macroscopic changes and correlate them to maternal and fetal clinical events in HDP.

Materials and Methods

A prospective study was conducted in a public maternity hospital which is a reference center for high-risk pregnancies, in the city of Goiânia, central-west Brazil, between August 2009 and July 2010. The study population consisted of pregnant women clinically-diagnosed with HDP and healthy women (control group) undergoing vaginal delivery or cesarean section with the outcome of live births, stillbirths, and fetal death during the study period.

Hypertension was defined as an increase in systolic blood pressure equal to or greater than 140 mm Hg or in diastolic pressure equal to or greater than 90 mm Hg in two separate measurements made no more than one week apart. This classification follows the criteria established in the National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy [9]. Preeclampsia occurs beyond 20 weeks of gestation and is characterized by high blood pressure accompanied by proteinuria (protein ≥ 0.3 g in 24-hour urine collection). In the absence of proteinuria, preeclampsia is suspected when high blood pressure is accompanied by signs and symptoms including headache, blurred vision, epigastric pain, abnormal laboratory test results, specifically low platelet counts (platelets ≤ 100,000 mm³) and abnormal liver enzymes (increased aspartate aminotransferase or alanine aminotransferase).

Pregnant women who had autoimmune diseases or other diseases that could lead to immunological changes, and those who received corticosteroid therapy during labor were excluded from this study. The control group comprised of pregnant women with no maternal complications during pregnancy, normal laboratory tests, and clinically normal newborns.

The authors evaluated all macroscopic changes on the fetal and maternal surfaces of the placenta and umbilical cord. All lesions were examined, recorded, and photographed.

Data was collected from medical records and transcribed into study forms. Collected information included socio-demographic variables (age, birthplace, level of education, occupation, and household income), underlying conditions, obstetric history, potential neonatal/fetal and maternal obstetric complications, neonatal anthropometric measurements, 1- and 5-minute Apgar scores, laboratory tests for evaluation of HDP, parity, and gestational age which was determined by the date of the last menstrual period, first-trimester ultrasound examination, and Capurro method. When gestational age assessment results were inconsistent, the results of the Capurro method [10] were used. Maternal underlying conditions were grouped according to the 10th Revision of International Statistical Classification of Diseases and Related Health Problems (ICD-10) [11] criteria; neonatal/fetal underlying conditions were grouped following Cartlidge and Stewart criteria [12].

Statistical analyses were performed with the use of SigmaStat® version 2.0. An electronic spreadsheet was created. Quantitative data were summarized using frequency distributions, means, and standard deviations. Parametric and non-parametric tests were used to assess associations identified in the statistical analysis.

The study project was approved by the Animal and Human Research Ethics Committee of Clinics Hospital of Goiás University (protocol number 101/2008). The research study followed all the recommendations of National Health Council Resolution 196/96 in Brazil, which provides guidelines and regulations for human research.

Results

The authors examined a total of 150 placentas: 30 placentas from each HDP group, totaling 120 and 30 placentas from the control group. All placentas were obtained from childbirths taking place at the public reference center for high-risk pregnancies in Goiânia (Brazil) between August 2009 and July 2010.

The mean maternal and gestational age in the HDP groups are shown in Table 1. The preeclamptic group had lower mean maternal age while the eclamptic group showed lower mean gestational age. The rate of premature birth (less than 38 weeks of gestation) was higher in the HDP group (42 cases, 35%), whereas four premature births (13.33%) were seen in the control group. No statistically significant difference regarding the rate of premature births was seen in the different HDP groups (Table 1).

Table 2 shows macroscopic placental measures. The mean placental weight in the control group was greater than in the HDP group. The maximal placental diameter and the lesser placental diameter were greater in the control group compared to the other groups. There was no statistically significant difference in lesser diameter, maximal diameter, and umbilical cord thickness between the groups studied. The mean cord thickness was greater in the control compared to the HDP group (Table 2).

Paracentral insertion of the umbilical cord was most common in the control group (27 cases, 90%). In the HDP group, paracentral insertion was also most common (87 cases, 72.5%), followed by central (16 cases, 13.3%), marginal (ten cases, 8.33%), and velamentous (seven cases, 5.83%).
Table 1. — Mean maternal and gestational age in the preeclamptic group evaluated in a public reference center for high-risk pregnancies in Goiânia, Brazil from August 2009 to July 2010.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>HDP group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years) X ± SD</td>
<td>23.8 ± 1.9</td>
<td>22.6 ± 1.8</td>
</tr>
<tr>
<td>Gestational age (weeks) X ± SD</td>
<td>39.5 ± 1.2</td>
<td>38.1 ± 1.1</td>
</tr>
</tbody>
</table>

Table 2. — Mean placental measures in control and HDP groups evaluated in a public reference center for high-risk pregnancies in Goiânia, Brazil from August 2009 to July 2010.

<table>
<thead>
<tr>
<th>Placental Measure</th>
<th>Control group</th>
<th>HDP group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g) ± SD</td>
<td>526.3 ± 95.6</td>
<td>435.5 ± 43.1*</td>
</tr>
<tr>
<td>Maximal placental diameter (cm)</td>
<td>20.2 ± 0.8</td>
<td>18.5 ± 1.2</td>
</tr>
<tr>
<td>Lesser placental diameter (cm)</td>
<td>17.5 ± 2.4</td>
<td>12.0 ± 1.2</td>
</tr>
<tr>
<td>Thickness of umbilical cord (cm)</td>
<td>1.5 ± 0.2</td>
<td>1.1 ± 0.4**</td>
</tr>
<tr>
<td>Length of umbilical cord (cm)</td>
<td>33.6 ± 0.9</td>
<td>31.8 ± 0.7</td>
</tr>
</tbody>
</table>

Table 3. — Anthropometric parameters and 1- and 5-minute Apgar scores of newborns of mothers in the control and HDP groups evaluated in a public reference center for high-risk pregnancies in Goiânia, Brazil from August 2009 to July 2010.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>HDP group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head circumference (cm)</td>
<td>33.5 ± 0.4</td>
<td>33.3 ± 0.1</td>
</tr>
<tr>
<td>Chest circumference (cm)</td>
<td>31.9 ± 0.5</td>
<td>31.8 ± 0.1</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2.889 ± 12.54</td>
<td>2.816 ± 16.71</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>50.1 ± 0.8</td>
<td>49.5 ± 0.5</td>
</tr>
<tr>
<td>1-min Apgar score</td>
<td>7.1 ± 1.2</td>
<td>7.4 ± 0.9</td>
</tr>
<tr>
<td>5-min Apgar score</td>
<td>8.8 ± 0.3</td>
<td>8.8 ± 0.7</td>
</tr>
</tbody>
</table>

Table 3. — Anthropometric parameters and 1- and 5-minute Apgar scores of newborns of mothers in the control and HDP groups evaluated in a public reference center for high-risk pregnancies in Goiânia, Brazil from August 2009 to July 2010.

<table>
<thead>
<tr>
<th>Anthropometric parameters</th>
<th>Control group</th>
<th>HDP group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head circumference (cm)</td>
<td>33.5 ± 0.4</td>
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</tr>
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</tr>
<tr>
<td>1-min Apgar score</td>
<td>7.1 ± 1.2</td>
<td>7.4 ± 0.9</td>
</tr>
<tr>
<td>5-min Apgar score</td>
<td>8.8 ± 0.3</td>
<td>8.8 ± 0.7</td>
</tr>
</tbody>
</table>

Discussion

HDP are disorders specific to pregnancy and postpartum period that mainly affect primiparous women at extremes of reproductive life. This was corroborated by the study results for gestational age. Women in the control group delivered at 39.5 ± 1.2 weeks of gestation while those in the HDP group, especially eclamptic cases, delivered at 36.7 ± 1.8 weeks, of which 35% were premature newborns.

Several studies have investigated the relationship between placental changes, maternal age, and HDP [13-15]. The authors found that the mean age of normotensive patients was higher than HDP patients. In the Bazaga et al. study [16], the patients with chronic hypertension and preeclampsia had a statistically higher maternal age than non-hypertensive patients and lower maternal age (lower than 20) was seen in preeclamptic patients. The result of the present study corroborates with this finding as preeclamptic cases had a mean age of 21.9 ± 1.2 years.

The current study found a mean placental weight of 526.3 ± 95.6 g in the control group and 435.5 ± 43.1 g in the HDP group, with a statistically significant difference (p < 0.001). In a study conducted by Artico et al. [15], placentas of hypertensive and control patients had a mean weight of 461.1 and 572.1 g, respectively. Lower placental weight was positively-associated with small-for-gestational-age (SGA) newborns with mean placental weight of 402 ± 67.2 g, median 392.5 g [17].

Several factors including HDP can affect placental development and cause a low-weight placenta. This is explained by the fact that maternal hypertension may cause decreased placental blood flow, reducing the transfer of specific nutrients, such as glucose and amino acids, and may result in low fetal and placental weight [18].

There was no statistically significant difference between mean 1- and 5-minute Apgar scores in both groups, although 1-min Apgar score was lower in the HDP than in the control group. These data are in contrast to those reported in the literature. Placentas with changes consistent with low blood flow were associated with newborns with lower 1- and 5-minute Apgar scores. Low blood flow may have caused fetal harm with manifesta-
sections at birth such as low scores [19]. In addition, the lowest gestational ages, birth weights, and 1-minute Apgar scores were seen among patients with HDP [20]. HDP increase the risk of unfavorable perinatal outcomes, SGA, low 1- and 5-minute Apgar scores, neonatal infection, meconium aspiration syndrome (MAS), infant respiratory distress syndrome (IRDS), prematurity, hyaline membrane disease (HMD), and perinatal death [13].

Calciphylaxis was the most common placental macroscopic change found in both the control and HDP group, 90% and 98.3% respectively. There is an association between calcification and prematurity in hypertensive pregnant women is early calcification with placental insufficiency and no physiological maturit.

It has also been reported the association between grade 3 placenta in preterm fetuses and perinatal complications in 78% of cases, mainly related to pregnancy-induced hypertension (PIH), among others. Premature placenta is associated with increased rates of fetal distress, presence of meconium in the amniotic fluid, low Apgar scores, low birth weight, perinatal death, and consequently respiratory distress [13].

There is a relationship between placental infarction and PIH. In the present study, areas of infarction were detected in the placenta of one patient in the HDP group (0.8% of cases) who was diagnosed with eclampsia. Fibrin in the fetal surface of the placenta was found in 54.1% of cases with HDP and 60% in the control group. Hypertension only, preeclampsia and eclampsia can cause similar placental macroscopic changes, and their severity bears a relationship with the severity of clinical signs, especially the duration and severity of hypertension [2; 21]. A similar study found macroscopic infarctions in 65% of the cases studied with an area greater than about 5% of placental tissue in 16% [15]. There were 43% of ischemic placental changes and 17% of infarction [22].

A full-term umbilical cord is quite variable measuring 40 to 70 cm in most cases; less than 35 cm is considered short and more than 80 cm is considered long [13]. All cords evaluated in the current study were less than 35 cm; however, the HDP group had even shorter cords, especially in patients with eclampsia with measures as low as 26.6 cm.

The factors associated with the development of the umbilical cord are still unknown. It is believed to be related to movement and tension exerted by the fetus. Thus, any condition that may cause restriction of the uterine cavity or fetal movements can lead to reduced tension on the cord and consequently reduced development [2].

A short umbilical cord does not necessarily indicate any harm to the newborn, although it may be suggestive of poor intrauterine fetal movement, but are often associated with fetal distress, neonatal asphyxia, birth defects, poor labor progression, placental abruption, cord rupture, intrafunicular hemorrhage, puerperal uterine inversion, and even fetal death [2].

Umbilical cord thickness is determined by the amount of Wharton’s jelly varying from one to two cm [2]. The HDP group showed significantly lower measures, 1.1 ± 0.4 cm.

Comparative studies have shown that arteries of infants born to mothers who had preeclampsia had twice as much collagen, reduced elastin, and hyaluronate content, which is replaced by sulfated proteoglycans. The accumulation of collagen with simultaneous reduction in elastin content of umbilical cord arteries can decrease elasticity of arterial walls and decrease fetal blood flow. Wharton's jelly’s ability to retain water and prevent compression of the vessels is also impaired [23, 24].

Pregnancies that progress with HDP are characterized by early maturation of the umbilical cord. There is an imbalance of its contents affecting the elasticity of Wharton’s jelly, which helps protect against compression of the umbilical vessels and preserves umbilical blood flow, ensuring adequate nutrient supply to the fetus [5]. Studies have shown that an increase in umbilical cord thickness is an adequate surrogate indicator for assessing fetal growth and predicting intrauterine growth and perinatal outcome [5, 24].

The insertion of the umbilical cord in the placenta may be central, paracentral, marginal or velamentous. Abnormal insertions include marginal (also known as battledore placenta) and velamentous cord insertion (when the cord insertes into the membranes of the placenta), a rare condition with great impact to the fetus. The authors found abnormal insertions in 14.16% of the HDP group. The asymmetrical insertion of the umbilical cord leads to inadequate vascular supply and thus decreased functional and metabolic efficiency of the placenta, which affects oxygen and nutrient transport through the vessels of the cord and results in SGA newborns [25].

The pathogenesis of these abnormal insertions of the umbilical cord is not well-understood but is often associated with placental disorders, single umbilical artery, fetal malformations, labor complications, low birth weight, prematurity, abortion, and perinatal asphyxia [2].

The false knots of the umbilical cord found in the HDP group included segments of folded and overlapped vessels due to their uneven growth and generally have no fetal impact [25].

Placental macroscopic examination is not routinely performed as part of clinical obstetric practice. Studies have shown that only placentas associated with major events including premature birth, 5-minute Apgar score ≤ 6, multiple pregnancies, suspected placental abruption, or any severe placental abnormality are sent for macroscopic and microscopic examination. Macroscopically-normal placentas without any clinical indications for examination are cold-stored for three days and are examined in the event of maternal and neonatal complications [1].

Several studies have stressed the importance of placental examination following birth [2, 24] as a way to detect changes that may have an impact on fetal/neonatal well-being and reduce fetal/neonatal complications caused by
HDP. A protocol for placental examination is required to improve the quality of care provided to newborns born to mothers with HDP.

Conclusion

Pregnant women with HDP were relatively younger. In addition, due to low blood flow seen in HDP, the macroscopic changes found included lower placental weight, calciphylaxis in the maternal surface of the placenta, and fibrin in the fetal surface of the placenta. Umbilical cords were shorter and thinner in the HDP group compared to the control group. Because of all complications associated, most women with HDP had preterm infants who developed respiratory problems and had shorter umbilical cords.

Acknowledgments

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Experience improves performance of hysterosalpingo-contrast sonography (HyCoSy): a comprehensive and well-tolerated screening modality for the subfertile patient

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Summary

Purpose: To investigate the clinical observations, provider experience, safety, and tolerance of the hysterosalpingo-contrast sonography (HyCoSy) procedure. Materials and Methods: A retrospective study design in which data was collected from ninety-six subfertile women who underwent the HyCoSy procedure at the University of Louisville over a 16-month interval. Results: Ninety-six HyCoSy procedures were performed by a single investigator and contained complete records for review. The authors observed significant decreases in the quantities of saline and air utilized per procedure over time (p < 0.0001 and p > 0.0001). Results from the HyCoSy studies were more often non-diagnostic or non-patent in women with a body mass index (BMI) > 30. Reported pain scores did not statistically differ over the course of the study interval. There were no procedure-related complications noted. Conclusion: The HyCoSy procedure is a timely and minimally invasive study that can be implemented in an office setting with minimal prior operator experience that improves over time.

Key words: HyCoSy; Hysterosalpingo-contrast sonography; Hysterosalpingogram; Infertility; Tubal patency.

Introduction

The current established diagnostic tests for tubal patency are regarded as accurate but have significant disadvantages [1-11]. Laparoscopy with chromoperturbation is viewed as the “gold standard” test for tubal assessment and adding hysteroscopy to the procedure allows for concomitant evaluation of the intrauterine cavity [11-13]. These procedures, however, mandate regional and/or general anesthesia and incur operative costs and associated risks. An alternative and widely accepted screening test, hysterosalpingography (HSG), is regarded as an effective tool for assessing tubal patency and uterine cavity architecture; however, the HSG provides little information regarding myometrial or ovarian morphology [5]. Although the HSG is regarded as safe, the procedure exposes the patient to ionizing radiation and potentially allergenic contrast media [2, 7, 9, 14, 15]. Contrast sonohysterography, or saline-infusion sonography (SIS), accomplishes a simultaneous assessment of the uterine cavity and ovarian morphology, but the procedure fails to provide reliable information regarding tubal patency [16-20]. The introduction of hysterosalpingo-contrast sonography (HyCoSy) has become an increasingly popular alternative in countries outside of North America, combining the principles of SIS with those of HSG. This method has proven to be an acceptable, time-efficient, and well-tolerated alternative to HSG with comparable accuracy in the assessment of the uterine cavity and tubal patency [1, 8, 12-13, 21-27]. However, there is a paucity of data from the United States where obesity is more prevalent and may compromise the feasibility of performing the HyCoSy procedure. This paper examined the technical experiences, patient tolerability, and clinical outcomes of a newly implemented HyCoSy protocol at the University of Louisville from December 2009 through March 2011. Specifically, the authors investigated the parameters of the quantities of saline and air utilized per HyCoSy procedure as a marker of technical skill and procedure proficiency over time.

Materials and Methods

A comprehensive review of the literature regarding the HyCoSy procedure was completed to devise and implement a standardized protocol at the University of Louisville [21, 25, 28-30]. Initiated in December 2009, the HyCoSy procedures were completed in the Division of Reproductive Endocrinology and Infertility (REI) outpatient office setting utilizing the following methods:

Hysterosalpingo-Contrast Sonography (HyCoSy) protocol

Patients were selected as appropriate candidates for the HyCoSy procedure based on the clinical indications of irregular uterine bleeding, amenorrhea, suspected intrauterine synechiae, and/or infertility. Patients gave their signed informed consent for this clinically-indicated procedure. Patients presented to the outpatient office during the follicular phase of a spontaneous menstrual cycle, typically cycle days 5-10 [31]. If patients reported a history of anovulation or irregular menses, they were placed on combination oral contraceptives, medroxyprogesterone acetate, or norethindrone acetate for 10-21 days prior to their procedure to prevent pregnancy. This also permitted endometrial uniformity and stabilization for improved ultrasonic visualization of the uterine cavity [31]. All patients had a negative urine pregnancy test prior to initiating pre-procedural hormone suppression and prior to the procedure.
To date, there are no large studies that address the occurrence of post-HyCoSy pelvic infection. A review of the literature revealed inconsistencies in the use of prophylactic antibiotics [1, 16, 26, 30, 32-34]. Without a consensus opinion cited in the literature regarding the prevention of HyCoSy procedure-related infection, the decision was made to prophylactically treat all patients who underwent the HyCoSy procedure with Doxycycline, 100 mg administered orally twice daily for three days (initiated on the day prior to the procedure). If clinically indicated, patients were screened for neisseria gonorhea and chlamydia trachomatis prior to the procedure.

The HyCoSy procedure was performed with the patient placed in lithotomy position. A baseline transvaginal pelvic ultrasound, utilizing the General Electric Voluson E8 system (General Electric Healthcare, Milwaukee, WI) was completed to assess for uterine size, myometrial composition, hydrosalpinges, antral follicle count, and ovarian morphology. The vaginal transducer was then removed and an open-sided vaginal speculum inserted. Cervical preparation was completed utilizing povidone-iodine solution; chlorhexidine gluconate was an alternative for patients with iodine allergies. Occasionally, when needed, a tenaculum was positioned slowly on the cervix for stabilization and uterine positioning. A standard 5 Fr, latex-free Redi-HSG catheter (Redi Medical, Goldsboro, NC) was inserted through the endocervix, with or without the aid of the stabilizing sheath. In rare cases, at the discretion of the provider, the balloon tip was slowly inflated to limit efflux of media and spontaneous expulsion of the catheter. Prior to insertion of the HSG catheter, the catheter lumen was flushed with sterile saline utilizing a pre-filled 30 ml syringe secured to the end of the catheter. This step was done to avoid insertion of air bubbles during the initial uterine cavity assessment. The speculum was then removed and the vaginal ultrasound transducer re-inserted. Sterile saline was instilled into the uterine cavity during simultaneous ultrasound imaging. Images were obtained and stored to document uterine cavity architecture in both two- and three-dimensional fields. Once the uterine cavity assessment was completed, the 30 ml syringe was removed from the HSG catheter, filled with approximately 15 ml air and 15 ml saline, and re-fastened to the catheter. The syringe was intermittently tilted to allow an alternating, slow inflation of air and saline in small increments (1-3 ml at a time) [21, 25, 28-29]. Hyperechoic “scintillations” were made possible on real time (b-mode) ultrasound imaging by the positive pressure flow of echogenic air bubbles as they traversed the path of least resistance, from the uterine cavity into the pelvis via patient Fallopian tubes. In a few cases, the catheter balloon was inflated to prevent excessive vaginal efflux of air and saline. Tubal patency was distinguished by visualization of proximal intratubal flow of echogenic contrast for at least five to ten seconds, followed by flow extending from the distal end of the Fallopian tube and the adjacent ovary.

For standardization purposes, real time ultrasound imaging was first directed to document bilateral proximal scintillations, accomplished best while viewing the uterine fundus in a transverse plane (Figure 1). The vaginal transducer was then guided to document the presence or absence of proximal and distal scintillations. The amounts of instilled saline and air required to complete the uterine cavity and tubal patency assessment were noted.

Once the HyCoSy procedure was completed, all vaginal instruments were removed. Patients were monitored for the occurrence of adverse symptoms for at least 15 minutes prior to discharge. Patients completed a Wong-Baker FACE and/or numerical 1-10 pain scale evaluation, noting the duration of experienced pain or other side-effects [35-37]. Patients were contacted within seven to ten days to review the results of their test and address any procedure-related side effects or concerns.

Statistical analysis of data

A retrospective review was conducted of all HyCoSy procedures performed between December 2009 and March 2010 under the approval of the University of Louisville institutional review board (IRB). The study conclusions were made upon analysis of the entire group and the goal was to provide data for improvements in evidence-based practice. Data of 96 patients was collected and analyzed in the current study. Demographic data included age, gravidity, parity, body mass index (BMI), experienced pain score, and clinical indication for the HyCoSy procedure. Outcome variables included the total amount of saline (ml) and air (ml) utilized during each HyCoSy procedure.

Information on the total amount of saline and air required for each procedure was investigated to determine if the utilized quantities decreased over time. Initially, the patients (n = 96) were stratified into tertiles (the first 33 women, the second 32 women, and the last 31 women) to investigate if the mean amount of saline/air used in the procedure decreased over time. Subsequently, the amount of saline/air used in the procedure was made a function of case number (first patient was case #1, second patient was case #2, up until the last patient that was case #96) to predict the effect experience had on amount of saline/air used in the procedure.

The data in each tertile group was tested for normality (α = 0.05). One-way analysis of variance (ANOVA) techniques were used to test for differences in patient age and BMI between the tertiles. The mean quantities of utilized air (ml) and saline (ml) between the three tertiles were also compared using one-way ANOVA techniques. This was done separately for both air and saline. When a statistical difference was noted, post-hoc comparisons (Tukey’s pairwise comparisons) were performed to identify where the group differences existed. Additionally, two separate linear regression models were developed to evaluate if the quantities of air (ml) and saline (ml) per HyCoSy procedure decreased over time (with increased experience); adjusting for age, gravidity, and BMI.

Additional analysis was performed to detect differences in patient-reported pain scores between and among tertiles utilizing one-way ANOVA techniques. A separate analysis was completed comparing HyCoSy procedure patency results, “patent” or “non-patent,” to patient BMI using the Mann-Whitney U (i.e., Rank-Sum Test) to test for differences.

All statistical calculations were computed using IBM SPSS, version 19, (IBM Corps, Armonk, New York) and GraphPad Prism-5 statistical software (GraphPad, La Jolla, CA, USA).

Results

Ninety-six total HyCoSy procedures were initiated at the University of Louisville between December 2009 and March 2011. These procedures were performed by a single, primary investigator and contained complete records for review. Two of the 96 procedures were aborted sec-
Experience improves performance of hysterosalpingo-contrast sonography (HyCoSy): a comprehensive and well-tolerated etc.

Table 1.—Mean patient demographics and characteristics among tertiles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1st Tertile</th>
<th>2nd Tertile</th>
<th>3rd Tertile</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33 (4.886)</td>
<td>32 (5.034)</td>
<td>31 (5.192)</td>
<td>0.339</td>
</tr>
<tr>
<td>BMI</td>
<td>29.4 (8.369)</td>
<td>28.9 (8.083)</td>
<td>29.4 (8.183)</td>
<td>0.967</td>
</tr>
<tr>
<td>Pain Score (1-10)</td>
<td>5 (2.401)</td>
<td>5.5 (1.934)</td>
<td>6 (2.874)</td>
<td>0.232</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.

Table 2.—Mean quantity of saline and air utilized per HyCoSy procedure.

<table>
<thead>
<tr>
<th>Contrast media</th>
<th>1st Tertile</th>
<th>2nd Tertile</th>
<th>3rd Tertile</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (ml)</td>
<td>39.04 (2.2)</td>
<td>31.17 (2.0)</td>
<td>26.11 (2.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Air (ml)</td>
<td>33.27 (3.3)</td>
<td>29.73 (3.1)</td>
<td>18.04 (2.4)</td>
<td>&lt; 0.002</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.

Table 3.—Linear regression analysis of saline and air quantities utilized per HyCoSy procedure.

<table>
<thead>
<tr>
<th>Contrast media</th>
<th>β</th>
<th>95% Confidence interval</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (ml)</td>
<td>-0.189</td>
<td>-0.395 -0.110</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Air (ml)</td>
<td>-0.252</td>
<td>-0.289 -0.089</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.

Patient tolerability and side-effects

Over the 16-month study interval, a mean reported pain score value of five was noted utilizing the Wong-Baker FACE and numerical 1-10 pain rating scales. The duration of experienced pain ranged from 15 to 120 seconds of maximum discomfort which rapidly subsided to a reported “mild cramping” or “menstrual-like feeling” following the procedure. Using one-way ANOVA statistical analysis, the mean pain scores and duration of reported pain did not differ significantly between or among tertiles (p = 0.232). Moderate side-effects included two reported episodes of nausea with emesis and 19 reported incidences, or 20 percent, of shoulder discomfort immediately following the procedures. One patient experienced a vasovagal reaction with near syncope. She was monitored for approximately 35 minutes post-procedure with complete resolution of symptoms. There were no uterine perforations, post-procedure infections, post-procedure hospitalizations, or other severe side-effects reported throughout the study interval.

Effect of BMI on HyCoSy procedure results

Fallopian tube assessment during the HyCoSy procedure was stated to have unilateral or bilateral “non-patency” if distal scintillations were unable to be visualized in one or both tubes, respectively. The term “non-patency” represented both true tubal occlusions as well as non-diagnostic results (when patency could not be determined) due to poor visibility. Anecdotally, the HyCoSy investigator noted increased difficulty in determining tubal patency in obese patients secondary to poor ultrasound visibility. Thus, further analysis of the data was undertaken to examine trends in HyCoSy patency results compared to patient BMI.

In this study population, obesity (BMI ≥ 30) was noted in 38 percent of patients and morbid obesity (BMI ≥ 40) in 13.5 percent of patients. The mean patient BMI for patients with patent tubal findings was 28; whereas, the mean patient BMI for all non-patent results was 32.
Eighteen of the 94 completed HyCoSy procedures, or 19 percent, revealed unilateral (n = 8) or bilateral (n = 10) non-patency. Of the eighteen non-patent results, nine, or 50 percent, occurred in patients with BMI greater than 30; five, or 28 percent, occurred in patients with a BMI greater than 40. Examined in another way, 15 percent of patients with a BMI < 30 yielded non-patent results, 25 percent of patients with a BMI ≥ 30 yielded non-patent results, and 38 percent of patients with a BMI ≥ 40 yielded non-patent results. Despite an apparent trend of increasing non-patient findings with increasing patient BMI (Figure 5), no statistically significant differences were noted when patency results were compared to obese and non-obese patient categories using a Mann-Whitney U test (p = 0.214).

**Additional findings**

Over the course of the study interval, the following additional pelvic findings were noted: endometrial polyps (n = 8), submucosal fibroid (n = 6), intrauterine synechiae (n = 2), and adnexal masses (n = 8). Of the eight adnexal masses, three appeared to be endometriomas and two appeared to be hydrosalpinges. Combined, a total of 24 patients, or 25 percent, were noted to have incidental pelvic pathology during HyCoSy imaging.

Abnormal findings from HyCoSy studies were further investigated by HSG as/or laparoscopy with or without hysteroscopy. As of March 2011, 19 of the HyCoSy patients have undergone laparoscopic and/or hysteroscopic procedures for further subfertility evaluation and treat-
ment. There were no incongruent findings between those noted at the time of surgery and the HyCoSy findings of pelvic pathology (e.g.: endometrial polyps, submucous myomas, ovarian cysts) and/or tubal patency. In fact, seven of the ten patients with bilateral, non-patent HyCoSy results were subsequently evaluated by laparoscopy. All seven laparoscopic assessments were congruent with bilateral tubal occlusion. Two of the remaining three bilateral, non-patent HyCoSy results were followed with HSG imaging, one of which was congruent with bilateral proximal tubal occlusion and the other was discordant with bilateral tubal patency. The remaining patient with a bilateral, non-patent HyCoSy result declined further investigation or treatment and was lost to follow-up.

Other notable findings were nine post-HyCoSy conceptions with documented, viable intrauterine pregnancies. All nine patients conceived within six months of their HyCoSy procedure. Three patients conceived spontaneously, two conceived with oral-agent ovarian stimulation and intrauterine insemination, and four conceived with in vitro fertilization and embryo transfer.

Discussion

The implementation of the HyCoSy procedure at the University of Louisville has dramatically streamlined the office evaluation of the uterine cavity and the assessment of tubal patency. Combining the principles of SIS and HSG, the HyCoSy accomplishes a comprehensive assessment of the uterine myometrium, cavity contour, adnexal pathology, antral follicle count, ovarian morphology, and tubal patency. Several studies have proven this method to be time-efficient and safe with comparable patient-perceived discomfort compared to HSG [28, 32, 36]. The present report examined a single individual provider’s experience with the HyCoSy procedure in a North American population with an average BMI of 29.2. This was not meant to be a definitive study in comparing the accuracy of this procedure with other modalities of tubal patency assessment. The findings are an estimate of the relative ease in implementing a new technique in the office setting with no prior experience in performing the procedure. The data provides insight into the anticipated clinical limitations and patient tolerability in an overweight or obese sample of patients.

Examination of the quantities of air and saline utilized per HyCoSy procedure demonstrates the relative ease at which methodological certainty and efficiency was achieved. In this review, a nadir mean of 26 ml of saline utilized per HyCoSy procedure occurred in the third tertile of total HyCoSy procedures. Extrapolated from the linear regression analysis, the data suggests that approximately 48 consecutive studies were required for the provider to reach a consistent use of less than 30 ml of saline per HyCoSy procedure. Meanwhile, a nadir mean of 18 ml of air per HyCoSy procedure occurred in the third tertile of patients. When extrapolated, the data reveals that approximately 54 studies were required for the provider to reach a consistent use of less than 20 ml of air per HyCoSy procedure. Thus, after approximately fifty studies, the provider was able to reach an objective plateau of mechanical ease and efficiency that accompanied subjective feelings of procedural expertise. The authors acknowledge that this analysis only demonstrated the learning curve on a single provider to reach individual technical efficiency. A much larger study evaluating the performance of several providers would be required to make broad assumptions on the number of consecutive HyCoSy procedures required before mastery of its technical skills.

With regard to patient complaints of procedure-related discomfort and other adverse effects, the results of this study are consistent with the findings in the literature [28, 32, 38]. Adverse events like referred shoulder pain and/or vasovagal symptoms occurred with a frequency that is similarly seen during hysterosalpingogram and sonohysterography procedures [28, 32]. Examination of patient-reported, HyCoSy-related pelvic pain did not yield a significant difference across the tertiles despite improved provider performance. Certainly, this analysis is limited as it did not address the patient’s reported baseline pain, history of previous pelvic surgery, sexual assault, anxiety or difficult pelvic exams. The infrequent use of a cervical tenaculum (estimated at less than 5%) and/or balloon inflation (estimated at less than 25%) was not consistently documented. The retrospective nature of the study did not allow for critical assessment of the effect of tenaculum and/or balloon inflation on patient reported pain. Such confounders may have influenced trends in patient-reported pelvic pain but no trend was seen in procedure tolerability over the course of the study. Other considerations might include the possibility that any small amount of saline and/or air might incite a pain response and that perhaps no correlation exists between the amount of utilized media and perceived pain.

Although comparisons of patency results across BMI categories were not statistically significant, an apparent
trend was noted of increased non-patient findings in patients with a BMI greater than 30, a discovery that was even more pronounced in patients with a BMI greater than 40. This trend of increased non-patient HyCoSy results in heavier patients likely represents a higher proportion of non-diagnostic studies rather than true tubal occlusions. Poor ultrasound penetration and image resolution, and thus possible non-diagnostic HyCoSy findings, are logically more likely to occur in the obese patient secondary to body habitus limitations. A perceived obesity-related hindrance on image visibility and technical performance is consistent with the observations made by Hamilton and colleagues [28]. Other ultrasound image limitations might occur in women with large uterine leiomyomas or severely retroflexed uterine positions.

The ability of the HyCoSy procedure to accurately and reliably assess tubal patency has been well established in the literature [8]. The diagnostic outcomes with the HyCoSy procedure are comparable to those of traditional HSG and laparoscopy, with tubal assessment concordance rates ranging from 80 to 93 percent [8, 12-13, 21-22, 24-27, 34, 39-45]. With several randomized and controlled trials comparing the HyCoSy procedure to the alternative HSG procedure and to the gold-standard laparoscopy, this paper did not aim to investigate the sensitivity, specificity, or predictive values of the HyCoSy results in our patient population. However, it is interesting to note, that seven of the ten bilateral, non-patient HyCoSy results were confirmed as such with subsequent laparoscopy. Furthermore, there were no other discrepant findings of pelvic pathology when HyCoSy was followed by laparoscopy or hysteroscopy (n = 19). As a direct result of the HyCoSy procedure, 24 patients had newly diagnosed abnormal pelvic pathology that may have altered their subfertility treatment strategies. These incidental findings may not have been otherwise detected using an alternative diagnostic method to assess tubal patency.

In the present study, nine patients conceived following their HyCoSy procedure suggesting a fertility-enhancing effect. However, the sample size is small and findings await confirmation from a larger prospective study.

Conclusion

This paper has reviewed the initial experience of a single investigator implementing the HyCoSy procedure at the University of Louisville over a 16-month interval. The HyCoSy procedure is well-suited to the outpatient office setting. Implementation of the HyCoSy procedure afforded minimal technical challenges, satisfactory patient tolerability, and swift attainment of provider-perceived ease and efficiency.

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References

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Evaluation of adhesions after laparoscopic myomectomy using the Harmonic Ace and the auto-crosslinked hyaluronan gel vs Ringer’s lactate solution

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Introduction
Adhesion formation is a common sequela of pelvic surgery and may cause several complications, such as bowel obstruction [1], chronic pelvic pain [2], and infertility [3-5]. Adhesiolysis increases pregnancy rate from 38% to 52% [6, 7] and reduces pain in 60%-90% of cases of chronic pelvic pain [8, 9].

In addition to a careful surgical technique, to a reduction of surgery time, of blood loss, and the risk of infection, a number of approaches have been proposed to prevent intraperitoneal adhesions. However, despite microsurgical techniques and the adoption of laparoscopic approaches, the problem of adhesions persists [10]. Therefore, other prophylactic measures have to be sought [10, 11].

Among the adhesion preventive agents developed in the last decades, hyaluronan (or hyaluronic acid - HA) based products have been frequently used in different application forms. Hyaluronan-based agents seem to prevent adhesions not only by producing a temporary barrier to fibrin-bridge formation but also through their biological actions. Indeed sodium hyaluronate has been reported to increase the proliferation rate of human peritoneal mesothelial cells, enhancing peritoneal tissue repair [12].

Uterine fibroids represent the most common pelvic tumor of the female reproductive system and myomectomy is traditionally the primary treatment in women with symptomatic fibroids who wish to retain their reproductive potential [13-15].

The accomplishment of myomectomy through laparoscopy has often been questioned due to the excessive blood loss and due to the increase of the operating time owing to hemostasis, which requires a meticulous time-consuming technique [16, 17].

The Harmonic Ace is an ultrasonic surgical instrument that enhances the blade’s ability to cut and coagulate blood vessels. The present authors previously demonstrated that the use of the Harmonic Ace for laparoscopic myomectomy is associated with lower operating time and intraoperative blood loss in comparison with conventional electrosurgery [18].

In this regard, the objective of the present study was to prospectively assess adhesion formation following laparoscopic myomectomy using the Harmonic Ace and an auto-crosslinked HA gel vs Ringer’s lactate.

Materials and Methods
Between February 2008 and 2010, 50 fertile women desiring pregnancy, underwent single laparoscopic myomectomy and were enrolled in the present study. Patients were divided into two groups (A and B) of 25 women each. At the end of the surgical procedure, group A patients received an application of auto-crosslinked HA gel (five ml) on the injured uterine surface, while in the control Group B Ringer’s lactate solution was used.

The type of gel used is sterile, transparent, and highly viscous gel, obtained by condensation of HA through an auto-crosslink-
Evaluation of adhesions after laparoscopic myomectomy using the Harmonic Ace and the auto-crosslinked hyaluronan gel etc.

Table 1. — Clinical parameters compared between Group A (HA) and Group B (Ringer’s lactate).

<table>
<thead>
<tr>
<th>Patients</th>
<th>Group A</th>
<th>Group B</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.6 ± 5.1</td>
<td>33.0 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Operating time (min.)</td>
<td>77.1 ± 43.7</td>
<td>70.5 ± 31.3</td>
<td>NS</td>
</tr>
<tr>
<td>Estimated blood loss (cc)</td>
<td>114.0 ± 105.8</td>
<td>105.0 ± 87.0</td>
<td>NS</td>
</tr>
<tr>
<td>Size (cm)</td>
<td>7.2 ± 1.0</td>
<td>7.2 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Classification according to Munro</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subserous (type 5) – n. (%)</td>
<td>14 (63.6%)</td>
<td>8 (36.4%)</td>
<td>NS</td>
</tr>
<tr>
<td>Intramural (type 4) – n. (%)</td>
<td>12 (54.5%)</td>
<td>10 (45.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior wall - n. (%)</td>
<td>5 (22.7%)</td>
<td>13 (59.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Posterior wall - n. (%)</td>
<td>4 (18.2)</td>
<td>7 (31.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Fundus uteri - n. (%)</td>
<td>10 (45.5%)</td>
<td>5 (22.7%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2. — Adhesions score.

<table>
<thead>
<tr>
<th>Adhesions</th>
<th>&lt; 1/3</th>
<th>1/3 - 2/3</th>
<th>2/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus anterior wall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filmy</td>
<td>1*</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Dense</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Uterus posterior wall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filmy</td>
<td>1*</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Dense</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

Site-specific modified scoring:
*0-1 is assigned when either no adhesions or anatomically non-significant adhesions were found.

Table 3. — Description of site-specific uterine adhesions (Group A vs Group B, p < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>9</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (22 pts)</td>
<td></td>
<td>8</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.05 ± 1</td>
</tr>
<tr>
<td>Group B (22 pts)</td>
<td></td>
<td>8</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>2.27 ± 2.5</td>
</tr>
</tbody>
</table>

Table 4. — Adhesions after laparoscopic myomectomy according to the number of patients (Group A vs Group B, p = 0.05).

<table>
<thead>
<tr>
<th>No.</th>
<th>%</th>
<th>Post-surgical adhesions</th>
<th>a 2</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>15/22</td>
<td>68.2</td>
<td>7/22</td>
<td>31.8</td>
</tr>
<tr>
<td>Group B</td>
<td>10/22</td>
<td>45.4</td>
<td>12/22</td>
<td>54.6</td>
</tr>
</tbody>
</table>

Table 5. — Histological detection of fibrosis (Group A vs Group B, p < 0.01).

<table>
<thead>
<tr>
<th>Fibrosis</th>
<th>0+</th>
<th>++/++++</th>
<th>Total fields</th>
<th>% fields</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>47</td>
<td>33</td>
<td>80</td>
<td>41</td>
</tr>
<tr>
<td>Group B</td>
<td>37</td>
<td>63</td>
<td>100</td>
<td>63</td>
</tr>
</tbody>
</table>

Table 6. — Histological detection of inflammation (Group A vs Group B, p < 0.01).

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>0+</th>
<th>++/++++</th>
<th>Total fields</th>
<th>% fields</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>73</td>
<td>7</td>
<td>80</td>
<td>8</td>
</tr>
<tr>
<td>Group B</td>
<td>45</td>
<td>55</td>
<td>100</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 7. — Histological detection of vessels (Group A vs Group B, p < 0.01).

<table>
<thead>
<tr>
<th>Vessels</th>
<th>0+</th>
<th>++/++++</th>
<th>Total fields</th>
<th>% fields</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>59</td>
<td>21</td>
<td>80</td>
<td>26</td>
</tr>
<tr>
<td>Group B</td>
<td>34</td>
<td>66</td>
<td>100</td>
<td>66</td>
</tr>
</tbody>
</table>

The absence or presence of (filmy) adhesions which were minimally related with the myometrial scar (< 1/3 of its length) were considered anatomically non-significant and were assigned, respectively, a score of 0-1, whereas anatomically significant adhesions were assigned a score of ≥ 2 [23-25] (Table 1). The adhesions at the second-look laparoscopy were sent out for histological analysis. The specimens were stained with haematoxylin-eosin and the assessment was based on the

formed by using a five-mm Harmonic Ace. The technique consisted of transverse incision of the perimetrium, highlighting pseudocapsule of myoma, traction with myoma-drill promoting myoma enucleation, and contemporary section of connectival bridges. Removal of myomas was performed using Steinert electrical (10-15 mm) morcellator. Sutting was always done in double layer (subserous-intramural myomas), intracorporeal single stitches were placed using 0 PDS thread (adsorbable, monofilament polydioxanone). The intraperitoneal cavity was asscetly explored and irrigated in order to remove any myoma remnants and blood [18].

The second-look operations were performed in postoperative days 45 to 60 and consisted in a diagnostic mini-laparoscopy (five-mm umbilical optic and two three-mm ancillary trocars) performed under general anaesthesia using a laryngeal mask airway in association with chromosalpingoscopy in order to assess tubal patency and function. The description of the type of adhesions and the assessment of their severity took into account whether they were: de novo adhesions (with the exclusion of uterine surgical wounds) or adhesions formed on myometrial scars.

Adhesions were assessed according to the classification given by the American Fertility Society (AFS). Adhesions formed on the uterine scar sites were assessed using site-specific modified scoring [20-22].

The absence or presence of (filmy) adhesions which were minimally related with the myometrial scar (< 1/3 of its length) were considered anatomically non-significant and were assigned, respectively, a score of 0-1, whereas anatomically significant adhesions were assigned a score of ≥ 2 [23-25] (Table 1). The adhesions at the second-look laparoscopy were sent out for histological analysis. The specimens were stained with haematoxylin-eosin and the assessment was based on the
absence (0/+) or presence (++/+++) of fibrosis, inflammation, and vascularization. Fibrosis was coded when more than 50% of the field was filled with collagenous fibres and fibroblasts, inflammation when the field contained more than 20 leukocytes and vascularization when the field contained more than five vessels.

Ethical approval was obtained from the Local Ethical Committee, and informed consent was obtained from patients before enrolment.

Results

The study was successfully conducted in 44 patients. One patient was excluded from the analysis because of pelvic adhesions found during myomectomy, whereas five patients refused second-look laparoscopy (10% drop-out rate).

Patient, fibroid, and surgical procedure characteristics are shown in Table 1. During the postoperative period, no allergic reactions were noted or adverse effects were assessed following the administration of HA.

The second-look operation took place after 44.7 ± 23.7 days, (48.2 ± 24.9 days for Group A and 41.2 ± 22.4 days for Group B).

The authors did not find any adherence involving the adnexa or involving extrauterine organs both in Groups A and B, with the exclusion of omental tissue, although they did not result statistically significant. On the contrary, anatomically significant adhesions (score ≥ 2) developed in seven patients receiving HA and 12 receiving no treatment. Similarly, the average site-specific modified score of adhesions was 1.05 ± 1 in Group A and 2.27 ± 2.5 in Group B.

Adhesions were more frequent in case of larger myomas (7.2 ± 1.0 cm), regardless of presence of HA (p < 0.001, Figures 1A-B).

The histological analysis of adhesion tissues performed in Group B revealed higher incidence of fields containing fibrosis, leukocytes, and vessels than in Group A (p < 0.01, Tables 6, 7, and 8).

Discussion

Myomectomy is a treatment procedure intended to preserve fertility in cases of uterine myomas. Any postoperative adhesion of the uterus, the adnexa or bowel may reduce fertility or increase post-operative pain.

The present study confirmed that laparoscopic myomectomy is associated with the development of uterine postsurgical adhesions, although their incidence is affected by the use of HA. In particular, the present series confirmed previous analysis, whereas the incidence of post-surgical adhesions after laparoscopic myomectomy varies from 29% and 64% [26, 27] and is consistently lower than with traditional surgery where it has been reported to be 90% [28, 29].

With the exclusion of sites of the uterine surgical wound, the present authors did not evidence any new adhesion formation involving the bowel or between the uterine adnexa. Interestingly, this finding appears to be unrelated with the use of HA. On the contrary, a previous series evidenced at least 8.9% of patients developed de novo adhesions of the uterine adnexa, although only the diameter of the largest myoma enucleated was identified as a factor influencing the development of these adhesions [30]. Dubuisson et al. reported 12% incidence of adhesions of the uterine adnexa, although enucleation of posterior myomas increased this risk [27].

Traditionally, the surgical technique of laparoscopic myomectomy is borrowed by laparotomy and it is performed by vertically incising the perimetrium using a monopolar or CO2 laser. In the present series, the perimetrium was always incised transversally, even in case of posterior myomas and the Harmonic scalpel was used to incise and to enucleate the myoma. Although the number of posterior myomas is limited (only ten in this series), the absence of de-novo adnexa adhesions appears to be remarkable and, to date, no studies, evaluating whether the type of perimetrium incision might influence the rate of adhesions after laparoscopic myomectomy, are available.

Surgical perioperative strategies have a great impact on
the frequency of development of postoperative adhesions. The present authors previously found that the use of the Harmonic Ace in myomectomy is associated with shorter global operative time and less intraoperative blood loss than epinephrine with electrosurgery [18]. Indeed, at least theoretically, the ultrasound Ache might confer some advantages in the surgical strategy to reduce formation of post-surgical adhesions, although the rate adhesions of the present series resulted in the same range of values available in other studies where conventional electrosurgery was used.

In addition the present findings support the efficacy of HA in reducing the rate and severity of post-surgical adhesions. Although the number of adhesions resulted the same between the two groups, the rate of anatomically significant adhesions for myomectomy site was lower in patients treated with HA. This finding was also confirmed by the histological evaluation of a lower grade of fibrosis, inflammation, and vessels in the sample tissue removed from adhesions of women receiving HA.

No complications or adverse events were reported after gel administration, and no clinically meaningful differences in haematological parameters were observed between the patients treated with the gel and the controls either after surgery or at second-look procedure. Therefore, no safety considerations were raised in any case.

The gel utilized is a reabsorbable adhesion-prevention gel barrier formed of auto-crosslinked HA, which is a natural component of the extracellular matrix and synovial fluid. It is highly biocompatible, possesses increased in situ residency time compared with native and unmodified HA, and may also have positive biological effects on healing, as would native HA [31, 32].

The safety and efficacy of this auto-crosslinked HA gel in adhesion prevention in different gynaecological surgery settings has also been investigated by other authors [12]. The present series confirms previous findings and the effects of the gel are not modified by the choice of a different surgical technique and by the use of the Harmonic Ace.

In conclusion, although laparoscopic surgery is less invasive, it is still associated with post-surgical adhesion formation with potential critical consequence on fertility preservation. The use of auto-crosslinked HA gel confirms a protection on adhesion formation on myometrial wounds, although the degree of this effect appears to be weak. The absence of adnexa adhesions using the harmonic scalpel and a modified uterine incision appear remarkable, although a larger number of patients is necessary to confirm the present findings.

References


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Relevance of thrombophilia and impact of office hysteroscopy on recurrent in vitro fertilization failures: a case series

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Department of Obstetrics and Gynecology, Istanbul University School of Medicine, Istanbul (Turkey)

Introduction
Recurrent in vitro fertilization (IVF) failures have been attributed to either embryo quality or endometrial receptivity, but remain unexplained in most cases. Benign endometrial pathologies may have a negative effect on pregnancy rate. Congenital or acquired thrombophilia have been associated with recurrent IVF failures as well. Office hysteroscopy (OH) allows a reliable visual assessment of the cervical canal and uterine cavity while providing the opportunity to perform treatment if needed.

Materials and Methods
Fifty-one infertile patients, who could not conceive naturally after one year of having regular unprotected sex and subsequently, had undergone two or more failed IVF cycles, and admitted to the Infertility Clinic of Istanbul University School of Medicine, were selected for this study. The authors performed OH as an outpatient procedure. When intrauterine pathologies were detected, treatment was performed immediately. Patients were screened for the presence of congenital or acquired thrombophilic factors (factor V Leiden, prothrombin G20201A mutation, protein C, protein S, antithrombin III, anticardiolipin IgM antibody, anticardiolipin IgG antibody, lupus anticoagulant, and homocysteine). Afterwards, they were followed-up for pregnancy outcome.

Results
OH revealed that 39 patients (76.5%) had a normal uterine cavity, seven patients (13.7%) had endometrial polyps, and five patients (9.8%) had intrauterine adhesions. Screening for the presence of congenital or acquired thrombophilic factors presented: 15 (29.5%) congenital, 11 (21.6%) acquired, and four (7.8%) unclear causes of thrombophilia (Table 1). Out of 51 patients, 49 attempted another IVF and two dropped out. In 49 patients, 14 (28.6%) were beta-human chorionic gonadotropin (βhCG) positive; clinical pregnancy was positive in 11 patients (22.4%); ten patients (20.4%) had live births. Four out of ten patients with live births had intrauterine pathologies detected and treated by OH which represented 33.3% of patients with intrauterine pathologies.

Discussion
The etiology of recurrent IVF failures is still unclear and probably dependent on multiple factors. Studies on the impact of congenital and acquired thrombophilia on IVF failures have provided inconclusive results. The common belief is that thrombophilia may cause recurrent implantation failures by impairing the initial vascularization process occurring at implantation, which is necessary for a successful pregnancy [1-5]. Some of the studies showed that at least one thrombophilic factor was positive in patients with recurrent IVF failures [2, 4-6]. The results in this study also indicated that at least one positive test resulted for congenital or acquired thrombophilia in nearly 60% of the patients that were screened for such factors. In a very recent study on inherited thrombophilias and adverse pregnancy outcome, the findings did not support a significant association between inherited thrombophilia and the pregnancy outcome [7]. On the other hand, as the present researchers indicated themselves, this study had a small number of patients in the subgroups (ten stillbirths and 16 placental abruption cases) and wide confidence intervals.

In a recent meta-analysis, women experiencing assisted reproductive technology (ART) failures suggested to be more frequently positive for factor V Leiden and anti-phospholipid antibodies, although the evidence is inconclusive [8]. The findings in this study also indicated factor V Leiden as the most frequently seen thrombophilic factor in women experiencing IVF failures.

A normal uterine cavity is important for a successful IVF outcome. In the literature, it is generally argued that
an increased rate of intrauterine pathologies is found in infertility patients, albeit with varying results. Researchers that evaluated OH’s relevance on patients who had recurrent IVF failures reported somewhat similar findings. In one study, 26% of 210 patients with recurrent IVF failures had intrauterine pathologies [6, 9]. In another similarly formatted study, 37% of 520 patients had intrauterine pathologies [10]. The researchers also recommended a routine usage of OH on patients with recurrent IVF failures. In a review on the role of OH in women with recurrent IVF failures, the authors concluded that the pregnancy rates increased when OH was performed, yet they also indicated the need for further studies [11]. The follow-up finding in this study revealed that patients with detected/treated intrauterine pathologies by OH, one-third had a live birth.

**Conclusion**

Ultimately, this limited study suggests that women experiencing recurrent IVF failures are more frequently positive for thrombophilic factors, yet this evidence is inconclusive. Additionally, although the usage of OH in patients with a history of recurrent IVF failures remains debatable, it may be a valid method due to the high detection/treatment rate of intrauterine pathologies that can adversely affect pregnancy rates. Nevertheless, further cohort studies with a larger sample size have to be carried out to confirm and support both findings.

**References**


**Table 1. — Overview of thrombophilia screening.**

<table>
<thead>
<tr>
<th>Congenital thrombophilia factors</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden</td>
<td>8</td>
<td>15.7</td>
</tr>
<tr>
<td>Prothrombin G20210A mutation</td>
<td>3</td>
<td>5.9</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>3</td>
<td>5.9</td>
</tr>
<tr>
<td>Antithrombin III deficiency</td>
<td>0</td>
<td>–</td>
</tr>
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</table>

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Anti cardiolipin lgM</td>
<td>9</td>
<td>17.7</td>
</tr>
<tr>
<td>Anti cardiolipin lgG</td>
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<td>–</td>
</tr>
<tr>
<td>Lupus anti-coagulant</td>
<td>2</td>
<td>3.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unclear thrombophilic factors</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated homocysteine levels</td>
<td>4</td>
<td>7.8</td>
</tr>
</tbody>
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Dihydrotestosterone may contribute to the development of migraine headaches

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2The 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 and Infertility, Camden, NJ
3Philadelphia College of Osteopathic Medicine, Department of Obstetrics and Gynecology, Philadelphia, PA (USA)

Summary

Purpose: To evaluate the possibility that dihydrotestosterone (DHT) may play a role in the etiology of some people’s migraine headaches. Methods: Finasteride 5 mg daily was given to a young woman with chronic migraines. Results: The chronic migraine headaches almost completely disappeared shortly following therapy. However, symptoms returned shortly after stopping the finasteride due to dry eyes. Conclusions: DHT may be an etiologic factor in causing migraines since finasteride suppresses DHT secretion. Alternatively, the benefit could be related to some other property of finasteride possibly by increasing testosterone which may compete with estrogen at the blood vessel level.

Key words: Migraine headaches; Finasteride; Dihydrotestosterone; 5 alpha reductase inhibitor.

Introduction

The obstetrician-gynecologist is frequently also considered the primary care physician for women. Thus the gynecologist is frequently asked for advice about conditions outside of the pelvic cavity. For example it is not unusual for a woman to complain about premenstrual headaches.

Headaches could also be caused by medication prescribed by the gynecologist. It is well known that oral contraceptives may cause headaches. In some instances this symptom may be eliminated or at least alleviated by reducing the dosage of estrogen and if this is ineffective then stopping the oral contraceptive and switching to some other mode of contraception.

Certainly the gynecologist can take the prerogative to refer the woman to a neurologist or internal medicine specialist for evaluation in order to exclude more serious etiologies and even for treatment. Frequently the therapy prescribed will be ergotamine derivatives, beta-blockers or topiramate or other similar drugs sometimes used for seizures. It would not be expected that all gynecologists would treat with these medications, though since primary care physicians do not automatically refer to a specialist unless an appropriate response to treatment fails to occur, it would not be wrong for the gynecologist acting as the primary physician to render this treatment.

However, just as the dermatologist has to be aware of medical conditions that present with certain skin disorders, the gynecologist has a similar responsibility, especially if headaches may be related to a disorder that would be more familiar to the gynecologist. One such entity is the defect related to sympathetic nervous system hypofunction known as the sympathetic neural hyperalgesia edema syndrome leading the infusion of chemicals and toxins into tissues that are normally impervious by the diminished permeability state rendered by inappropriate sympathetic tone.

This sympathetic nervous system disorder is the main etiologic factor for pelvic pain including chronic pelvic pain, mittelschmerz, dyspareunia, dysmenorrhea, vulvar pain, and interstitial cystitis [1]. It is associated with pain in many other areas of the body along with chronic fatigue syndrome, skin disorders, e.g., chronic urticaria, eczema, edema, and weight gain [2]. Frequently headaches, especially migraines, are related to this disorder [2-5].

Similar to pelvic pain disorders, migraine headaches respond very well to treatment with sympathomimetic amines even when they have failed to respond to the “standard” aforementioned therapies. The gynecologist should be aware that other pain syndromes may exist without pelvic pain being present. The gynecologist should also be aware that the predominant number of publications concerning this disorder has been in the gynecologic literature and not in neurology or internal medicine journals. Thus it is very likely that the treating neurologist will be unaware of this condition and prescribe less effective therapies with more side-effects or subject the woman to painful and expensive and sometimes risky diagnostic procedures. Thus it is reasonable for a gynecologist to provide first-line therapy for this sympathetic nervous disorder, and only if the headaches do not resolve, then the patient should be referred to a neurological expert.
The case described below is another woman with migraines who responded fairly well to treatment with dextroamphetamine sulfate, the sympathomimetic amine of choice for treating these disorders. Her response was less complete than many other women previously treated before her. However in attempting to treat her androgen symptomatology, a new insight into a hormone involved in headaches may have been discovered and a possible new treatment for some individuals.

Case Report

A 21-year-old female presented with a desire to treat various symptoms including secondary amenorrhea, alopecia, hirsutism, and acne. She had previously been prescribed oral contraceptives but despite changing brands, multiple times she could not find any combination that did not cause weight gain and enlarged painful breasts.

Though the oral contraceptive exacerbated weight gain, she continued to gain weight despite dieting. Based on ultrasound showing the classic polycystic ovarian syndrome (PCOS) characteristics, a high luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio (LH 12 mIU/ml and FSH 5.0 mIU/ml) and a slightly elevated serum testosterone level of 58 ng/dl she was diagnosed with the PCOS syndrome.

Another complaint was chronic headaches that were worsened by oral contraceptives. She was advised that treatment with dextroamphetamine sulfate would likely improve her headaches and also help her to lose weight by inhibiting fluid retention [6]. However for the acne, hirsutism and alopecia spironolactone was advised.

She responded well to both dextroamphetamine amine sulfate (30 mg daily), lost weight, and her breasts were no longer tender. In addition she noted a marked improvement in her constipation and no longer had shortness of breath when climbing a flight of stairs. Her anxiety was also improved, and her energy markedly improved. The headaches improved by 50%. Eventually the headaches were 80-90% improved on the dextroamphetamine sulfate but did not completely disappear. Sometimes they were only 50% better. Hair loss also improved after the use of spironolactone.

The acne had improved on the spironolactone but was not completely gone. We added 5 mg of finasteride daily. The patient stopped the finasteride after one month because of the side-effects of dry eyes. She noted that during the month she took the finasteride her headaches which had been listed as a range of 1-10 were 50% better. Hair loss also improved after the use of spironolactone.

The headache intensity resumed to a 5 while she was taking the finasteride. The headache intensity resumed to a 5 within a few days of stopping the finasteride.

Discussion

The patient was questioned as to whether any time during the 1 ¾ years she was treated with the dextroamphetamine sulfate there had been a period of time when the headaches abated to the degree she had following finasteride. Her answer was no.

Though the improvement in headaches could be coincidental, this case report suggests that dihydrotestosterone may have an etiologic role in some headaches in some people. Thus medications that are 5 alpha reductase inhibitors, e.g., finasterade or possibly dutasteride, may be tried in patients who are refractory to standard therapy or have side-effects from standard therapy.

The first time the benefit of sympathomimetic amines was to alleviate migraine headaches refractory to standard therapy the possibility that the improvement was merely fortuitous was considered [3]. However, it has subsequently been found to almost invariably improve migraine symptoms despite years of suffering and failure to respond to standard therapy. Indeed this young woman responded sufficiently well to sympathomimetic amine therapy that she resisted staying on the finasteride in an attempt to alleviate her dry eyes. She also resisted increasing the dosage of dextroamphetamine sulfate being satisfied with the improvement in headaches, energy, weight, and breast tenderness.

Finasteride suppresses the conversion of testosterone to dihydrotestosterone (DHT). Thus the response suggests that DHT may be an etiologic factor in some headaches in at least some patients. Possibly 5 alpha reductase inhibitors may prove to be another drug to use for treatment of refractory migraines.

The possibility exists that too much DHT was not the factor causing headaches. Instead by raising testosterone with dextroamphetamine sulfate there had been a period of time when the sympathetic nervous system was an etiologic factor for a wide variety of chronic treatment-refractory pathologic disorders which all respond to therapy with sympathomimetic amines". Med. Hypoth., 2011, 77, 717.


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Colostrum in menopause effects on vaginal cytology/symptoms

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Summary

The aim of this study was to assess the effects of three weeks of daily colostrum cream on vaginal cytology and local symptoms related to menopause. Genito-urinary symptoms and cell morphology were analyzed at time 0 (T0) and after three weeks (16 ± days since the end of treatment) at time 1 (T1). Dyspareunia, vaginal dryness, and maturation index (MI) reached a statistically significant difference between T0 and T1. The results proved to be an alternative treatment for vaginal distress caused by lack of hormones in patients in which hormonal treatment is contraindicated.

Key words: Colostrum; Menopause; Vaginal cytology.

Introduction

A normal vaginal epithelium is made of pluristratified cells; the basal cells lay on a basal membrane in the inner layer; the cells are rounded in shape, small (10-12 microns), with dense homogeneous and basophilic cytoplasm, and have a large centrally-located nucleus. They are typical of postmenopausal Papanicolaou (Pap) smears characterized by intense atrophy [1]. Vaginal atrophy and related symptoms (vaginal dryness, soreness, and itching, dyspareunia, and dysuria) are caused by a drop in the estrogen level, as seen during menopause. Atrophy is characterized by several cell modifications resulting in a slower cellular turnover. In menopause, topical treatment based on estrogen and lubricant creams is the main remedy currently used. Literature reports many studies on local hormonal treatment, while studies on medication other than hormones are much less [2-4]. The aim of this study was to assess the effects of colostrum on vaginal cytology and local symptoms related to menopause.

Material and Methods

Between February 2010 and June 2010, 38 patients with physiological menopause with a negative Pap smear within one year, were enrolled at the Outer Gynecology Department of Sapienza Faculty in Rome. Genito-urinary symptoms related to menopause (vaginal dryness, soreness, itching dyspareunia, and dysuria), and cell morphology were analyzed at time 0 (T0) and after three weeks of one daily application of a colostrum vaginal cream. Patients were evaluated 16 ± days before the end of treatment at time 1 (T1). Colostrom is what is actually called “first milk”, a thick, yellowish serum that provides the newborn with the essential vital substances following delivery. Table 1 shows all the components derived from colostrum and its related activities.

Cytological assessment: scraping of the lateral vaginal wall at the fornix was made using an Ayre spatula; the samples were then fixed on a glass using a cytological fixative. An expert cytologist manually evaluated all samples previously inked with a Pap method. The main parameter for cytological evaluation was the cell maturation index (MI) corresponding to a rate between parabasal, intermediate, and superficial cell numbers. On 100 cell counts, the number of parabasal cells was multiplied by 0.0, while the number of intermediate cells was multiplied by 0.5, and the number of superficial cells was multiplied by 1.0. The total number was then divided by the total number of cells: a high total number corresponded to the most estrogenic effect on cells (maximum = 1.0). Clinical symptoms assessment: patients were asked to answer a questionnaire at T0 and after one daily treatment with a colostrum vaginal cream for three weeks. A score ranging from 0 to 3 was associated to severity of symptoms (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Statistical assessment: Student’s t-test was used to compare the results obtained at T0 and T1 either for cytological assessment or for clinical symptoms.

Results

Nine patients dropped out from follow-up; three patients had inflammation at T0 (two of them were not evaluated). Eight patients were evaluated just for symptoms because they had a high MI at T0. Three patients were excluded for side-effects (intense burning) and two for comorbiditity (vulval intraepithelial neoplasia - VIN1, lichen sclerosus). All patients complaining of clinical symptoms at T0 improved after treatment. Dyspareunia and vaginal dryness reached a statistically significant difference between T0 and T1 results, respectively (p = 0.006358) Table 1 (p = 0.000683), and Tables 2 and 3. MI reached a statistically significant difference between T0 and T1 results (p = 0.00195097) Table 4.

Interestingly, 78.5% of patients had cytological signs of aspecific inflammation on T1 samples (18% discrete, 45% mild, and 36% severe inflammation) (Table 5). Thirty-three percent of patients complained of symptoms besides a high MI at T0; in these patients an improvement in the severity of symptoms was also seen after treatment.
Discussion

In menopause, the vaginal epithelium shows a prevalence of basal cells and almost an absence of superficial cells resulting in a thinner epithelium. This fact leads to a lower exfoliation process of vaginal cells; usually during apoptosis, cells release glycogen that is hydrolyzed to glucose. Lactobacillus found in the vagina converts glucose to lactic acid. The conclusion is that in menopause, there is a change in vaginal pH resulting in a more basic vaginal pH value. This condition can lead to inflammation and infection caused by an imbalance of the vaginal flora. When estrogen levels drop, as seen during menopause, there is also lack of water tissue content, decreased blood tissue supply, and increased connective tissue despite elastin. Cell atrophy can correspond to physical symptoms related to the genito-urinary system. In this pilot study, besides the small numbers provided, the authors found a statistically significant difference in dyspareunia, vaginal dryness, and in MI, in patients treated with a daily application of colostrum vaginal cream for three weeks. The authors could not find a direct association between physical symptoms and cytological assessment; in fact even when the MI was high, patients would complain of symptoms as well. On the other hand, in 33% of these patients, the authors observed improvement of symptoms after treatment suggesting a placebo effect. This evidence strengthens the contribution of physician in explaining and supporting the transition process of finding a new balance after menopause. In this study, 78.5% of patients showed cytological signs of aspecific inflammation without symptoms after treatment. This finding did not correlate with MI improvement in the specific case but did correlate in the overall media samples, signifying that inflammation could represent the explanation key for interpreting the results. As stated, cytological signs of aspecific inflammation in T1 samples of this study, did not have a negative correspondence in terms of symptoms related to menopause. It must be taken in account that there is a physiological role of inflammatory cytokines that are
mandatory for many important conditions (i.e. follicle rupture, relaxation of the birth canal at term, etc.) [5-7]. This is the perspective from which the authors portray their results. In a study of Greendale et al., a relationship between examination characteristics believed to represent inflammation and inflammation biomarkers was not upheld [8]. Triggers of inflammation are a result of infectious agents, trauma, immune system activation, etc. The authors documented the presence of granulocytes, macrophages, and lymphocytes in 78.5% of T1 samples that appears to be independent of infection. Many factors can instead either stimulate or modulate synthesis of inflammatory mediators (i.e. hypoxia, environmental pollutants, exercise, etc.). Emerging literature highlights the active role of tissue recovery through biochemical activation triggered by irritative stimuli, against the old concept that a regained tissue homeostasis is only due to exhaustion of the inflammatory process. The authors focused their attention on some colostrum constituents (epidermal growth factor (EGF), immunoglobulin, and cytokines) involved in inflammation cascade [9]. The role of EGF in the skin process of repair is well known, otherwise different studies discuss its role on mucosal cell as well. Chao et al. reported that oral administration of EGF (60 mg/kg/day) can increase EGF content in the duodenal mucosa and promote the healing of rats with duodenal ulcer by its mitogenic action [10]. Immune mediators included in colostrum could elicit a receptor-mediated signal favoring inflammation through immune system activation and synergistically work with growth factors to promote cell maturation. The epithelium of barrier organs (respiratory tract, oral cavity, vagina, etc.) works constantly with the immune system to guarantee protection from the outside environment. This complex network of cells not only monitors and regulates immune homeostasis, but also has a role in epithelial homeostasis, development, and cell integrity. Particularly, many studies highlight the interaction between epithelial cells (EC) and intraepithelial lymphocytes (IELs) in maintaining epithelial integrity. Local γδ T cells are necessary for the differentiation and maintenance of intestinal crypt epithelia [11]. Some effector peptides produced by ECs and leukocytes can induce chemotaxis of neutrophils as well as stimulate epithelial wound closure [12, 13]. Leukocytes sensing any kind of cell injury create a PGE2 rich environment that favors proliferation of colonic epithelial progenitors [14]. As discussed above, colostrum components could have raised a local process involving inflammation that brought vaginal epithelial cells into the beginning of a new cell cycle that resulted in a MI improvement. Mitosis is a process developing in hours, as shown in Mori experiment, whereas the mitotic rate can change based on different hormonal cell status and cell age [15]. The hormonal topical treatment for genito-urinary symptoms was recently confirmed in a meta-analysis including 19 randomized clinical trials that enrolled 4,162 women [2]. The problem arises when women treated for estrogen-related cancer in premenopause, like breast cancer, complain of the same symptoms. Research should direct to find alternative treatments for this type of patient.

Conclusions

In this pilot study, besides the small numbers provided, the authors found a statistically significant difference in dyspareunia, vaginal dryness, and in MI, in patients treated with a daily application of colostrum vaginal cream for three weeks. These results proved to be an alternative treatment for vaginal distress caused by lack of hormones that can include patients in which hormonal treatment is contraindicated.

References


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Superselective uterine arterial embolization combined with transcatheter intra-arterial methotrexate infusion in 40 cases with fallopian tube ectopic pregnancy

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Summary

Purpose: To evaluate the therapeutic results of superselective uterine artery infusion and embolization in 40 patients with fallopian tube ectopic pregnancy, and to explore the role of this minimally invasive treatment as an alternative to surgery. Materials and Methods: Superselective catheterization of uterine artery through cannulation of femoral artery was achieved in 40 patients with fallopian tube ectopic pregnancy (EP). Location of the lesions involved feeding arteries and active bleeding were observed through angiography. Methotrexate (MTX) diluted in saline water was slowly infused into the target artery. Small gelatin spongy particles were used to embolize the uterine artery until its branches were totally obliterated. Follow-up was undertaken to observe the results of the treatment. Results: Superselective uterine arterial infusion and embolization were successfully performed in all 40 patients without any related complications. Active bleeding in the peritoneum in 33 cases ceased soon after embolization. The embryos in 13 patients were confirmed to have died by ultrasound three days after the procedure. Beta-human chorionic gonadotropin (β-hCG) value dropped to below 15 IU/L at three to 21 days. Hemorrhage in the peritoneum dissolved after seven days in all cases. Mixed mass disappeared after one month. Hysterosalpingography was performed three months after the procedure in 19 patients and patent fallopian tubes were found in 18 patients. Conclusions: Superselective uterine arterial infusion and embolization is a minimally invasive procedure, which can be used to effectively treat EP by disabling the ectopic embryo and leaking arteries with the advantages of preserving the fallopian tubes.

Key words: Fallopian tube ectopic pregnancy; Superselective uterine artery infusion and embolization; Methotrexate; interventional radiology.

Introduction

Approximately 1/100 pregnancies are ectopic, with the conceptus usually implanting in the fallopian tube (95.5%), which is higher in women with damage to the fallopian tubes due to pelvic infections, surgery, or previous ectopic pregnancy, and in smokers [1]. Few ectopic pregnancies (EP) resolve spontaneously, but most continue to grow and lead to rupture of the tube, and may seriously compromise women’s health and future fertility [2, 3].

The treatments of EP include expectant treatment, medical management with methotrexate (MTX), and surgical interventions such as salpingotomy and laparotomy [3]. Salpingotomy by laparoscopy becomes more common in the treatment of EP, because it may lead to fewer complications and shorter recovery times compared with laparotomy, but may also be less likely to remove all the trophoblast, may eventually leading to persistent EP [4]. Surgical intervention, including laparoscopic surgery and laparotomy, is usually performed under emergency conditions, with both the risks of surgery and general anesthesia, and eventually leading to damage to fertility [5].

Several conservative methods for pregnancy termination have been suggested to avoid bleeding, preserve the uterus, and maintain fertility, such as systemic and local administration of MTX. Administered to properly selected patients, it has a success rate of up to 94% [6]. However, the adverse effects such as nausea, vomiting, gastritis, diarrhea, abdominal pain, oral mucositis, pneumonitis, bone marrow suppression, and abnormal liver function are prevalent after intravenous injection of MTX [7, 8]. It is distressing that the time for the serum beta human chorionic gonadotropin (β-hCG) concentration to decline to less than 15 IU/L is 33.6 days on average, but may be up to 109 days [9], which is needed by the patient to comply with post-treatment monitoring. Interestingly, the local MTX injection has been reported that it can decrease the adverse effects and is suggested as an alternative to surgery in selected cases of early unruptured tubal pregnancy [10].

Since the late 1960s, transcatheter arterial embolization, as an interventional radiology treatment, has been used for the control of pelvic hemorrhage resulting from malignancy, trauma, and radiation [11]. More recently, use of transcatheter embolization of the uterine arteries has been described for treatment of abdominal pregnancy and cervical EP [12-14]. Therefore, the authors’ hypothesis is that the combination of the transcatheter intra-arterial MTX infusion and the embolization of uterine artery which feed the gestational sac may be an alternative to surgery.
To address these issues, a retrospective study was performed evaluating the superselective uterine artery with transcatheter intra-arterial infusion and embolization (UAIE) in selected patients with fallopian tube EP, proving that UAIE is a minimally invasive procedure, which can be used to effectively treat EP by disabling the ectopic embryo and leaking arteries with the advantages of preserving the fallopian tubes.

Materials and Methods

Patients

Between February 2007 and March 2011, 40 patients with fallopian tubal EP entered this study. All patients willing to accept the interventional radiology and desired future fertility; the time of suppressed menstruation < 70 days; the gestational sac has unruptured; the gestational sac has ruptured or resolved spontaneously, with abdominal bleeding, but the vital signs were still stable; transvaginal ultrasound and serum β-hCG test supported the diagnosis of tubal EP; the patients with shock were excluded. The demographic and clinical features of 40 patients are summarized in Table 1.

Operation protocol

The 5F uterine artery catheter punctured the right femoral artery following the Seldinger method, and it was inserted selectively into the uterine artery of sick side. Then, the superselective uterine artery angiography was carried out to observe the feeding uterine arteries to gestational sac and active bleed-
Detection of serum \( \beta \)-hCG

A \( \beta \)-hCG test kit was used in the detection of serum \( \beta \)-hCG, and the manufacturer’s instructions were followed. The venous blood samples were obtained from the subjects and allowed to clot at room temperature and aliquots of serum were obtained by centrifugation. Serums were collected and stored until analysed. Serum levels of \( \beta \)-hCG were measured by chemiluminescence.

Assessments

Patients were followed up with a review of medical records and telephone interviews. The follow-up periods after UAE ranged from three days to 12 months (median, four months). The authors evaluated the UAIE technique, complications, vaginal bleeding, serum levels of \( \beta \)-hCG, ultrasound examination, menstruation function, pregnancy, and delivery. Technical success was defined as disappearance of uterine arterial flow on bilateral iliac arteriography and disappearance of active vaginal bleeding on gynecologic examination after UAIE. Clinical success was defined as the disappearance of uterine arterial angiography stopped bleeding after embolization. No major complication related to UAIE was detected. Vaginal bleeding was controlled after UAIE. Ten patients had a small amount of vaginal bleeding during the three weeks after UAIE. Eighteen patients with active abdominal bleeding which showed obvious contrast agent extravasation around the annex district from the uterine artery angiography stopped bleeding after embolization.

Statistical analysis

Categorical data are presented as absolute values and percentages, and continuous data are summarized as median and range or mean and SD. The Wilcoxon test was used for comparison of continuous variables. A value of \( p < 0.05 \) was regarded as significant.

Results

The 40 patients all underwent UAIE procedures. The bilateral uterine artery arteriograms were obtained before UAIE to observe the gestational sac of blood supply (Figure 1). In all sessions, enlarged tubal branches to the uterus were identified (Figure 1, L1 and R2). Then, UAIE was performed with gelatin sponge particles. The bilateral uterine arteriogram obtained after UAIE shows absence of opacification of both distal uterine arteries, suggesting successful embolization (Figure 1, L3 and R3).

The clinical success was obtained in the 37 patients, among them, one patient had lesions removed because of the EP in the left fallopian tube after UAIE at one year, whose previous conceptus implanted in the right fallopian tube, and the right accessories were confirmed as well during laparoscopic surgery. Three patients finally underwent surgical treatment, two patients whose serum \( \beta \)-hCG levels that did not decline significantly had symptoms that increased, and one patient because of no willingness to comply with post-treatment monitoring.

Table 1. — Demographic and basal clinical features.

<table>
<thead>
<tr>
<th>Patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age in years (range)</td>
<td>28 (17-38)</td>
</tr>
<tr>
<td>Median ( \beta )-hCG value U/l (range)</td>
<td>2182 (269-4821)</td>
</tr>
<tr>
<td>Survival of embryos (no.) and Among embryos</td>
<td>13, 0.8 - 2.5</td>
</tr>
<tr>
<td>gestational sac and its diameter (cm)</td>
<td>42 - 52</td>
</tr>
<tr>
<td>Suppressed menstruation (days)</td>
<td>18</td>
</tr>
<tr>
<td>Active bleeding (no.)</td>
<td>42</td>
</tr>
</tbody>
</table>

Figure 2. — The detection of serum \( \beta \)-hCG was carried out before and after UAIE at seven, 14, and 21 days.
Nineteen patients underwent hysterosalpingography after UAIE at six months, and the result displayed a tubal patency rate of 84.2%. So far there were six patients with normal pregnancy and birth.

Discussion

The tubal branch of uterine artery bears more than 85% of the tubal blood supply, and the ipsilateral uterine artery mainly feeds the ectopic embryo occurring in the fallopian tube, which is the anatomical basis of the transcatheter intra-arterial drugs to perform abortions and embolization to stop bleeding.

Ectopic pregnancies are today often diagnosed before the patient’s condition has deteriorated such that surgical intervention is inevitable. This is partly due to an increased knowledge and awareness of risk factors among both clinicians and patients. Reliable diagnostic algorithms integrating transvaginal ultrasound and serum $\beta$-hCG measurement, that enable to make an accurate non-invasive diagnosis [15, 16]. As a result, the conservative management such as MTX, has been more and more accepted.

MTX, a folic acid antagonist, inhibits DNA synthesis in actively dividing cells, including trophoblasts [6]. Many researchers believe that MTX is necessary when the ectopic mass less than 3.5 cm in diameter and/or when serum $\beta$-hCG level exceeds no more than 3,000 IU/l [3]. Can patients without this range undergo the conservative treatment?

The frequency of MTX complications is similar to those with laparoscopy [17]. For this reason, the authors used the technology for transcatheter intra-arterial MTX infusion. The data showed that this technology effectively reduced the complications and adverse effects. In addition, as shown in Figure 2, the time for the serum $\beta$-hCG value to decline to normal was about 14-21 days, which greatly shortened post-treatment MTX monitoring time. The transcatheter intra-arterial MTX infusion had been used in the treatment for the cervical EP [18]. The authors believe that the transcatheter intra-arterial MTX infusion improves MTX benefits for EP by increasing the amount of MTX delivered to the site of the gestational sac.

At three days after embolization, the ultrasonography of five patients with more pelvic blood volume (about 1,500-2,000 ml) showed that pelvic hemorrhage was about 300 ml, and then underwent puncture of posterior fornix of vagina; the blood removed was about 150 ml. At seven days after embolization, ultrasonography showed that pelvic hemorrhage was all absorbed. The ultrasonography of these five patients showed that gestational sac was ruptured. The authors embolized the abnormal side of uterine artery, which not only can arrest bleeding, interrupt the blood supply of gestational sac, but can also extend the residual time of MTX. Therefore, the authors believe that the combination uterine artery embolization with local infusion of MTX can be used to treat the patients which have an abortion, whose gestational sac has ruptured and/or in which bleeding has occurred, but the patients can still tolerate.

The initial purpose of embolization is to control the bleeding caused by abortion or rupture. The authors chose gelatin spongy particles with a size of 0.5 x 0.5 x 0.5 mm³ as the embolic agent, which are the temporary embolic agents. With the pelvic interventional clinical research increased, more and more literatures report that the ovarian artery participates in the abnormal blood supplying to the pelvic focus of infection [19, 20]. The ovarian artery if ignored, may lead to treatment failure caused the rebleeding. More small embolic particles can more effectively achieve the peripheral distal, and can reach the ovarian distal artery, blocking the ovarian artery, while controlling bleeding. In addition, the embolic particles within one to three mm in diameter usually cannot cause necrosis of the normal uterus.

However, one patient had active vaginal re-bleeding 45 days after UAIE and she eventually underwent salpingotomy through laparoscopy. The cause of rebleeding after UAIE is considered to be as follows: the establishment of extensive collateral circulation; the recanalization of embolized uterine arteries after UAIE using temporary
embolic agents, such as gelatin sponge, might have once again supplied blood to the uterus; some gestational tissues may have remained alive after short-time ischemia.

In conclusion, UAIE is a safe, minimally invasive, and effective procedure with a low rate of complications for tubal EP. However, the risk of vaginal rebleeding after UAIE should be recognized. Furthermore, the results of the present study show that UAIE allows the preservation of the uterus and the potential for future fertility. These results are encouraging, but the authors cannot draw firm conclusions due to the small number of patients in this study.

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Sympathomimetic amines effectively control pain for interstitial cystitis that had not responded to other therapies

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Introduction

A common condition relatively unknown to the medical community exists where a defect in the sympathetic nervous system leads to a wide variety of symptoms that are generally refractory to standard medical therapies, but responds quickly and effectively to treatment with sympathomimetic amines [1]. Prior publications have shown that small dosages of dextroamphetamine sulfate have efficiently and effectively controlled urticaria, joint pain, fibromyalgia, chronic fatigue syndrome, inability to lose weight despite dieting, gastrointestinal motility disorders, inflammatory bowel disease, chronic pelvic pain, dysmenorrhea, vulvovaginitis, and vasomotor symptoms [1]. There is also a published case report that demonstrated prompt marked improvement of bladder pain following dextroamphetamine sulfate therapy in two women with long-standing suffering who failed to respond to traditional therapy [2]. The present study evaluated sympathomimetic amine therapy for painful bladder syndrome/interstitial cystitis in a series of six additional cases of chronic painful bladder syndrome refractory to standard therapy.

Materials and Methods

The study was an observational case series without placebo controls. Only women were selected for this study, since this disorder of the sympathetic nervous system occurs predominantly in women. All subjects had to have painful bladder for over a year and had failed to have adequate improvement from standard therapies. For inclusion in the study, the women had to have bladder pain urgency and frequency, despite negative urine cultures for at least 12 months. Furthermore, cystoscopy findings had to be consistent with interstitial cystitis. Dextroamphetamine sulfate extended release capsules were begun at 15 mg daily and increased depending on tolerance and response to a maximum of 30 mg per day in one or two divided doses. To evaluate longevity of treatment benefit, the study only included those women responding to sympathomimetic amines who continued the medication for at least six months. If a woman failed to respond to this treatment, it was suspended; the patient, however, was still included in the study.

Results

Prior to therapy, four of the six women had such severe symptoms that they could not function in daily society. Five of the six women, in addition to dysuria, had nocturia (at least five times per night), frequency, and urgency. Commencing at 15 mg of dextroamphetamine sulfate extended release capsules, all six women showed significant relief in dysuria, urgency, frequency, and nocturia. All patients increased the dosage to either 25 mg or 30 mg per day usually after the first month (with one exception). Within two to six months, their urinary symptoms were either completely gone or so mild as to be very tolerable. Four out of six patients decreased nocturia to once per night and two women had two urinations during the night. All symptoms remained almost completely relieved or cured both at six months and one year evaluations.
Discussion

This was not a controlled study, so one could argue that the sympathomimetic therapy perhaps worked as a placebo. However, all patients did not show any placebo response to pentosan polysulfate sodium, or pelvic floor physical therapy. Thus with the quick and long-lasting benefits with dextroamphetamine sulfate, it seemed highly unlikely that the symptomatic remission was from either placebo effect or spontaneous remission. Further evidence supporting specific benefit from sympathomimetic amine drug therapy was the fact that three women who ran out of medication briefly for a few days (the schedule II drug did not allow refills), the bladder symptoms returned immediately. Resuming dextroamphetamine sulfate once again improved symptoms within 24 hours.

Although none of the women had postural syncope, evidence of a disorder of the sympathetic nervous system was demonstrated by abnormal water load tests in all six patients [1]. The sympathetic nervous system is responsible for maintaining normal intravascular fluid volume in response to the orthostatic position which, because of an increase in hydrostatic pressure, would tend to cause water to extravasate from intravascular to extravascular space were it not for a signal by the sympathetic nervous system causing precapillary sphincters to constrict. An abnormal water load test is determined when following ingestion of 1,500 ml of water over a half-hour period of time, a woman urinates ≥ 75% of the ingested water load over four hours supine, but the next day fails to excrete at least 75% of the water load over four hours while remaining erect.

The autonomic nervous system innervates the mucosal epithelium [3]. It is believed that a diminished sympathetic tone, possibly related to antibodies against ganglionic acetylcholine receptors, leads to diminished function of this mucosal epithelium especially in its role of preventing absorption of toxins from the lumen to the epithelium [4]. The toxins stimulate inflammatory response. Also the sympathetic nervous system innervates lymphoid tissue possibly facilitating inflammatory response [5]. These pain syndromes are not limited to the bladder and do not always include the bladder depending on which sympathetic nerves are involved. Nevertheless, almost all of the various pain syndromes responded quickly and effectively to sympathomimetic amine therapy. The common link is the usual abnormal water load test with ≥ 75% excreted of the water load supine but < 75% standing.

Dextroamphetamine therapy is without dependence or withdrawal symptoms when used in this small dosage. Patients with other types of pain disorders have been treated with this drug for over 30 years without problems [1]. It is generally well-tolerated and if side-effects such as insomnia, palpitations or personality change occur, they are usually transient.

Thus the authors have now demonstrated that in eight consecutive patients (including two from the original case report), severe protracted interstitial cystitis had dramatically responded to this benign therapy. No other patients with painful bladder syndrome had failed to respond to this therapy. Hopefully this case series will generate more widespread interest in evaluating dextroamphetamine sulfate therapy and controlled trials are welcome. Perhaps other novel therapies may be generated, based on the responses seen to sympathomimetic amines for bladder pain. It would be interesting to determine if sympathomimetic amines can also improve bladder pain in male patients.

References


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Dyslipidemia is a persistent problem in puerperium with or without preeclampsia

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Summary

Purpose of investigation: To compare serum levels of triglycerides and cholesterol and the dyslipidemic factor (DLF): (triglycerides (mg/dl)/150) X (cholesterol (mg/dl)/200) among puerperal women with or without preeclampsia. Materials and Methods: Three groups of puerperal women were formed: group A uncomplicated deliveries, group B deliveries complicated with preeclampsia and that had attended the OICU; and group C puerperal women complicated with preeclampsia and that had attended the OICU. Results: The authors studied a total of 47 puerperal women, 14 without complications, 11 complicated with preeclampsia, and 22 complicated with preeclampsia requiring attention at the OICU. Thirteen (92.8%) puerperal women without complications and 100% of puerperal women complicated with preeclampsia had triglycerides higher than 150 mg/dl at least three days post-delivery. Furthermore, six puerperal women without complications (42.8%), one puerperal woman complicated with preeclampsia (9%), and eight puerperal women complicated with preeclampsia requiring attention at the OICU (36.3%) had levels in crescendo. Conclusions: Hypertriglyceridemia is a persistent problem in puerperal women who suffered preeclampsia and the DLF could be a useful tool to evaluate a mixed lipemic state. Finally, preeclampsia and dyslipidemia might be considered as risk factors to develop chronic endothelial disease (CED).

Key words: Dyslipidemia; Preeclampsia; Puerperium.

Introduction

Dyslipidemia includes a number of different pathological conditions whose common element is a disorder of lipid metabolism, and their subsequent alteration of the concentrations of lipids and lipoproteins in the blood. This is a common metabolic disorder during pregnancy that persists in the postpartum period (until 40 days following childbirth) and even later.

Triglycerides constitute 90% of our body fat and their normal value is 150 mg/dl. Cholesterol acts on the composition of molecules of vitamin D, hormones, and bile acids; its normal value is 200 mg/dl. Whatever the type, a lipidic disorder increases the risk of atherosclerosis and heart disease [1].

During the first two quarters of pregnancy, there is an increased lipid storage with a lipolysis similar to non-pregnant women and in later stages, as fetal nutritional demands have significantly increased, the maternal reserves decrease. The low-density lipoprotein (LDL) reaches its maximum at about 36 weeks due to liver effects of estradiol and progesterone. The high-density lipoprotein (HDL) reaches its maximum at about 25 weeks, decreases at about 32 weeks, and holds steady during the rest of pregnancy [2].

A significant percentage of pregnant women go on to develop hypertension associated with preeclampsia, dyslipidemia, gestational diabetes, and maternal obesity. The long-term consequences of these conditions have not been adequately investigated in controlled clinical studies [3]. Unfortunately, there are limited treatment options for the possible complications to the binomial mother-child and most studies on the subject are still at an early stage [4].

In Mexico, the prevalence of metabolic diseases is rising and our concern about the high rates of maternal and infant mortality has led the authors to study the female population in the process of puerperium with persistent dyslipidemia. In the Obstetric Intensive Care Unit (OICU) of the Materno Perinatal Hospital “Mónica Pretelini” (HMPMP), State Health System of the State of Mexico (ISEM), levels of cholesterol and triglycerides were monitored in all patients.

Preeclampsia, defined as the increase in blood pressure accompanied by edema, proteinuria or both, that occurs after the 20th week of gestation, is considered a diffuse endothelial disorder, which complicates 6%-10% of pregnancies [5]. This entity is characterized by increased vascular resistance, vasoconstriction, metabolic changes in nitric oxide (NO), lipids and prostaglandins, in addition to clotting abnormalities [6].

Eclampsia is defined as the development of seizures due to hypertensive encephalopathy in a preeclamptic patient, not attributed to other causes. Its incidence is close to one per 2,000 births. Seizures, which are the sign of eclampsia, are preceded by manifestations of preeclampsia, although 20% of seizures can occur up to six days after delivery [7].

Surprisingly, despite its high prevalence, information related to dyslipidemia in puerperium is scarce. Thus, further examination of the implications of dyslipidemia in puerperium is mandatory.
Dyslipidemia is a persistent…

The authors’ principal aim was to compare serum levels of triglycerides and cholesterol and the dyslipidemic factor (DLF): (triglycerides (mg/dl)/150) X (cholesterol (mg/dl)/200), among puerperal women complicated or not with preeclampsia.

Materials and Methods

This was a cohort, observational, prospective, comparative and longitudinal study developed in the HMPMP, Toluca, Mexico, during the period from August 1st, 2009 to March 30th, 2010.

Participants

Women in postpartum were between 16 and 46-years-old. Those with premature membrane rupture, with a previous known chronic-metabolic disease (diabetes, pregestational dyslipidemia, and hypertension), or with incomplete medical records were excluded from this study.

Three groups were conformed: group A included puerperal women without complications (physiological puerperium), group B puerperal women complicated with preeclampsia, and group C puerperal women complicated with preeclampsia requiring attention at the OICU (Figure 1).

Preeclampsia was defined as gestational hypertension (systolic blood pressure more than 140 mm Hg and diastolic blood pressure more than 90 mm Hg) and proteinuria (at least 300 mg of urine protein in 24 h). Women requiring attention at the OICU were those with severe criteria: organ damage [8] or severe hypertension requiring i.v. drugs (sodium nitroprusside or nitroglycerin).

Data and measures

The data accessed in the clinical records of each patient was entered in an Excel spreadsheet previously designed by the researchers.

Blood pressure was measured with electronic monitor in the Triage room and during hospitalization.

The SPSS version 16 was used for statistical analysis. Continuous variables were expressed in means ± standard deviation (SD). As there was no a normal distribution, the authors compared the groups using the Kruskal Wallis test and the Mann-Whitney U test for differences between two groups. A p ≤ 0.05 was considered statistically significant.

| Table 1. — Laboratory results from the three groups analyzed. |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Group A (n = 14) | Group B (n = 11) | Group C (n = 22) |
| Age (years)                    | 27.2 ± 10.1      | 22.9 ± 6.5       | 27.8 ± 7        |
| Albumin (mg/dl)*               | 3.1 ± 0.49       | 2.5 ± 0.6        | 2.9 ± 0.7       |
| Calcium (mg/dl)†               | 7.8 ± 0.9        | 7.9 ± 0.7        | 7.6 ± 0.6       |
| Cholesterol (mg/dl)*           | 162.4 ± 4        | 206.6 ± 80.7     | 181.3 ± 54.4    |
| Creatinine (mg/dl)†            | 1 ± 0.7          | 0.6 ± 0.1        | 1 ± 0.6         |
| DLF*                           | 1 ± 0.6          | 2.4 ± 2.1        | 1.5 ± 0.7       |
| Glucose (mg/dl)                | 97.1 ± 35        | 105 ± 49.6       | 108.4 ± 49.2    |
| Magnesium (mg/dl)              | 2.9 ± 0.9        | 2.2 ± 1          | 2.5 ± 0.9       |
| Triglycerides (mg/dl)          | 227.9 ± 66       | 304.4 ± 162.7    | 244.4 ± 60.4    |
| Uric acid (mg/dl)†             | 5.3 ± 2.4        | 5.1 ± 1.1        | 5.7 ± 1.5       |
| VLDL (mg/dl)                   | 46 ± 13.3        | 60.8 ± 32.5      | 48.8 ± 12       |
| Hemoglobin (g/dl)              | 10.1 ± 1.5       | 11 ± 2.2         | 10.4 ± 1.8      |

Group A: puerperal women without complications; Group B: puerperal women complicated with preeclampsia; Group C: puerperal women complicated with preeclampsia requiring attention at the OICU; DLF: dyslipidemic factor = (triglycerides (mg/dl)/150) X (cholesterol (mg/dl)/200); OICU: Obstetric Intensive Care Unit; VLDL: very low density lipoproteins.

Laboratory

After delivery and for three days, with a fasting period of eight hours, the authors measured albumin (mg/dl), calcium (mg/dl), cholesterol (mg/dl), creatinine (mg/dl), glucose (mg/dl), magnesium (mg/dl), triglycerides (mg/dl), uric acid (mg/dl), and hemoglobin (g/dl). Very low-density lipoproteins (VLDL) were calculated as triglycerides (mg/dl)/5.

This study was approved by the Ethical and Research Committee of the HMPMP (date: July 2, 2009).

Results

For the eight month period, the authors compiled complete laboratory results of 14 puerperal women without complications (median age 27.2 ± 10.1 years), 11 puerperal women complicated with preeclampsia (median age 22.9 ± 6.5 years), and 22 puerperal women complicated with preeclampsia requiring attention at the OICU (median age 27.8 ± 7 years). Table 1 shows the laboratory characteristics of the three groups. There was a statistical significant difference in albumin and cholesterol levels between puerperal women without complications and puerperal women complicated with preeclampsia, as well as in albumin, creatinine, and uric acid between puerperal women complicated with preeclampsia and puerperal women complicated with preeclampsia who attended the OICU.

When analyzed more precisely, 13 (92.8%) puerperal women without complications, 100% of puerperal women complicated with preeclampsia, and puerperal women complicated with preeclampsia requiring attention at the OICU had triglycerides higher than 150 mg/dl at least three days post-delivery. Furthermore, six (42.8%) puerperal women without complications, one (9%) puerperal woman complicated with preeclampsia, and eight (36.3%) puerperal women complicated with preeclampsia requiring attention at the OICU had levels in crescendo.
Dyslipidemia is a persistent problem in puerperium with or without preeclampsia

In relation to cholesterol, two (14.2%) puerperal women without complications, six (54.5%) puerperal women complicated with preeclampsia, and 11 (50%) puerperal women complicated with preeclampsia requiring attention at the OICU had serum values higher than 200 mg/dl at least one day during post-delivery. Furthermore, five (35.7%) puerperal women without complications, two (18.1%) puerperal women complicated with preeclampsia, and nine (40.9%) puerperal women complicated with preeclampsia requiring attention at the OICU had levels in crescendo. The \( p \) value was of 0.027 between puerperal women without complications and puerperal women complicated with preeclampsia.

Only two (14.2%) puerperal women without complications, six (54.5 %) puerperal women complicated with preeclampsia, and 11 (50%) puerperal women complicated with preeclampsia requiring attention at the OICU had both triglycerides and cholesterol levels above normal limits at least once. DLF showed a statistically significant difference between puerperal women without complications and puerperal women complicated with preeclampsia (\( p \leq 0.05 \)).

In relation to glucose, two (14.2%) puerperal women without complications, one (9%) puerperal woman complicated with preeclampsia, and two (9%) puerperal women complicated with preeclampsia requiring attention at the OICU had levels higher than 100 mg/dl. Besides the results previously commented, the anemic state of most of the women was brought to the authors’ attention as Toluca City is 2,500 m above sea level and the minimum value for hemoglobin should be 12.3 g/dl [9].

Discussion

In a normal pregnancy, serum cholesterol and triglycerides rise 25%-40% and 200%-400%, respectively. A supraphysiologic rise in plasma triglycerides’ concentrations in late pregnancy may serve as a marker of pre-lipemia [10]. The metabolic syndrome, defined by abdominal obesity, elevation of blood pressure, fasting glucose and triglycerides, and low levels of HDL, may play an important role in the pathogenesis of unsuccessful pregnancy, by including a pro-inflammatory and pro-thrombotic state [11]. Inflammation in women with spontaneous preterm birth might be related to their metabolic profile, such as lipids [12]. Unfortunately, lipid profiles are not routinely measured in all pregnant women, which might indicate that the true prevalence of dyslipidemia is under-estimated [13].

Several studies have examined the development of the metabolic syndrome in pregnant women [14] and there are several criteria and indexes to evaluate the repercussions of metabolic disturbances during pregnancy, such as insulin-like growth factors (IGFs), insulin-like growth factor binding proteins (IGFBPs), leptin, homeostasis model assessment (HOMA), maternal insulin sensitivity, etc. [15], but the information related to puerperium is scarce [16]. Considering the context of other work, the authors agree that women should be followed up until 42 days postpartum [17]. Moreover, every postpartum visit should include the evaluation components of the metabolic syndrome and not only glucose intolerance [18]. In the present study the authors analyzed the post-pregnancy presence of dyslipidemia in women with physiological or complicated puerperium. The importance of this issue is emphasized by the continuous increase in the prevalence of dyslipidemia [19].

The atherogenic index of plasma (\( AIP = \log (\text{triglycerides}/\text{HDL}) \)), correlates with the size of pro- and antiatherogenic lipoprotein particles. Clinical studies have shown that AIP predicts cardiovascular risk [20]. Although AIP is a useful cardiovascular risk marker and measure of response to treatment, it is not common to check HDL in all pregnant and puerperal women. According to the present authors’ point of view, DLF proposed in this study could be a useful tool to evaluate a mixed lipemic state with a good cost-benefit approach. Certainly a study designed to obtain ROC curves for the factor suggested by the present authors to be considered in clinical practice is still missing.

Systolic hypertension and hypercholesterolemia are both considered other variables to determine the Framingham score, moreover, arterial coronary disease or equivalents or diabetes are considered as coronary risk factors. Preeclampsia, as well as the other diseases previously mentioned, produce endothelial dysfunction, in fact, preeclamptic women have an increased risk to develop this complication in a subsequent pregnancy. The authors suggest, therefore, that preeclampsia and dyslipidemia might be considered as risk factors in developing a chronic endothelial disease (CED).

Finally, several limitations to this study need to be mentioned. Firstly, this was a study with a low number of patients. Secondly, puerperal women were not systematically followed up after their delivery to take blood samples to monitor triglycerides and cholesterol levels.

Conclusion

HDL is not usually measured in all pregnant and puerperal women. According to the authors’ point of view, DLF could be a useful tool in evaluating a mixed lipemic state with a good cost-benefit approach.

References


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Low-dose estrogen and drospirenone combination: effects on metabolism and endothelial function in postmenopausal women with metabolic syndrome

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Introduction

The association of insulin resistance, abdominal obesity, dyslipidemia, hypertension, inflammatory and prothrombotic state, defined as metabolic syndrome, is associated with a significant increase in cardiovascular disease (CVD), the leading cause of morbidity and mortality in Western countries [1]. The prevalence of this condition increases during postmenopause in relation to the endocrine-metabolic changes induced by hypo-estrogenism. In addition to classical cardiovascular risk factors, endothelial dysfunction is a relevant factor of cardiovascular damage [2].

In postmenopausal patients, hormone replacement therapy (HRT) should be used with extreme caution; all the latest clinical recommendations indicate the opportunity to treat postmenopausal symptoms with the lowest effective dose in the shortest possible time [3]. Low-dose estradiol (E2) may be prescribed in a continuous combined regimen both with different types of progestins. In women with cardiovascular risk, in order to maximize the benefits and reduce the risks of HRT, a careful selection of patients and choice of progestin is mandatory because of its potential detrimental impact on metabolic cardiovascular risk [4]. To date, progestins derived from progesterone (P) are preferred to the androgenic progestins used in contraception therapy for their lower metabolic effect [5].

Drospirenone (DRSP) is a progestin with anti-androgenic and anti-mineralocorticoid, recently used in combination to hemihydrate E2 for the therapy of climacteric disorders. Preliminary studies suggest beneficial effects of DRSP on the cardiovascular profile in relation to the antialdosterone activity able to counteract the estrogen-induced activation of the renin-angiotensin system, and then the retention of sodium and water, resulting in reduction of blood pressure in postmenopausal women with hypertension [6]. It has been speculated that the ability to preserve the homeostasis Na-K could counteract the pro-inflammatory activity of the endothelium [7], while the antiandrogen properties may counteract the android distribution of adipose tissue and the atherogenic lipid metabolism occurring in the postmenopause [8]. In a previous study, a low-dose hemihydrate E2/DRSP treatment did not reveal any negative effect in healthy postmenopausal women on carbohydrate metabolism, acting in a neutral way on insulin sensitivity, while the treatment induced favorable changes in lipid profile and showed a significant improvement of vascular reactivity [9]. To date, data on the impact of low-dose hemihydrate E2/DRSP in women with metabolic syndrome are lacking. The aim of this study was to evaluate the effects two mg of DRSP in combination with one mg hemihydrate estradiol one mg + drospirenone two mg. At recruitment and after six months, clinical and laboratory parameters of metabolic syndrome were evaluated. Endothelial function was assessed measuring the flow-mediated dilatation of the brachial artery and the intima-media thickness of the common carotid artery. Results: After six months an overall improvement of metabolism was observed in both groups reaching statistical significance for triglycerides, total cholesterol, and systolic pressure in group B. A trend to lower baseline flow-mediated dilatation was also found in group B. Conclusions: Drospirenone improves cardiovascular risk factors and does not impair endothelial function in menopausal women with metabolic syndrome.

Materials and Methods

Twenty-eight healthy patients (group A) and 28 patients with metabolic syndrome (group B) were treated with hemihydrate estradiol one mg + drospirenone two mg. At recruitment and after six months, clinical and laboratory parameters of metabolic syndrome were evaluated. Endothelial function was assessed measuring the flow-mediated dilatation of the brachial artery and the intima-media thickness of the common carotid artery. Results: After six months an overall improvement of metabolism was observed in both groups reaching statistical significance for triglycerides, total cholesterol, and systolic pressure in group B. A trend to lower baseline flow-mediated dilatation was also found in group B. Conclusions: Drospirenone improves cardiovascular risk factors and does not impair endothelial function in menopausal women with metabolic syndrome.

Key words: HRT; Drospirenone; Metabolic syndrome; Cardiovascular risk; Endothelial dysfunction.
ment of menopausal symptoms were enrolled in the study protocol. Before beginning the study, assessment of plasma follicle-stimulating hormone (FSH) (> 40 IU/l) and E2 (< 30 pg/ml) concentration, mammography, cervical cytology, and transvaginal ultrasound (TVUS) examination were performed. These parameters were found to be normal and compatible with the menopausal status.

Patients of group A were healthy, did not show signs of diabetes or impaired glucose tolerance, breast cancer, liver or kidney parameter alterations, history of major thromboembolism, thyroid disease, uncontrolled or treated hypertension (systolic blood pressure > 140 mmHg or diastolic > 90 mmHg), and were not smokers. Patients of group B had metabolic syndrome defined by the presence at least three of the following disorders: waist circumference > 88 cm, fasting blood glucose > 110 mg/dl, triglycerides > 150 mg/dl, high-density lipoprotein (HDL) cholesterol < 50 mg/dl, blood pressure (BP) > 130/85 mmHg (National Institutes of Health - NIH, 2001) (References for the NHI 2001). Represented exclusion criteria established diabetes, triglycerides > 200 mg/dl, total cholesterol > 300 mg/dl, BP levels > 150/90 mmHg, smoking, alcohol intake (> 40 g/day), contraindication to HRT, use in the three months prior to the study of hormonal therapies for cholesterollowering drugs, antidiabetic, anti-hypertensives, aspirin, Nons-teroidal antiinflammatory drugs (NSAIDs), and antioxidant vitamins.

All patients were treated for six months with two mg DRSP in combination with one mg hemihydrate E2. At enrollment (T0) and at six months (T1) the following parameters were evaluated:
- clinical parameters (waist-to-hip ratio, WHR, blood pressure);
- fasting laboratory parameters (triglycerides, HDL cholesterol, total cholesterol, low-density lipoprotein (LDL) cholesterol, apo-A, apo-B, blood glucose);
- endothelial function (EF) through the measurement of brachial reactivity as changes of diameter and the flow of the artery at rest and after compression (flow-mediated dilation - FMD); these data were expressed in percent value;
- atherosclerosis progression by B-mode ultrasonography measuring the intima-media thickness of the carotid arteries [2].

Table 1 describes the characteristics the two study groups at baseline and after six months therapy. An overall improvement in lipid profile and blood pressure was observed in both groups reaching statistical significance only for triglycerides, total cholesterolemia, and systolic blood pressure in patients of group B. With regards to the endothelial function, a lower baseline FMD was observed in group B and a trend to a better response to compression in healthy patients (from 6.1% to 9.3%) compared to those with metabolic syndrome (from 4.7% to 6.6%), although not statistically significant (Figure 1). No change was observed in the intima-media thickness of the carotid arteries.

### Results

Table 1 describes the characteristics the two study groups at baseline and after six months therapy. An overall improvement in lipid profile and blood pressure was observed in both groups reaching statistical significance only for triglycerides, total cholesterolemia, and systolic blood pressure in patients of group B. With regards to the endothelial function, a lower baseline FMD was observed in group B and a trend to a better response to compression in healthy patients (from 6.1% to 9.3%) compared to those with metabolic syndrome (from 4.7% to 6.6%), although not statistically significant (Figure 1). No change was observed in the intima-media thickness of the carotid arteries.

Table 1. — Clinical, laboratory, and instrumental data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A Baseline</th>
<th>Group A After six months</th>
<th>Group B Baseline</th>
<th>Group B After six months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.9 ± 4.8</td>
<td>51.6 ± 4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years of menopause</td>
<td>3.5 ± 2.0</td>
<td>3.2 ± 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82.7 ± 7.9</td>
<td>83.0 ± 7.8</td>
<td>90.8 ± 11.0</td>
<td>90.3 ± 9.8</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>88.8 ± 9.9</td>
<td>90.1 ± 9.7</td>
<td>103.2 ± 14.7</td>
<td>92.8 ± 13.9</td>
</tr>
<tr>
<td>Triglyceridemia (mg/dl)</td>
<td>88.4 ± 23.8</td>
<td>88.9 ± 28.0</td>
<td>119.3 ± 48.8</td>
<td>110.2 ± 50.3</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>216.9 ± 24.3</td>
<td>215.9 ± 22.6</td>
<td>214.9 ± 14.5</td>
<td>194.4 ± 26.0</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>46.3 ± 7.0</td>
<td>46.8 ± 7.8</td>
<td>51.1 ± 6.5</td>
<td>59.2 ± 12.4</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132.7 ± 11.2</td>
<td>136.9 ± 15.6</td>
<td>136.9 ± 13.7</td>
<td>120.5 ± 12.8</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>85.9 ± 8.2</td>
<td>84.7 ± 7.0</td>
<td>83.4 ± 6.7</td>
<td>78.6 ± 5.9</td>
</tr>
<tr>
<td>Thickness of the carotid arteries (mm)</td>
<td>0.39 ± 0.11</td>
<td>0.39 ± 0.13</td>
<td>0.38 ± 0.12</td>
<td>0.39 ± 0.1</td>
</tr>
</tbody>
</table>

*p ≤ 0.05 vs group B; *p ≤ 0.05 vs group A.

### Discussion

CVD is the leading cause of death in women in Western countries and its incidence is highest in postmenopausal women when the prevalence of the metabolic syndrome increases and contributes significantly to the alteration of the parameters of cardiovascular risk induced by estrogen deficiency [10]. It is believed that alterations of lipid metabolism, central obesity, insulin resistance, and hypertension, that are the most important features of metabolic syndrome, represent a substantial component of the cardiovascular risk, worsened also by hypoestrogenism. Endothelial dysfunction is undoubtedly one of the key elements in the pathophysiology and progression of vascular damage.

HRT is the gold standard for the treatment of symptomatic menopausal patients [11], and is also considered the gold standard therapy for menopausal osteoporosis even if recent studies suggest new potential target for osteoporosis therapy as modulators of the endovanillloid/endocannabinoid system [12, 13]. In women in which HRT is contraindicated, phytoestrogens are the most used alternative therapy [14, 15]. Nowadays, the impact of HRT on cardiovascular risk is controversial [16]: HRT is not currently indicated for the prevention of CVD, but it can play a role of primary prevention in healthy subjects in perimenopause or early postmenopause before endothelial damage occurs [9]. The literature today suggests the use of estrogens and progestins used at the lowest dosages and with lowest metabolic impact [4].

DRSP is a novel synthetic progestin structurally similar to spironolactone, which differs from classical progestin because it has both antiandrogenic and anti-mineralocorticoid effects. Such action could contribute to the control of endothelial dysfunction because it may antagonize the renin-angiotensin-aldosterone system by blocking the action of aldosterone at the receptor level resulting in renal reuptake of sodium and fluids, by promoting NO activity resulting in smooth muscle relaxation and vasodilation. It is also hypothesized that the ability to preserve Na-K homeostasis could counteract the pro-inflammatory endothelial activity [6-8].

Clinical evidence on the impact of DRSP on cardiovas-
cascular risk in healthy postmenopausal women show a favorable change in the lipid profile and an improvement of vascular reactivity [9], but data on women at risk of CVD are lacking. The present study shows that the two mg DRSP + one mg hemihydrate E2 has a good impact on metabolic cardiovascular risk factors because it improves the metabolic parameters in patients with metabolic syndrome compared to healthy controls, with a greater impact on blood pressure, while endothelial function as measured by brachial artery reactivity is not impaired. These results may be due to the new profile of activity of DRSP which seems to be preferable to other progestins for the association in HRT in postmenopausal patients at risk of CVD, even if the influence of E2 cannot be excluded. In conclusion, this study, although carried out in a selected and limited population, and lasting only a brief period, indicated an overall remarkable beneficial effect of DRSP on several determinants of cardiovascular diseases and encourage further investigations.

References


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Maternal mortality in Serbia

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Summary
Maternal mortality related to obstetric events is still high today. The main components of reproductive health are evaluated. Early diagnosis of the obstetric risks can significantly reduce maternal mortality.

Key words: Pregnancy; Mortality; Obstetric risk.

Introduction
Maternal mortality refers to deaths of women due to complications of pregnancy, childbirth, and puerperium (the first six weeks after delivery). The maternal mortality ratio is used, as a more stable indicator which monitors deaths per 100,000 live births. The most common complications that lead to maternal deaths are: infection, excessive bleeding after childbirth or abortion, and other complications of pregnancy, including eclampsia and postpartum sepsis [1].

Worldwide, more than 350,000 women die annually from complications during pregnancy and childbirth, 99% occur in developing countries [2].

Materials and Methods
Our state adopted the Millennium Declaration in 2000. The Millennium Development’s fifth goal is: improving maternal health. As maternal health is an indicator of quality of healthcare of women of childbearing age (women aged 15 to 49 years), this is taken into account when adjusting the Millennium Development’s fifth goal in the Republic of Serbia with the following tasks:
1. Reduce by 75% the maternal mortality ratio since 1990 by 2015. For the Republic of Serbia until 2015, reduce the maternal mortality ratio to 4.9.
2. By 2015, preserve and improve women’s reproductive health by maintaining the fertility rate at the current level, while reducing the abortion rate by doubling the percentage of women using modern contraceptive methods.
3. To reduce mortality in the group of women of childbearing age by one-third between 2000 and 2015.

Discussion
There are many problems in the quality of registration of maternal deaths, as reported by sightings and by an expert in the Republic of Serbia. It occurs that these deaths are registered under other causes, especially if the woman was diagnosed with some chronic health problems before pregnancy [3]. In some cases, registration under other causes of death can occur if death take place in another hospital in which the patient was admitted due to other medical complications. It is estimated that as much as ten percent of maternal deaths occur after the 42nd day of delivery, in which case the death is not registered under the same cause, therefore there is underregistration [4]. Since there 2007, triangulation methodology is used for collecting data: death certificate, registration of birth, and hospitalisation list. Sources of data for this analysis were: the Statistical Office of Serbia [5] and the Institute of Public Health of Serbia "Dr. Milan Jovanovic Batut" [6].

Based on comparisons of the five-year average at the beginning of the Millennium period for monitoring of maternal health (1990-1994), with the five-year average based on the analysis performed in 2005 (for period 2001-2005), it is evident that the maternal mortality ratio decreased significantly from 13.9 to 6.5, therefore it was considered realistic to reach the proposed national value of five deaths due to complications of pregnancy, childbirth, and puerperium per 100,000 live births [7] by 2015.


In our population causes of maternal mortality in the period from 2007 to 2011 were: hemorrhage (four), eclampsia (six), embolism (seven), sepsis (eight), influenza H1N1 (eight), adult respiratory distress syndrome (four), acute heart failure (seven), leukemia (one), and unknown cause (two). In 2009 and 2010, the highest maternal mortality ratio was recorded, and the incidence of influenza H1N1 as the cause of death was 28.5% and 33.3%, respectively.

Maternal mortality in Serbia for years has been low and has been reduced to sporadic cases, as in most Western countries. The incidence of births that take place in the presence of trained health workers in the Republic of Serbia is very high; since 2002, more than 99% (up to 99.5% in year 2005). Certain prerequisites for the reduc-
tion of maternal mortality are still unsatisfactory, such as coverage of antenatal women’s healthcare.

Conclusion

To promote and preserve the health of women of child-bearing age, it is necessary to ensure greater social and economic security of all women, especially in the period of maternity, pro-natal policy of the state, and the protection of the family. Analysis of main components of reproductive health: fertility, safe motherhood, family planning, prevention of unwanted pregnancies and abortions, as well as specific disease in women of childbearing period [8], is necessary. A significant reduction in maternal mortality can be achieved by early diagnosis, prompt treatment, and rehabilitation after certain illnesses.

References


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Operative treatment of gynaecologic diseases in puberty: seven years of experience

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Summary

Purpose of investigation: The aim of this study is to present the incidence and surgical management of gynaecologic pathology in adolescence in the 1st Obstetrics and Gynecology Department of Aristotle University of Thessaloniki. Methods: After a retrospective review of the medical records of over a seven year period (2004-2011), 32 adolescent patients with reported surgical gynaecologic procedures were identified and analysed. Results: Fourteen out of the 16 adolescents with ovarian masses (eight neoplastic and eight non-neoplastic) were treated by laparoscopy. Congenital anomalies were diagnosed in seven patients and only one of them was treated by laparotomy. The rest were surgically treated for uterine leiomyoma (1), ectopic pregnancy (2), pelvic abscess (1), mesosalpingeal cysts (2), mesenterian cyst (1) and investigation of chronic pelvic pain. Discussion: Although benign ovarian cysts and congenital anomalies represent the major indication for operative treatment of gynaecologic diseases in puberty, laparoscopy and/or hysteroscopy should be the gold standard procedure after careful preoperative investigation.

Key words: Adolescent; Laparoscopy; Ovarian cyst; Congenital anomalies.

Introduction

Surgical treatment of gynaecologic disease in puberty presents differences from those in adulthood. The treatment modalities are further complicated by considerations of fertility. Psychological issues of puberty are also of central importance. The diagnosis of an ovarian mass is a stressful experience at every age; in puberty it is considered to be traumatic to the patient and family. There are numerous studies involving considerable populations in adolescence. These studies suggest that ovarian masses compromise the most common type of tumour of the reproductive system at adolescence, of which 65% are benign while 35% present malignancy [1, 2]. Tumours of the uterus are very rare in puberty. The most common presenting symptom for malignant tumours is vaginal bleeding [3]. Tumours of the vulva also present with decreased incidence while cervical and vaginal tumours in puberty are very rare.

The aim of this study is to present the experience of the 1st Department of Obstetrics and Gynecology at Aristotle University of Thessaloniki in the management and treatment of surgical gynaecologic pathology in adolescence.

Material and Methods

A retrospective study of adolescent patients from 13-18 years of age, that presented and were treated surgically at the 1st Obstetrics and Gynecology Department of Aristotle University of Thessaloniki with gynaecologic pathology during the period 2004-2011. The common presenting symptoms were abdominal pain, palpable abdominal mass and/or abdominal distention, menstrual disturbances and amenorrhea. Twenty-one were treated with laparoscopy, eight with laparotomy and three with vaginal procedures. Table 1 presents the 32 cases classified according to diagnosis. Sixteen ovarian masses were treated. Eight were neoplastic (6 were mature cystic teratomas and 2 serous ovarian cystadenomas) and eight non neoplastic (3 were follicular ovarian cysts and 5 luteal ovarian cysts). Fourteen were treated by laparoscopy and two by laparotomy. Of 16 ovarian masses, only three oophorectomies were performed, two by laparoscopy and one by laparotomy. In two cases of luteal cyst rupture, haemostasis was performed – one by laparoscopy and one by laparotomy, and in the remaining 11 cases laparoscopic removal of the ovarian mass was performed.

Congenital anomalies of the reproductive tract were diagnosed in seven patients of which one was treated by laparotomy, while the rest were treated transvaginally with hysteroscopy and laparoscopy. The most common congenital anomaly was Rokitansky-Mayer-Kuster-Hauser syndrome which was treated by the neovagina formation-Davidoff procedure.

One uterine tumour (leiomyoma) was removed by laparoscopy. The two ectopic pregnancies were treated by laparoscopic salpingectomy. One pelvic abscess was treated by laparoscopic drainage. Two mesosalpingeal cysts and a mesenterian cyst were laparoscopically removed. Finally for the two cases with chronic pelvic pain diagnostic laparoscopy was performed and revealed no pathological findings.
Table 1. — Thirty-two cases of gynaecologic pathology in puberty classified depending on the final diagnosis.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplastic ovarian tumors</td>
<td>8</td>
</tr>
<tr>
<td>Mature cystic teratoma</td>
<td>6</td>
</tr>
<tr>
<td>Serous ovarian cystadenoma</td>
<td>2</td>
</tr>
<tr>
<td>Non neoplastic ovarian cysts</td>
<td>8</td>
</tr>
<tr>
<td>Follicular ovarian cysts</td>
<td>3</td>
</tr>
<tr>
<td>Luteal ovarian cysts</td>
<td>5</td>
</tr>
<tr>
<td>Congenital anomalies of reproductive tract</td>
<td>7</td>
</tr>
<tr>
<td>Vaginal diaphragm</td>
<td>1</td>
</tr>
<tr>
<td>Rokitansky syndrome</td>
<td>2</td>
</tr>
<tr>
<td>Uterine diaphragm</td>
<td>1</td>
</tr>
<tr>
<td>Didelphys uterus</td>
<td>1</td>
</tr>
<tr>
<td>Gonadal dysgenesis</td>
<td>1</td>
</tr>
<tr>
<td>Cervical atresia</td>
<td>1</td>
</tr>
<tr>
<td>Uterine tumours</td>
<td>1</td>
</tr>
<tr>
<td>Leiomyoma</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>8</td>
</tr>
<tr>
<td>Ectopic pregnancy</td>
<td>2</td>
</tr>
<tr>
<td>Pelvic abscess</td>
<td>1</td>
</tr>
<tr>
<td>Mesosalpingeal cyst</td>
<td>1</td>
</tr>
<tr>
<td>Mesenterian cyst</td>
<td>1</td>
</tr>
<tr>
<td>Diagnostic laparoscopy for chronic pelvic pain</td>
<td>2</td>
</tr>
</tbody>
</table>

Discussion

Whether benign or malignant, functional or organic, fluid or solid, ovarian masses are the most common gynaecologic tumours, with benign tumours and functional cysts predominating. The annual incidence of ovarian neoplasms is estimated at 2.6 per 100,000 girls and they are very rarely malignant, representing only 1% of all cancers in children and adolescents [4, 5]. In our study ovarian masses represent 50% (100% benign) from the overall pathology in adolescent patients that were treated operatively.

The most common presenting symptom is abdominal pain: pain may be subacute, abdominal and pelvic, sometimes recurrent and/or chronic. In other cases, a palpable mass may be detected after a complaint of pelvic heaviness with varying degrees of tenderness [6]. Torsion is the most common complication of ovarian masses with frequency ranging from 3% to 33% [7, 8]. When the ovarian mass manifests with acute pain, torsion is highly probable 42% [8].

The means of diagnosis included: imaging studies (US, CT), laboratory studies such as pregnancy test, blood count, erythrocyte sedimentation rate, C-reactive protein [9], hormonal investigations and serum tumour markers. Information obtained from those imaging and laboratory studies are very useful. Nevertheless, the use of intraoperative biopsy is of great importance to decide how radical the operation should be. The central concern of the surgeon should be fertility preservation. For this reason a conservative approach to the adolescent patient is preferred [10].

In the current study non-neoplastic ovarian masses represented 25%. According to the international literature, follicular cysts represent the most common histologic diagnosis [11]. In our series, they accounted for 37.5%. The main presenting symptom was acute pelvic pain due to rupture or torsion. The most common procedure performed as indicated was laparoscopy.

Surgical procedures for congenital anomalies of the reproductive tract are diagnosed most usually at younger ages. The diagnosis is most commonly based on evaluation for primary amenorrhoea [12]. In our study the mean age of diagnosis of congenital anomalies of the reproductive tract was at 15.1 years and they represented 21.8% of the overall pathology in adolescent patients treated operatively. Obstructive abnormalities are more likely to be associated with pelvic pain and endometriosis. Diagnosis of an obstructive mullerian abnormality can be difficult and may require a combination of history, examination, radiologic imaging (US and/or MRI), laparoscopy and hysteroscopy [13]. Furthermore investigation of the urinary tract should be performed due to the common embryologic origin and the usual coexistence of anomalies in both [12].

Laparoscopy represents the gold standard procedure with the benefits of shorter hospital stay and future fertility preserving potential, especially in the absence of contraindications such as suspicious masses.

In conclusion, the target group of adolescent gynaecologic disorders requiring operative intervention differ substantively from those in adulthood in means of incidence and management.

References


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Eclampsia with neurological complications: a five-year experience of a tertiary centre

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2Department of Neurology, Yuzuncu Yil University, Van (Turkey)

Summary

Purpose: The neurological signs and symptoms in 107 pregnant women with eclampsia in the last five years at the Department of Obstetrics and Gynecology at the Yuzuncu Yil University School of Medicine are presented. Materials and Methods: The medical records of 107 pregnant women with eclampsia in the Clinic of Obstetrics and Gynecology at the Yuzuncu Yil University consulted with neurology clinic from September 2005 to December 2010, were evaluated. Results: The most common symptoms of the patients were seizure, headache, and seeing spots of light. Although neurologic examination was normal in 81 patients, 26 had pathological signs. The most common pathological signs determined were alterations in consciousness. Conclusion: In eclamptic patients, brain scanning might reveal pathological results in spite of normal neurological examination. With neurological examination and brain scanning, it may be possible to diagnose and treat severe complications that may otherwise result in maternal mortality.

Key words: Eclampsia; Pregnancy; Neurologic signs; Brain imaging.

Introduction

Preeclampsia is a disease that may occur in the period from 20th week of pregnancy until the 6th week in the post-partum period; it is characterized by proteinuria (300 mg/24 h and over), and hypertension (140/90 mmHg and over). If this scenario is complicated with seizure, then it is called eclampsia [1]. Eclampsia is a relatively common complication of pregnancy, which leads to significant maternal and fetal morbidity and mortality [2]. Common neurologic symptoms are headache, visual disturbances, hyperreflexia; in eclampsia, convulsions and sometimes coma are accompanying neurologic symptoms. Rarely, transient blindness may occur in preeclampsia-eclampsia cases [3].

In the present study, we studied the neurologic signs and cranial scanning results of pregnant women who were managed and monitored with the diagnosis of eclampsia in the last five years in the Department of Obstetrics and Gynecology at Yuzuncu Yil University, School of Medicine.

Materials and Methods

The medical records of 107 pregnant women who were hospitalized with the diagnosis of eclampsia in the Department of Obstetrics and Gynecology at Yuzuncu Yil University School of Medicine and consulted with the Neurology Department in the period from September 2005 to December 2010, were evaluated retrospectively. Socio-demographic characteristics, neurologic examination findings, and brain imaging results were obtained from the medical charts.

Results

In this study, the medical records of 107 pregnant women with eclampsia were evaluated. The age of the patients ranged from 18 to 47 years with a mean of 27.4 years. The average gestational age was 33 weeks. The average blood pressure was 170/98 mmHg; 24-h proteinuria level was calculated as 2163.2 mg/l/day. Cesarean delivery was performed in 81 patients, whereas 26 patients had a normal spontaneous vaginal delivery.

The most common symptoms of the patients were seizure, headache, seeing spots of light, swelling in the body, epigastric pain, and visual disturbances. Fifty-two patients (48.5%) had the complaint of headache prior to seizure; 12 patients (11.2%) had seizures in the postpartum period. Neurologic examination was evaluated as normal in 81 patients (~76%); while neurologic examination revealed pathologic signs in 26 patients (~24%). The most common signs determined on neurologic examination were alterations in consciousness, e.g. confusion, not responding to a painful stimulus, and status of coma (Table 1).

Cranial scanning was performed on patients who had abnormal neurologic signs and severe complaints such as persistent headache, nausea and vomiting without any pathologic signs. Magnetic resonance imaging (MRI) was used to scan the cranium. We found pathologic signs in 14 (~53.8%) of those patients, who had pathologic signs in neurologic examination. Thirty-two patients (39.5%) had pathologic signs on cranial scanning despite a normal neurologic examination. In this study, for cranial scanning, MRI was performed in a total of 85 patients. In 46 patients (54.1%), pathologic signs were recognized on cranial scanning (Table 2).

All patients, who were consulted at the Department of Neurology were recommended to undergo blood pressure regulation and monitoring of consciousness. All eclamptic patients were given anti-convulsive treatment with 2
Eclampsia with neurological complications: a five-year experience of a tertiary centre

Table 1. — Neurological examination signs.

<table>
<thead>
<tr>
<th>Signs</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered consciousness (confusion-coma)</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Loss of vision</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Bilateral restriction of lateral eye movement</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Loss of strength</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Normal</td>
<td>81</td>
<td>76</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>107</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. — Distribution of findings of brain MRI.

<table>
<thead>
<tr>
<th>Signs</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRES</td>
<td>24</td>
<td>28.2</td>
</tr>
<tr>
<td>Edema</td>
<td>16</td>
<td>18.8</td>
</tr>
<tr>
<td>Hematoma</td>
<td>4</td>
<td>4.7</td>
</tr>
<tr>
<td>Infarct</td>
<td>4</td>
<td>4.7</td>
</tr>
<tr>
<td>Hypertensive encephalopathy</td>
<td>2</td>
<td>2.3</td>
</tr>
<tr>
<td>Subarachnoid hemorrhage</td>
<td>2</td>
<td>2.3</td>
</tr>
<tr>
<td>Sinus vein thrombosis</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Bifrontal glial mass</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Hypoxic encephalopathy</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Normal</td>
<td>30</td>
<td>35.4</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>100</td>
</tr>
</tbody>
</table>

PRES: posterior reversible encephalopathy syndrome.

g/h of magnesium sulfate, and 6 mg/d of diazepam in that order. In addition to anti-convulsive treatment, anti-edema treatment was done with 0.5-1.5 g/kg/d of mannitol in 31 patients who were unconscious and with 4 x 4-8 mg/d of dexamethasone in two patients. One patient was given 2 x 0.6 cc of low molecular weight heparin treatment because of subacute infarct; one patient was transferred to the Department of Neurosurgery due to the finding of a frontal mass. Three patients were monitored in the intensive care unit after intubation since they developed respiratory failure.

Discussion

Eclampsia is a severe complication of pregnancy, which may affect various organs and systems. Scanning techniques may help in disclosing the pathogenesis of this complication and are able to identify other brain lesions, as well. Among scanning methods, brain MRI is the most commonly used technique [4].

In eclamptic patients, early diagnosis before the development of neurologic complications is essential. During monitoring of preeclamptic and eclamptic pregnant women, visual disturbances, altered mental status or seizures should alert the physician for brain lesions. Therefore, these patients should have cranial scanning performed [5, 6]. In this study, cranial scanning was done in 85 patients.

In eclamptic patients, changes consistent with cytotoxic edema may be seen, especially in posterior parts of cerebral hemispheres, but less frequently in the brain stem, cerebellum, and basal ganglia on brain imaging studies. If it is not treated, vasogenic edema and irreversible brain damage may develop [6]. In our study, in accordance with the literature data, pathologic changes were located in the cerebral hemispheres in 43 patients, in the brain stem in one patient, in the cerebellum in one patient, and in the basal ganglia in one patient.

Digre et al. determined pathologic signs on scanning images in 50% of 16 severe preeclamptic patients [7]. In another study, this rate was reported to be 75% [8]. Topuz et al. found the rate to be 47% among patients with severe eclampsia, eclampsia, and HELLP syndrome [9]. In another study, no pathologic scanning sign was determined in patients with atypical eclampsia [10]. In our study, 46 patients (54.1%) had pathologic findings.

Tamam et al. [5] identified pathologic scanning signs in 79.3% of patients with pathologic findings on neurologic examination, while the authors found pathologic scanning signs in 14% of the patients who had a normal neurologic examination. In our study, the results of brain scanning studies were pathologic in 14 patients (53.8%) with pathologic neurologic examination while, 32 patients (39.5%) with normal neurologic examinations had pathologies on brain scanning images.

In eclamptic patients, with appropriate treatment of hypertension, the lesions and clinical symptoms are expected to regress [3]. In our study, 94 patients, who were able to regulate their blood pressure, were discharged. Two patients died because of intracranial bleeding and one patient died because of cerebral edema and respiratory failure. Nineteen patients were managed by the Department of Neurology because of posterior reversible encephalopathy syndrome; they were discharged after regression of clinical symptoms.

Conclusion

In conclusion, eclampsia is a severe complication of pregnancy which may or may not cause severe abnormal neurological signs. In eclamptic patients, brain scanning might reveal pathological results in spite of a normal neurological examination. With neurological examination and brain scanning, it may be possible to diagnose and treat severe complications that may otherwise result in maternal mortality.

References

[6] Topuz et al. identified pathologic signs in 50% of 16 severe preeclamptic patients [7]. In another study, this rate was reported to be 75% [8]. Topuz et al. found the rate to be 47% among patients with severe eclampsia, eclampsia, and HELLP syndrome [9]. In another study, no pathologic scanning sign was determined in patients with atypical eclampsia [10]. In our study, 46 patients (54.1%) had pathologic findings.


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Accuracy of the cytopathology, bacterioscopy, and vaginal flora culture


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Introduction

The vaginal tract features a microbiota that varies according to the biological development phase of female organism and that follows the different phases of the menstrual cycle.

Peixoto [1] defined as vaginal fluid, the absolute prevalence of lactobacilli in respect to other morphotypes of microorganisms, in addition to the presence of epithelial cells in variable amount, higher than the number of leukocytes, and the absence of target cells, Trichomonas vaginalis and fungi.

During post-menopause, the decrease of estrogen levels leads to a lower deposit of glycogen and consequently to a decrease of lactobacilli controlling the excessive growth of potentially pathogenic bacteria.

The growth of usual flora and/or the colonization of new microorganisms introduced through sexual intercourse is liable to lead to infections characterized by vaginal discharge, frequent complaint in medical offices, in addition to the development of pelvic inflammatory disease [2]. The inflammatory process in the vaginal tract renders it difficult to properly assess the cytopathological exam intended to screen precedent lesions. The events of usual microbiota should not alter the diagnostic capacity of cytopathology.

Materials and Methods

A transversal and observational cohort study was performed. During the Joint-Action for Gynecological Disease Prevention offered to the female employees of Hospital São Paulo-Unifesp complex, 118 women were selected, with 59 of them in menacme and 59 in post-menopause phase. All participants signed the free and informed consent letter and the project was approved by the Ethics Research Board of Hospital São Paulo – UNIFESP.

Results: Bacterioscopy and culture proved to be better than the cytopathologic exam in featuring the bacilli and cocci. The bacterioscopy provided a better detection of the presence of bacilli (p < 0.001); no statistical difference was seen between both exams with respect to the detected cocci. The β-hemolytic Streptococcus group was of significance in post-menopausal women (p < 0.05).

Conclusion: In this study, the bacterioscopic and culture exams of the vaginal fluid were more effective in assessing the vaginal flora and in the detection of bacilli, compared to the cytopathological exam.

Key words: Cytology; Bacterioscopy; Culture; Vaginal content.
schedule with antibiotics for whatever infections processes, were excluded from the study.

The participants were submitted to a focused anamnestic interrogatory and a gynecological exam with vaginal speculum exam. With the aid of a long swab, a sample of vaginal fluid was collected and a smear was made on two glass slides, for a stained bacterioscopic exam (GRAM). The corresponding reports were supplied by members of the staff of microbiologists at the Central Laboratory of HSP. The reports featured the number of epithelial cells, leukocytes, and microorganisms in cocci, bacilli, yeasts, and the form of groups in each of them.

Another sample of vaginal contents was properly packed for transportation for the yeast organism culture and sent to the Central Laboratory of HSP for seeding in an appropriate culture means for positive aerobic pathogenic microorganisms, whether pathogenic or not. The culture means in use were: chocolate agar for positive gram growth, negative gram and growth, blood agar for positive gram and negative gram growth, and teague agar (eosin methylene blue) for negative gram growth. Biochemical tests such as the use of the EPM/MILI system and immunological tests were performed. The corresponding reports were supplied by a team of microbiologists from the Central laboratory of HSP.

The collection of material for the performance of cytopathological smear analysis was made with an endocervical canal, ectocervix, and vaginal sac material, using a canal brush and wood spatulas (Ayre), respectively. The material was deposited on glass slides, identified and fixed through fixing liquid vaporization (Colpofix). It was then sent to the Cytopathology Laboratory of the Gynecology Department, to be processed, stained by the modified method of Papanicolaou, and submitted for interpretation, by an experienced cytopathologist of the service.

For the comparison of three methods, the microorganisms identified were split into three groups: cocci, bacilli, and yeasts. The analyses were performed using the statistical package SPSS - Statistical Package for Social Sciences (v16.0). For the assessment of which of the three exams managed to identify the largest number of microorganisms, the average of organisms detected through each method was assessed, by subdividing the sample into patients according to menacme and post-menopause. For the comparison of qualitative data, the Kruskal-Wallis and the Mann-Whitney tests were used, for non-parametric data. The statistical significance value was established as 5%, or p < 0.05.

Results

The joint work gave priority to women aged 30 to 60 years; the average age of those in menacme was 41 years and 55 years for those in post-menopause.

The microorganisms identified in the cultures are shown in Table 1. The most frequently found bacteria comprised: Döderlein bacilli (66% in menacme and 50.8% in post-menopause), Staphylococcus coagulase-negative (40% of each hormonal situation), beta-hemolytic Streptococcus group B (7% of women in menacme and 27% in post-menopause), Enterococcus spp (10% of each hormonal situation), and Escherichia coli (8.5% in menacme and 12% in menopause). These microorganisms were then divided into two groups: cocci and bacilli. Some yeasts were also identified in the culture.

In the cytopathological report, microorganisms found were Döderlein bacilli, gram-positive bacilli, gram-negative bacilli, labile grams bacilli, gram-positive cocci, and yeasts. In addition, the number of epithelial cells, leukocytes and microorganisms were featured as rare, some, and numerous.

When the averages of agents identified in patients were analyzed through the different methods studied (through the Kruskal-Wallis method), a statistical difference was noticed among the three methods, according to the number of bacilli and cocci (p < 0.05). In order to identify which of the three groups would differ from each

<table>
<thead>
<tr>
<th>Yeasts</th>
<th>Menacme</th>
<th>Postmenopause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cocci</td>
<td>1,268.50</td>
<td>0.003</td>
</tr>
<tr>
<td>Total bacilli</td>
<td>1,495.50</td>
<td>0.13</td>
</tr>
<tr>
<td>Total cocci</td>
<td>1,495.50</td>
<td>0.13</td>
</tr>
<tr>
<td>Total bacilli</td>
<td>1,209.50</td>
<td>0.001</td>
</tr>
<tr>
<td>Beta-hemolytic Streptococcus group B</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Enterobacter</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Staphyloccus aureus</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Haemophilus spp</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Streptococcus Group D non-enterococcus</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>CESP</td>
<td>1.153.00</td>
<td>0.18</td>
</tr>
<tr>
<td>Morganella Morganii</td>
<td>1.385.50</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 1 — List of 118 women at menacme and post-menopause, according to microorganism.
other, a “two by two” analysis was performed, through the Mann-Whitney method and the respective values of p appear in Tables 2 (menacme) and 3 (post-menopause). The results of multiple comparisons for each variable were submitted to Bonferroni correction in order to eliminate possible bias concerning false positives, and the p value was then significant, when below 0.02.

The authors could affirm that there was a significant difference in the averages of bacilli in the “cytological vs bacterioscopy” and “bacterioscopy vs culture” methods (p > 0.001). When the averages of cocci were considered, a significant difference was noticed among the “cytological vs bacterioscopy” and “cytological vs culture” methods (p < 0.01). The averages of cocci can be considered equal between the “bacterioscopy vs culture” methods, since the value of p was p > 0.02.

For the detection of yeasts, no statistical difference was noticed between the number of microorganisms and the utilized method (p > 0.05).

Discussion
Bacterioscopy proved to be a worthy examination tool, since it informs the physician about the number of Döderlein bacilli of epithelial cells and leukocytes in respect to the other microorganisms, which facilitates to identify the flora as normal or abnormal, as shown by Espiegel [4]. Gram-staining was also the method that better identified the bacilli, in addition to being comparable to the culture for identification of cocci. This type of identification and the option of fresh exam are the best forms for the diagnosis of vaginitis caused by aerobics [5]. Nugent et al. [6] demonstrated that the diagnosis of vaginosis through bacterioscopy is liable to be reproduced among different diagnostic centers and microbiologists, with no great discrepancies. As shown in the reports, when the clinical diagnosis is doubtful, the culture can differentiate vaginosis from vaginitis caused by aerobic flora and can aid in the differentiation of infection by multiple microorganisms [5]. Considering that the analysis of aerobics is focused, the results in the culture have shown the presence of bacteria such as beta-hemolytic Streptococcus Group B in a larger group of women and isolated cases such as Citrobacter, Proteus sp which described as present in floras and deemed to be normal [7], can however be the cause of inflammatory processes. The same authors also described the Döderlein bacilli and coagulase-negative Staphylococcus, as it also occurred in the present study.

The significant difference between the floras in both hormonal situations was the identification of beta-hemolytic Streptococcus Group B in women at post-menopause (p < 0.05), which occurred in the Hillier and Lau [8] paper that also demonstrates that gram-negative anaerobics and gram positive cocci are the microorganisms most found in this hormonal phase. Dicaciatti et al. [9] demonstrated that the cytopathological exam can be very useful in the identification of bacterial vaginosis – a vaginal ecosystem unbalance. However, the fact that cytopathologists focus their attention on alterations of the epithelium decreases the sensitivity of the method to assess the vaginal flora. In this study, the cytopathological exam was less effective than bacterioscopy and culture in identifying the flora.

The reports demonstrates and discusses the complexity of the vaginal flora and its changes according to the hormonal stage of each woman, similarly to this study, with the three exam being discussed. In view of these facts, the different microorganisms causing inflammatory process and discharges cannot always be treated the same way. As already seen, the abnormal flora can lead to a difficult cytopathological assessment. The present authors can then conclude that the aids in bacterioscopy proved to better identify the bacilli. Culturing might help the physician to understand what is occurring in this complex flora, treating the patients in a more specific and individual form, and thus allowing a better screening of precedent lesions to be made.

Acknowledgement
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References

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Comparison of hysterosonography and hysteroscopy for diagnosing perimenopausal bleeding

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Clinic of Gynecology and Obstetrics “Narodni Front”, Belgrade (Serbia)

Summary
This investigation was a prospective study performed at the Gynecological Clinic “Narodni Front” in Belgrade. In the investigated group, endometrial hyperplasia, endometrial polyps, and uterine myoma were diagnosed as the most frequent causes of bleeding during perimenopause. The test group of patients was then subjected to hysterosonography in order to diagnose bleeding etiology, followed by hysteroscopy to confirm its etiology based on hysterosonography. Material was sent for histopathological analysis to definitely confirm the diagnosis. The aim of this study was to compare the findings of hysterosonography and hysteroscopy to evaluate the sensitivity and efficacy of hysterosonography for diagnosing the etiology of uterine bleeding in perimenopausal women. In the diagnostics of submucosal myoma, endometrial hyperplasia, and endometrial polyps, hysterosonography has proven to be a good screening method. By its use for diagnosing intracavitary uterine pathology, and thus also the pathology of bleeding in perimenopausal women, in many cases hysteroscopy can be avoided. This is a method that is easy to perform, less invasive, less costly to perform, and is well-tolerated.

Key words: Perimenopausis; Endometrial hyperplasia; Endometrial polyp; Uterine myoma; Hysteroscopy; Hysterosonography.

Introduction
The most common causes of bleeding during perimenopause are endometrial hyperplasia, endometrial polyps, and uterine myoma. In a retrospective study of endometrial curettage in patients with irregular endometrial hyperplasia, endometrial carcinoma was found in one percent of cases with simplex hyperplasia, in three percent of cases with complex hyperplasia, in eight percent of cases with atypical simplex hyperplasia, and in 29% of cases with atypical complex hyperplasia [1, 2]. Endometrial polyps are the most common cause of irregular bleeding outside the menstrual cycle. Myomas are very frequent benign tumors of the uterus. Approximately 30% of women have a fibroid tumor, and 20%-30% of myomas exhibit clinical symptoms [3]. Saline infusion sonohysterography (SIS) is a method enabling the diagnosing of certain pathological states in the uterine cavity and thus also to discover the causes for perimenopausal bleeding [4]. One of the benefits also relates to differential diagnostics between a focal lesion and generalized endometrial thickening, as well as a polyp and submucosal myoma. Hysteroscopy, as an endoscopic method for imaging the uterine cavity, simultaneously enables both the discovering and removal of the cause of perimenopausal bleeding [5-7].

Materials and Methods
This investigation was a prospective study performed at Gynecological Clinic “Narodni Front” in Belgrade. It comprised of 50 patients aged 37-50 years, suffering from perimenopausal bleeding. The majority of patients had a menstrual cycle, which was not abundant, and within a duration interval from a minimum of 15 days to a maximum of 55 days. Colposcopy, blood pressure (BP), complete blood cell count, ultrasonography investigation, hysterosonography, and hysteroscopy were performed in all patients. From January 1, 2010 to January 1, 2011, patients were checked by ultrasonography to evaluate perimenopausal bleeding. In the investigated group, endometrial hyperplasia, endometrial polyp, and myoma were diagnosed as the most frequent causes of perimenopausal bleeding. Check-ups were done using an ultrasonic apparatus with a five MHz transvaginal probe. In each patient, the uterine cavity was checked in the sagittal and the transversal planes. The test group of patients was then subjected to hysterosonography in order to diagnose bleeding etiology, followed by hysteroscopy to confirm the bleeding etiology based on hysterosonography. Hysterosonography was done in the outpatient department, with a previously checked cervical smear, colposcopy finding, and BP. Under speculum control, a pediatric intraumbilical catheter was placed transcervically in the uterine cavity, and the medium – physiological saline was injected, which separated the edges of the cavity and demonstrated its content [8, 9]. Then, using an ultrasonography apparatus with a five MHz transvaginal probe, the uterine cavity was checked in the sagittal and the transversal planes. The hysteroscopic examination was done using a rigid eight-mm diameter hystroscope, with previous cervical dilatation to Haegar no. 8. The uterine cavity was distended using physiological solution. Instruments for performing excision, biopsy, or electrocoagulation were inserted via the hystroscope. Material was sent for histopathologic analysis to definitely confirm the diagnosis. Anesthesia was mainly intravenous or endotracheal. Obtained data were processed using descriptive statistics methods (SV, SD, MIN, and MAX) and analytical statistics methods (Student T test, Chi-squared test, Wilcoxon test, Spearman, and Pearson correlations). The data base was created on a PC, using the SPSS 10.0 statistical package. The aim of this study was to compare hysterosonography and hysteroscopy findings in order to evaluate the sensitivity and efficacy of hysterosonography for diagnosing the etiology of uterine bleeding in perimenopausal women.
Comparison of hysterosonography and hysteroscopy for diagnosing perimenopausal bleeding

Results

The authors studied 50 patients suffering from perimenopausal bleeding. Their average age was $47 \pm 3.6$ years. Patients aged 40-49 years were the most common with $73.4\%$, followed by patients aged 50-54 years with $16.3\%$, while patients aged over 55 years were less common with $8.2\%$, and patients aged 35-39 years were the least common with $2.1\%$. Statistical analysis of obtained data showed a statistically highly significant difference ($\chi^2 = 25.388; p < 0.01$) in patients aged 40-49 years, compared to patients aged 35-39 years. Before hysterosonography and hysteroscopy, an ultrasound examination was done in all patients. A regular finding was diagnosed in twentypatients, endometrial hyperplasia in $46.9\%$, endometrial polyp in $40.9\%$, and myoma in $10.2\%$ of patients. Presented data showed a higher incidence of endometrial hyperplasia compared to submucosal myoma. Hysterosonography most frequently diagnosed endometrial polyp, in $55.1\%$ of patients, followed by endometrial hyperplasia in $30.6\%$, and submucosal myoma in $14.3\%$ of patients. Presented data shows that endometrial polyps are more frequent than submucosal myomas, with a statistically significant difference ($\chi^2 = 12.408; p < 0.01$). Hysteroscopy diagnosed endometrial polyps in $53.1\%$ of patients, endometrial hyperplasia in $32.6\%$ of patients, and submucous myomas in $14.3\%$ of patients. Presented data indicate a more frequent presence of endometrial polyps compared to submucosal myomas. The established difference was statistically significant ($\chi^2 = 11.061; p < 0.01$). Material obtained by biopsy during hysteroscopy was processed by histopathology. Histopathologically, endometrial polyps were found in $53.1\%$ of patients, endometrial hyperplasia in $32.6\%$ of patients, while submucosal myomas were present in $14.3\%$ of patients. Presented data indicated a more frequent presence of endometrial polyps compared to submucosal myomas. These results are presented in Table 1. Hysterosonographic findings show a high percentage of agreement with histopathological findings, in $98\%$ of cases, differing in only two percent of cases. The lack of agreement appeared in histopathological diagnosis of hyperplasia, where in two percent of patients hysterosonography showed endometrial polyp. Presented results show no statistically significant difference ($z \approx -1.000; p > 0.05$) between hysterosonographic and histopathological findings. These results are presented in Table 2. Hysteroscopic findings show an agreement with histopathological findings in $100\%$ of cases. Hysterosonographic findings show an agreement with hysteroscopic findings in $98\%$ of cases, with a difference in only two percent of cases. Lack of agreement was found in the hysteroscopic diagnosis of hyperplasia, where in two percent of patients, hysterosonography found an endometrial polyp. Presented results show no statistically significant difference ($z \approx -1.000; p > 0.05$) between hysterosonographic and hysteroscopic findings. These results are presented in Table 3. Hysteroscopy had the highest level of sensitivity of $100\%$. The SIS method also demonstrated a satisfactory sensitivity of $98\%$. Specificity of hysteroscopy as a diagnostic method was $100\%$, and for SIS was $97\%$. Statistical data processing established no statistically significant difference for specificity between hysteroscopy and hysterosonography. Hysteroscopy had the highest positive predictive value of $100\%$, while for SIS this was $96\%$. These results are presented in Table 4. For hysteroscopy, there were no false positive findings, while SIS had four percent false positive findings. Obtained data showed no statistically significant difference between hysteroscopy and hysterosonography. Hysteroscopy had no false negative findings, while SIS had three percent false negative results. Obtained data show no statistically significant difference between hysteroscopy and hysterosonography.

Discussion

This prospective study included 50 patients suffering from perimenopausal bleeding, and in order to diagnose the bleeding, after detailed laboratory and colposcopic preparation, they were checked by ultrasonography, hysterosonography, and hysteroscopy. Patients were between 37 and 50 years of age, with a mean age of $47$. The most common population were women aged between 40 and 49 years. The duration of the menstrual cycle was from a minimum of 15 days to a maximum of 55 days. The largest number of patients (as high as $61.2\%$) had regular cycles lasting 21-35 days. Most cases, approximately two-third of patients, did not have abundant menstruations, therefore a high number of patients had a normal blood count, with anemia present in only a small percentage. All patients were processed by colposcopy and BP, with a high percentage of regular findings, and pathological findings in only $4.1\%$ of cases. To contribute to resolving the dilemma regarding the use of various diagnostic techniques and to compare results, percentages for sensitivity, specificity, and positive and negative predictive values, were established for the applied diagnostic techniques. For all patients an ultrasonographic examination was performed, and in a high percent of cases, endometrial hyperplasia was found ($46.9\%$), followed by endometrial polyp ($40.9\%$), submucosal myoma ($0.2\%$), with a regular finding in only two percent of patients. In one study, 84 patients with abnormal bleeding were subjected to transvaginal ultrasound (TVUS), followed by hysteroscopy. Results were compared with results of biopsy, surgical hysteroscopy, and hysterectomy. Based on results obtained for sensitivity, specificity, positive and negative predictive values, hysteroscopy had higher sensitivity (TVUS $67.3\%$, hysterosonography $91.6\%$). It was concluded that TVUS is an excellent initial diagnostic method, but HS is a much more precise diagnostic method for evaluating abnormal bleeding in perimenopause. [10]. Subsequently, all patients were subjected to a sonohysteroscopic examination. This method diagnosed no regular findings, while the most common finding was endometrial polyp in approximately one-half of cases ($55.1\%$). In almost one-third of patients ($30.6\%$),
endometrial hyperplasia was found, while the rarest finding was myoma, in only 14.3% of cases. Other authors performed a comparative prospective study for transvaginal color Doppler (TVCD) and hysterosonography for diagnosing endometrial polyp. It included 51 patients between 27 and 75 years of age, mean age 51. Then all patients were subjected to hysteroscopy and endometrial biopsy, and the histopathologic finding was used as the gold standard. Forty-one endometrial polyps, three endometrial hyperplasias, four atrophic cystic hyperplasias, two proliferative endometriums, and one case of endometritis, were confirmed. Based on sensitivity and specificity for TVCD (95% and 80%) and hysterosonography (100% and 80%), it was concluded that TVCD and hysterosonography had similar performances in diagnosing endometrial polyps [11].

In this study, after sonohysteroscopy, hysteroscopic examination was also performed for this group of patients, and in most cases endometrial polyps (53.1%) were diagnosed, followed by endometrial hyperplasia (32.6%), with myomas in only 14.3% of cases. In a randomized study of 148 patients, subjected to hysteroscopy, pathological lesions were not diagnosed in 37 patients, in five patients both a polyp and a myoma were found, in 43 patients endometrial polyps, and in 20 submucosal myomas were diagnosed. The authors concluded that the pathology of abnormal bleeding is dominated by endometrial polyps and submucosal myomas, and that in some cases only hysteroscopy was the method of choice to diagnose the pathology of uterine bleeding [12]. All material obtained by biopsy during hysteroscopy diagnosis was sent for histopathological analysis. In the present investigation, histopathological findings confirmed the hysteroscopic diagnosis in 100% of cases, i.e. endometrial polyps were confirmed in 53.1% of patients, followed by endometrial hyperplasia in 32.6% of cases, and the least frequent, myomas in 14.3%.

Hysteroscopy proved to be also a good method of choice in diagnosing and typization of endometrial pathology in abnormal perimenopausal bleeding. As for ultrasonographic findings, when compared to the histopathological findings, they demonstrated an agreement in 79.6% of cases, with findings differing in 20.4% of cases. The highest percentage of disagreement was for histopathologically-diagnosed polyps, which were declared as regular findings by ultrasonography in two percent of cases, and as endometrial hyperplasia in 14.3% of cases. There was also a somewhat lower agreement in diagnosing myomas, which was seen as an endometrial polyp in 4.1% of patients. In a high percentage, in 98% of cases, hysterosonographic diagnostics agreed with histopathological findings. The highest disagreement was for histopathological diagnostics of endometrial hyperplasia, where in two percent of patients an endometrial polyp was seen. In one study in menopausal patients (419) with uterine bleeding, diagnostics of endometrial pathology were compared using TVUS and hysteroscopically with the hystopathology findings of the endometrium used as the basic standard. Based on obtained results, it was established that TVUS is the method of choice for investigating the endometrium limited to a thickness of four-mm (double layer technique) in postmenopausal patients with irregular bleeding, while hysteroscopy is a much more precise method than TVUS for all patients, especially for endometrium with a thickness of over four-mm. [13]. In the present investigation, hysterosonographic diagnostics, in 98% of cases agreed with histopathological findings. A study which performed a comparison between SIS and hysteroscopy diagnosis was sent for hystopathological findings. In the present study, the least agreement between hysterosonography and hysteroscopy was found for histopathological diagnosis of endometrial hyperplasia, where in two percent of patients an endometrial polyp was seen. One prospective study performed a comparison between TVUS, SIS, and hysterosonography as diagnostic methods in premenopausal patients with abnormal uterine bleeding. TVUS demonstrated a sensitivity of 60% in direct visualization of intracavitary abnormalities, with a specificity of 93%.

### Table 1. Compatibility of the hysterosonographic and histopathological findings in the study group of patients.

<table>
<thead>
<tr>
<th>Hysterosonography</th>
<th>Histopathological finding</th>
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<tbody>
<tr>
<td></td>
<td>Polyp</td>
</tr>
<tr>
<td>-------------------</td>
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</tr>
<tr>
<td>Polyp</td>
<td>53.1</td>
</tr>
<tr>
<td>Myoma</td>
<td>0</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>53.1</td>
</tr>
</tbody>
</table>

### Table 2. Compatibility of the hysteroscopic and histopathological findings in the study group of patients.

<table>
<thead>
<tr>
<th>Hysteroscopy</th>
<th>Histopathological finding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Polyp</td>
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<tr>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>Polyp</td>
<td>53.1</td>
</tr>
<tr>
<td>Myoma</td>
<td>0</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>53.1</td>
</tr>
</tbody>
</table>

### Table 3. Compatibility of the hysterosonographic and hysteroscopic findings in the study group of patients.

<table>
<thead>
<tr>
<th>Hysterosonography</th>
<th>Hysteroscopy</th>
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<tbody>
<tr>
<td></td>
<td>Polyp</td>
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</tr>
<tr>
<td>Polyp</td>
<td>53.1</td>
</tr>
<tr>
<td>Myoma</td>
<td>0</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>53.1</td>
</tr>
</tbody>
</table>

### Table 4. Sensitivity and specificity of implemented diagnostic methods.

<table>
<thead>
<tr>
<th></th>
<th>Hysterosonography</th>
<th>Hysteroscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>97%</td>
<td>100%</td>
</tr>
<tr>
<td>Positive predictive values</td>
<td>96%</td>
<td>100%</td>
</tr>
<tr>
<td>False positive values</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>False negative values</td>
<td>3%</td>
<td>0%</td>
</tr>
</tbody>
</table>

D. Dimitrijevic, M. Vasiljevic, R. Anicic, S. Brankovic

Histopathology findings of the endometrium were used as the basic standard. Based on obtained results, it was established that TVUS is the method of choice for investigating the endometrium limited to a thickness of four-mm (double layer technique) in postmenopausal patients with irregular bleeding, while hysteroscopy is a much more precise method than TVUS for all patients, especially for endometrium with a thickness of over four-mm. [13]. In the present investigation, hysterosonographic diagnostics, in 98% of cases agreed with histopathological findings. A study which performed a comparison between SIS and hysteroscopy diagnosis was sent for hystopathological findings. In the present study, the least agreement between hysterosonography and hysteroscopy was found for histopathological diagnosis of endometrial hyperplasia, where in two percent of patients an endometrial polyp was seen. One prospective study performed a comparison between TVUS, SIS, and hysterosonography as diagnostic methods in premenopausal patients with abnormal uterine bleeding. TVUS demonstrated a sensitivity of 60% in direct visualization of intracavitary abnormalities, with a specificity of 93%.
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...tivity was 88% and specificity 95%. Authors concluded that SIS is a more accurate method than TVUS for diagnosing intracavitary lesions in premenopausal patients. The use of TVUS and SIS to diagnose intracavitary lesions in an endometrium thicker than five mm can in most cases reduce the number of hysteroscopies [15]. In the present study, in the selected sample, hysteroscopic findings demonstrated the highest percent of agreement in 100% of cases, with hystopathological findings.

Conclusion

In the diagnostics of intracavitary uterine pathology in women with perimenopausal bleeding, hysteroscopy appears to be the best diagnostic method, followed by hysterosonography. In the diagnostics of submucosal myomas, endometrial hyperplasia, and endometrial polyps, hysterosonography has proven to be a good screening method. Through its use for diagnosing intracavitary uterine pathology, and thus also the pathology of bleeding in premenopausal women, in many cases hysteroscopy can be avoided. This is a method that is simple, less invasive, less costly, and is well-tolerated.

References

Diagnosis value of hysteroscopy for chronic endometritis

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²Department of Obstetrics and Gynecology, Taihe Hospital Affiliated Hubei Medical College Hospital, Shiyan (China)

Summary

Objective: To investigate the correlation of hysteroscopy in the diagnosis of chronic endometritis (CE) with histology and assess its reliability. Materials and Methods: Two hundred eleven patients with CE diagnosed by hysteroscopy were selected as the case group, and 30 cases without endometritis diagnosed by hysteroscopy were selected as the control group. Hysteroscopy and endometrial biopsy were carried out in all patients with endometrial hyperplasia. Results: Among 211 patients with CE diagnosed by hysteroscopy, 200 cases were confirmed histologically. There was a significant correlation (p < 0.001) between characterization of CE by hysteroscopy and pathological grading. In 173 cases (86.5%), both histological and hysteroscopic grading were consistent (Kappa value = 0.62). Conclusion: Hysteroscopy is reliable in diagnosing CE and it can assess clinical effectiveness of antibiotic therapy.

Key words: Chronic endometritis; Hysteroscopy; Endometrial biopsy.

Introduction

Chronic endometritis (CE) influences implantation of fertilized eggs, and is one of the important causes of recurrent spontaneous abortion and infertility [1-3]. As these kind of patients often have no symptoms or are only accompanied with a slight pelvic pain, abnormal leucorrhea, irregular vaginal bleeding, etc [4], pelvic examination and ultrasonography have no specific advantages, and endometrial biopsy will cause missed diagnosis, hence clinical diagnosis is a challenge.

Clinically, hysteroscopy is usually used to diagnose CE [1]. CE by hysteroscopy manifests endometrial diffuse hyperemia [3]. Its characteristics lie in the white spots that are at the center of red zone, and exists locally in the uterine cavity or may be dispersed in the entire uterine cavity, with typical “strawberry” characteristics [5, 6]. However, there are many factors influencing endometrial hyperemia. Therefore, the reliability of diagnosing CE by hysteroscopy is always contestable [7, 8]. This study analyzes and compares endometritis diagnosed by hysteroscopy in order to assess whether patients with severe CE is related to histology, and whether it is feasible to assess its severity by hysteroscopy. The final purpose is to assess the reliability of hysteroscopy in diagnosing CE, its evaluation, and its repeated detection.

Materials and Methods

Cases

Among the patients receiving hysteroscopy in the Gynaecology Department of Affiliated Taihe Hospital of Hubei Medical College from January 2007 to January 2010, 211 patients with CE diagnosed by hysteroscopy were selected as the study group, and vaginal bleeding, severe cardiovascular disease, endometrial lesions, and pregnancy related diseases were excluded. In addition, 30 patients without endometritis diagnosed by hysteroscopy were selected as the control group. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Taihe hospital Affiliated Hubei Medical College Hospital. Written informed consent was obtained from all participants.

Diagnosis criteria

Main manifestations of CE confirmed by hysteroscopy include [9]: (a) endometrial focal or diffuse hyperemia; (b) endometrial thickening, edema; (c) micro polyp existence; and (d) endometrial polyps complicated with interstitial edema or focal or diffuse periglandular hyperemia.

Endometritis severity grading criteria

Grade 0 (no inflammation); grade I (mild inflammation): focal or diffuse periglandular hyperemia with/without increase of mucosal thickness, no micro polyp; grade II (medium/severe inflammation): diffuse apparent hyperaemia complicated with endometrial thickening, micro polyps or endometrial polyps [10].

Endometrial histology inflammation grading criteria

Grade 0 (no inflammation); grade I (mild inflammation): edema and plasma cell infiltration; grade II (medium/severe inflammation): severe distortion and hyperplasia of inflammatory infiltration gland body [11-13].

Methods

Hysteroscopy was carried out in patients with endometrial hyperplasia (menstrual cycle of six to 12 days). A hysteroscopic lens with outer diameter of 2.7 mm and diagnostic sheath with outer diameter of 3.5 mm was utilized. Firstly, five percent mannitol was used to expand the uterus under pressure of 100 mmHg at a flow rate of 260 ml/min. During the hysteroscopic process, anterior and posterior walls of uterus were completely examined including the endometrial surface in order to confirm any irregular lesion on the latter. After hysteroscopy, endometrial biopsy was carried out in all patients. Histological examination adopted a double-blind principle. Moreover, all operations were conducted under non-narcotic non-anesthetic...
conditions. After histological examination, the patients were administered antibiotics for preventing infection.

Data analysis

The hysteroscopic and histologic findings in case of CE were compared using Fisher test. If \( p < 0.05 \), there was a significant difference. The consistence of the two methods in grading was calculated (Kappa index).

Result

Out of 211 patients with CE diagnosed by hysteroscopy, 200 cases were confirmed histologically (Table 1).

Based on the above criteria, 51 cases were regarded as patients with mild manifestations in histology (grade I), and 149 cases were diagnosed as patients with medium/severe inflammation (grade II). In the control group, only 2/30 (6.7%) cases presented significant histological inflammation \((p < 0.001)\). Between the characterization of CE by hysteroscopy and pathological grading, there was a significant correlation (Table 2).

In 173 patients (86.5%) both histology and hysteroscopy grading were consistent (Kappa value = 0.62).

Discussion

The results of this study showed that between different characterizations of endometritis by hysteroscopy and severity of histological inflammation, there was a good correlation, namely hysteroscopy and histology were consistent in evaluating the extent of endometritis. Especially, existence of endometrial polyps is related to endometritis severity, mucosal injury severity, and abnormal endometrial proliferative stimulation, and it is a reliable characterization of inflammation existence. Also, this characterization has a high predictive value [4].

Endometrial micro polyp is a new endometrial proliferation in histology, composed of organisms in small vessels coved by endometrium. Characteristics of micro polyps matrix include inflammatory cells (lymphocytes, plasma cells or eosinophilic granulocytes) and interstitial cells of normal gland body that gather around small blood vessels and glandular structures [14, 15]. In addition, as the percent of lymphocytes in endometrial cells is abnormal, leukocyte infiltration in CE not only presents quantitative changes, but also qualitative changes [14, 15].

Although endometrial polyp volume is very small, these subtle lesions in hysteroscopy are very easily detected. In the second examination, Kappa value of hysteroscopy and histology was 0.62. If the control group is included, Kappa value was 0.74. Kappa value of the two shows that the two techniques have a good consistency. Hysteroscopy and pathologic diagnosis of grade II inflammation had a better consistency than hysteroscopy and pathologic diagnosis of grade I (Table 3). Also, inconsistent ratios of detection results were respectively, 6.7% and 33%. Possible explanation for this is that endometrial biopsy uses blind curettage, and micro polyps are possibly missed due to the damage caused by treatment. Therefore, pathological examination is likely to underestimate inflammation severity. Hysteroscopy aims to conduct a comprehensive assessment under direct view and it can compensate for the inadequacy of pathological examination [6–9, 15].

In conclusion, not only is hysteroscopy reliable for diagnosis of CE, but can also reflect the severity. Therefore, hysteroscopy not only assesses inflammatory severity, but also evaluates the effectiveness of antibiotic therapy.

References


Table 1. — Indications in 211 women who were diagnosed with CE by fluid hysteroscopy and histology.

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>Hysteroscopic check and diagnosis</th>
<th>Histologic diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal uterine bleeding</td>
<td>74 (35%)</td>
<td>67 (33.5%)</td>
</tr>
<tr>
<td>Infertility</td>
<td>61 (29%)</td>
<td>64 (32%)</td>
</tr>
<tr>
<td>Endometrial polyp</td>
<td>39 (18.4%)</td>
<td>33 (16.5%)</td>
</tr>
<tr>
<td>Cervical polyp</td>
<td>15 (7.1%)</td>
<td>15 (7.5%)</td>
</tr>
<tr>
<td>Submucous myoma</td>
<td>15 (7.1%)</td>
<td>14 (7%)</td>
</tr>
<tr>
<td>Uterine malformation</td>
<td>7 (3.3%)</td>
<td>7 (3.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>211</td>
<td>200</td>
</tr>
</tbody>
</table>

Table 2. — Correlation between each sign of CE by fluid hysteroscopy and histological grading.

<table>
<thead>
<tr>
<th>Hysteroscopy</th>
<th>Grade I</th>
<th>Grade II</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestion lesions</td>
<td>45</td>
<td>11</td>
<td>0.001</td>
</tr>
<tr>
<td>Diffuse congestion</td>
<td>6</td>
<td>138</td>
<td>0.001</td>
</tr>
<tr>
<td>Interstitial edema</td>
<td>49</td>
<td>149</td>
<td>n.s.</td>
</tr>
<tr>
<td>Small polyps</td>
<td>28</td>
<td>9</td>
<td>0.001</td>
</tr>
<tr>
<td>Diffuse micropolyps/polyps</td>
<td>0</td>
<td>140</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 3. — Correspondence between hysteroscopic and histological grading (Kappa index = 0.62).

<table>
<thead>
<tr>
<th>Histological grade</th>
<th>Hysteroscopic grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>34</td>
</tr>
<tr>
<td>Grade II</td>
<td>10</td>
</tr>
</tbody>
</table>


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The prevalence of phenotypic subgroups in Greek women with polycystic ovarian syndrome


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Summary

Background: Since 2003, when the American Society for Reproductive Medicine (ASRM) and European Society of Human Reproduction and Embryology (ESHRE) sponsored consensus established criteria for polycystic ovarian syndrome (PCOS) diagnosis, the phenotypic spectrum of the syndrome has been significantly broadened. Purpose of the study: This survey makes an effort to distinguish PCOS according to phenotypic expression and to estimate its prevalence in a Greek population. Materials and Methods: Greek women from 18 to 35 years of age, who visited the outpatient department, claiming either irregular menstruation (oligo- or anovulation, OA) or clinical manifestations of hyperandrogenemia (HA) were recruited. They gave full disease history and underwent clinical examination, including transvaginal ultrasound (TVUS) scan to identify PCO morphology. Blood samples were collected to perform hormonal and metabolic analyses. Acute or chronic disorders were excluded. Finally, 266 PCOS women constituted the study population. Conclusions: The full-blown phenotype (HA+OA+PCO) is the predominant phenotype in this Greek population.

Key words: Polycystic ovary syndrome; Phenotype; Prevalence.

Introduction

Polycystic ovary syndrome or PCOS, is the most common endocrine disorder among women of reproductive age. Criteria for the diagnosis of the particular disorder have been proposed by two different organizations. The initial criteria developed in 1990 by the National Institutes of Health (NIH) conference [1] included 1) oligo- or anovulation (OA) to the exclusion of other disorders and 2) clinical and/or biochemical signs of hyperandrogenemia (HA). Another set of diagnostic criteria were proposed by the American Society for Reproductive Medicine and European Society of Human Reproduction and Embryology (ASRM/ESHRE) sponsored consensus [2] held in 2003 in Rotterdam according to which PCOS is diagnosed when two out of the following three characteristics are present: 1) OA, 2) clinical and/or biochemical signs of HA, and 3) polycystic ovaries as evidenced on ultrasound examination.

When the aforementioned second group of criteria is applied, it yields the following subgroups [3]: A) OA+HA+PCO, that is, the full-blown PCOS phenotype, B) OA+HA, with normal ovarian morphology, C) HA+PCO, with regular ovulation and menstruation, also called “ovulatory PCOS”, and D) OA+PCO, without hyperandrogenemia.

The aim of this study was to estimate the prevalence of the aforementioned phenotypes in a large sample of Greek reproductive women.

Materials and Methods

The present study was conducted from September 2005 to September 2009 in the Third Department of Obstetrics and Gynecology of Attikon University Hospital. The study protocol was in accordance with both Greek and European Union Legislations and was approved by the Hospital Ethics Committee. All patients gave informed consent.

Patients

All subjects were recruited from the Gynecological Endocrinology Ambulatory Clinic. The sample consisted of Greek Caucasian women (age range: 18 to 35 years), complaining of irregular menstruation or clinical signs of HA. Patients with positive pregnancy test, personal history of acute or chronic disease, and following treatment with compounds affecting sex hormones (oral contraceptives) within the past six months, were excluded from the study.

Study design

Disease history: A detailed questionnaire addressing subjects’ menstrual cycle characteristics (age of first menstrual cycle, frequency of menstruation, qualitative, and quantitative characteristics of menses) was completed by all study participants. Chronic anovulation was defined in the questionnaire as having fewer than eight menstrual cycles per year.

Lifestyle variables among others considered in the present study included: alcohol and tobacco use, extensive physical exercise, and use of hormonal treatment. Also recorded were participants’ family history of diabetes mellitus, hypertension or cardiovascular disease, and the presence of any first-degree relatives exhibiting irregular menses.

Clinical examination

Each woman in the present study underwent a physical examination conducted by two gynecologists with experience in reproductive disorders. Data were collected concerning women’s waist and hip circumference, body weight, height, and blood pressure, while body mass index (BMI-weight in kgs divided by the square of height in m²) and waist-to-hip ratio were also calculated. The amount of excess terminal hair growth was assessed by using the Ferriman-Gallwey scale (FG), based on whole body overview, with patients scoring 8 or higher considered as hirsute [4]. Finally, the presence of acne vulgaris, androgenetic alopecia or acanthosis nigricans, and a cutaneous sign of hyperinsulinemia, were also recorded, however with no particular scoring technique applied.

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Transvaginal ultrasound (TVUS) scans

Three-dimensional ovarian morphology and size was recorded on the sixth to eighth days of patients’ menstrual cycle by using the same operator [5]. The presence of ≥ 12 follicles with a diameter of two to nine mm or increased ovarian volume (> 10 cm³) established a sonographic diagnosis of PCO.

Table 1. — Prevalence of PCOS phenotypes in different study populations.

<table>
<thead>
<tr>
<th>Nationality</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsu et al. (2007) [6]</td>
<td>Taiwan</td>
<td>51.8</td>
<td>8.8</td>
<td>21.2</td>
</tr>
<tr>
<td>Barber et al. (2007) [7]</td>
<td>U.K.</td>
<td>61.8</td>
<td>0</td>
<td>24.6</td>
</tr>
<tr>
<td>Dewailly et al. (2006) [8]</td>
<td>France</td>
<td>60.6</td>
<td>6.7</td>
<td>16.5</td>
</tr>
<tr>
<td>Pehlivanov et al. (2007) [9]</td>
<td>Bulgaria</td>
<td>58.6</td>
<td>11.4</td>
<td>20</td>
</tr>
<tr>
<td>Chae et al. (2008) [10]</td>
<td>Korea</td>
<td>52.4</td>
<td>13.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Diamanti-Kandarakis et al. (2007) [11]</td>
<td>Greece</td>
<td>45.5</td>
<td>40.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Belosi et al. (2006) [13]</td>
<td>Italy</td>
<td>73.6</td>
<td>7.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Welt et al. (2006) [14]</td>
<td>U.S.A-Iceland</td>
<td>71.3</td>
<td>1.7</td>
<td>18.4</td>
</tr>
<tr>
<td>The present study</td>
<td>Greece</td>
<td>44.4</td>
<td>18</td>
<td>26.3</td>
</tr>
</tbody>
</table>

A: HA+OA+PCO; B: HA+OA; C: HA+PCO; D: OA+PCO; HA: Hyperandrogenemia; OA: Oligo-anovulation, PCO: polycystic ovarian morphology (by U/S).

Table 2. — Age, BMI, and BMI categories of PCOS subgroups.

<table>
<thead>
<tr>
<th>Phenotype A</th>
<th>Phenotype B</th>
<th>Phenotype C</th>
<th>Phenotype D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE, mean ± SD, median</td>
<td>25 ± 6</td>
<td>24 (21 - 29)</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>BMI, mean ± SD, median</td>
<td>27.1 ± 7.7</td>
<td>24 (21.1 - 32.7)</td>
<td>25.5 ± 6.9</td>
</tr>
<tr>
<td>BMI, categories</td>
<td>Normal</td>
<td>62</td>
<td>52.5</td>
</tr>
<tr>
<td></td>
<td>Overweight</td>
<td>18</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>Obese</td>
<td>38</td>
<td>32.2</td>
</tr>
</tbody>
</table>

Table 3. — Hormonal profile of PCOS phenotypes.

<table>
<thead>
<tr>
<th>Phenytopes A</th>
<th>Phenotype B</th>
<th>Phenotype C</th>
<th>Phenotype D</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mU/ml)</td>
<td>5.7 ± 1.9</td>
<td>5.4</td>
<td>6 ± 2.8</td>
</tr>
<tr>
<td>LH (mU/ml)</td>
<td>(4.2 - 6.7)</td>
<td>5.8</td>
<td>6.5 ± 3</td>
</tr>
<tr>
<td>PRL</td>
<td>(3.9 - 8.1)</td>
<td>14.9</td>
<td>15.9 ± 12.1</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>(10.3 - 21.3)</td>
<td>18</td>
<td>17.5 ± 18.4</td>
</tr>
<tr>
<td>Total testosterone (ng/dl)</td>
<td>54.3 ± 64.9</td>
<td>39.9</td>
<td>45.8 ± 29.5</td>
</tr>
<tr>
<td>Free testosterone (pg/ml)</td>
<td>(31 - 51)</td>
<td>60</td>
<td>62.7 ± 23.6</td>
</tr>
<tr>
<td>OHP17 (ng/ml)</td>
<td>(41 - 75)</td>
<td>12</td>
<td>1.1</td>
</tr>
<tr>
<td>DHEA-S (µg/dl)</td>
<td>(1.5 - 2.8)</td>
<td>1.1</td>
<td>1.3 ± 0.8</td>
</tr>
<tr>
<td>Δ4 Androstenedione (nmol/l)</td>
<td>(0.8 - 1.5)</td>
<td>2</td>
<td>2 ± 0.9</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>214.5</td>
<td>224.3</td>
<td>122.6</td>
</tr>
<tr>
<td>Cortisole (mg/dl)</td>
<td>183.7</td>
<td>156</td>
<td>18.4 ± 6.2</td>
</tr>
<tr>
<td>T3 (nmol/l)</td>
<td>(13 - 22.1)</td>
<td>17</td>
<td>18.4 ± 6.2</td>
</tr>
<tr>
<td>T4 (µg/dl)</td>
<td>(7 - 8.8)</td>
<td>7.8</td>
<td>7.6 ± 1.8</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>(1.6 - 2.6)</td>
<td>7.7</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Biochemical measurements

Venous blood samples were drawn from subjects early in the morning following an overnight fast between the third and sixth day after the onset of a spontaneous or progesterone-induced menstruation.

Blood samples were centrifuged, and serum was drawn off and frozen at -70°C until analyzed. In order to rule out abnormal thyroid function, hyperprolactinemia and Cushings syndrome, thyroid tests (FT3/FT4/TSH) were performed and levels of prolactin (PRL) and cortisone were estimated, respectively, as part of the differential diagnosis work-up. The 17-α-OH-progesterone (17-OHP) was also measured. For women who exhibited plasma levels higher than 1.5 ng/ml, a Synacthen test using tetracosactide was conducted (Novartis Pharma S.A.), enabling to exclude patients with congenital adrenal hyperplasia from the study.

Ultimately, participants enrolled in the study were 266 women.

The parameters below were also assessed in the sample, providing thus a basis for conducting comparisons between different phenotypic groups:

- follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2);
- total testosterone, free testosterone, Δ4-androstenedione (Δ4-A), and dehydroepiandrosterone-sulfate (DHEA-S);
- sex-hormone binding globulin (SHBG).

HA was defined as serum total testosterone or Δ4-androstenedione level higher than two nmol/l and 10.5 nmol/l, respectively.

Results

The final sample consisted of 266 women with a mean age of 25 years (± 5.6 years). The prevalence of the four different subgroups is shown in Table 1.

The predominant phenotype in this sample was the full-blown phenotype A with a prevalence estimated at 44.4%. Phenotype B represented 18% of the sample, while phenotype C at 26.3%. Phenotype D was the most rare phenotype in comparison with the three others, having a prevalence of 11.3%.

BMI rates differed significantly among the four subgroups. More specifically, the use of Bonferroni adjustment for the level of statistical significance revealed a statistically significant higher BMI for women of phenotype A in comparison with those with phenotype D (p = 0.009). In addition, women of normal weight were significantly more in phenotype C compared with those of phenotype A (p = 0.007) (Table 2).

Discussion

The prevalence of the different PCOS phenotypes vary according to the different study populations as shown in Table 1 [6-14]. Since these studies have involved women of different ethnicities, ethnic background may also be considered as an important confounding factor.

According to the present results, 44.4% of Greek PCOS women belong to phenotype A, 18% to phenotype B, 26.3% to phenotype C, and 11.3% to phenotype D. With specific reference to the latter phenotype, there is varying evidence in the relevant literature as to whether and to what extent can a woman without HA be diagnosed with PCOS? Other studies have illustrated that women categorized in this group share the same clinical and metabolic characteristics as those of the general population and do not require any treatment in order to modify their hormonal profile [8, 15-18].

This fact has led the Androgen Excess Society (AES) to the proposal of different criteria for the diagnosis of PCOS, according to which the diagnosis of the syndrome is set when a woman fulfills the following two diagnostic criteria: 1) HA, clinical or biochemical and 2) OA as shown by menstrual disorders or polycystic ovarian morphology. Although AES includes PCO as a characteristic of the syndrome, it is not considered as an autonomous criterion [19]. The application of AES criteria leads to the formation of three different subgroups, that is, the same with those of Rotterdam criteria with the exception of phenotype D.

There is a difference in the present results concerning prevalence compared to those of another study of a Greek population conducted by Diamanti-Kandarakis et al. [11]. This deviation may be attributed to differences in the study protocol. For the definition of biochemical HA, the authors employed not only the total testosterone levels above the 95th percentile of levels detected in normally-menstruating women, as the aforementioned study did, but also Δ4-androstenedione values greater than 10.5 nmol/l [8]. The latter fact highlights the need for adopting commonly-accepted definitions and standards, primarily for HA, hirsutism, and anovulation.

The sub-classification of PCOS into different subgroups seems clinically significant. According to the literature, not all phenotypes share the same metabolic and hormonal profile (Table 3), indicating that not all PCOS patients need the same therapy or intervention. Clinicians should be aware of the phenotypic expression of the syndrome in order to treat a patient individually and also recognize possible future cardiometabolic risks.

References


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Does Kruger’s strict criteria have prognostic value in predicting ICSI clinical results?

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2Zeynep Kamil Teaching and Researching Hospital, Department of Reproductive Endocrinology and Infertility, Istanbul
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Summary

Objective: The purpose of this study was to compare clinical results of ICSI for different sperm morphology subgroups divided according to Kruger’s classification system. Materials and Methods: This retrospectively study was conducted at Zeynep Kamil Training and Researching Hospital in Istanbul (Turkey). The study included 332 intracytoplasmic sperm injection (ICSI) cycles. The patients were under 37 years of age with primary infertility who were admitted to the Department of Reproductive Endocrinology and Infertility, from January 2005 to June 2009. The patients were divided in three groups based on Kruger’s strict criteria. Normal sperm morphology was less than 4% in group 1, between 4-14% in group 2, and greater than 14% in group 3. All patients underwent ICSI and embryo transfer (ET) following controlled ovarian hyperstimulation (COH). The groups were compared to the rates of fertilization, implantation, clinical pregnancy, abortion, and live birth. Results: Pregnancy occurred in 132 (39.7%) of all ICSI cycles. There was no statistically significant difference between regarding groups regarding the rates of fertilization, implantation, clinical pregnancy, biochemical pregnancy, abortion, and live birth. Conclusion: The authors concluded that the normal sperm morphology defined by Kruger’s strict criteria and sperm motility will not be able to predict prognosis of ICSI cycles.

Key words: Kruger’s criteria; ICSI; Normal sperm morphology.

Introduction

Infertility is a problem that affects approximately 10-15% of all reproductive-aged couples. During the recent years, significant developments have occurred in the treatment of infertility. The success rates have increased in the treatment of infertility through improvements of assisted reproductive techniques, although these are expensive, time-consuming, and stressful treatments for infertile patients. The selection of appropriate treatment techniques is essential for the success of assisted reproductive techniques. Therefore, ovarian reserve and semen quality should be determined before commencing treatment [1].

The importance of the male factor has been known for a long time in infertility etiology. Currently, intracytoplasmic sperm injection (ICSI) has been introduced worldwide in the treatment of male-factor infertility. ICSI is a viable option for men who have serious impairment of spermatogenesis, even of a single living sperm [2].

Semen analysis is the classic and routine test for evaluating male-factor infertility. However, standard semen analysis cannot show the full fertilization potential of sperm. According to Kruger, normal sperm morphology is classified according to three categories: “less than 4%”, “4-14%”, and “greater than 14%”. In the case of less than 4% normal morphology, the fertilization rate per each oocyte is 7.6% with in vitro fertilization (IVF). The fertilization rate however increases up to 63.9% in samples having more than a 4% normal morphology [3]. As a result of these findings, the World Health Organization (WHO) reduced the normal limits of morphology up to 30%.

The aim of present study was to compare the clinical results of ICSI for different sperm morphology subgroups divided according to Kruger’s classification system.

Materials and Methods

Patients

This retrospectively study was conducted at Zeynep Kamil Training and Researching Hospital in Istanbul (Turkey). The study included 332 ICSI cycles. The patients were under 37 years of age with primary infertility who were admitted to the Department of Reproductive Endocrinology and Infertility, from January 2005 to June 2009.

Patients who had sperm obtained by invasive method (TESE), laparoscopically-diagnosed severe endometriosis, polycystic ovarian syndrome, clinically significant systemic or endocrine disease, history of severe ovarian hyperstimulation syndrome in previous IVF practice, history of more than three failed assisted reproductive technology (ART) application, history of uterine surgery, presence of endometrial pathology (submucous myoma, synecchia, polyps, uterine septum), cases using frozen embryos, and cases with inadequate sperm for morphologic evaluation in semen analysis < 1x10^9/ml), were excluded from the study.

Revised manuscript accepted for publication June 7, 2012
Sperm morphology assessment

Sperm specimens were evaluated for total sperm count, motility, progression, and morphology in preparation for ICSI. Sperm specimens were stained using Diff-Quik (Dade Behring, Newark, Delaware) and evaluated on the basis of Kruger’s strict criteria. The rate of normal sperm forms was determined in each specimen after scoring 100 sperm. Uniformity of grading in the laboratory was routinely monitored for inter-technician variation. Density gradient centrifugation over a 90% layer of isopaque was used to prepare sperm. Sperm cells were classified as morphologically normal or abnormal under inverted microscope used for micromanipulation (x400 magnification, Hoffman modulation). A sperm cell was considered as morphologically normal if the head was normal (normal shape, normal size, having an acrosome, and lacking midpiece or tail defects). In addition, the rate of abnormal motility was accepted as less than 50%. The patients were divided into subgroups based on normal sperm morphology. Three subpopulations were created. Normal sperm morphology was determined as < 4% in group 1, 4-14% in group 2, and > 14% in group 3.

IVF procedure

Controlled ovarian hyperstimulation (COH) was performed with long protocol using gonadotropin-releasing hormone (GnRH) agonist (a) in 227 patients and antagonist (ant) protocol in 105 patients. In the long protocol, GnRHa was initiated on day 21 of the menstrual cycle. Recombinant follicle-stimulating hormone FSH (r-FSH) or human menopausal gonadotropin (hMG) was begun on day 2 of menstruation. In the antagonist protocol, the gonadotropin treatment was administered on day 3 of menstruation with r-FSH or hMG using 150-450 IU / day doses. The antagonist treatment was begun on day 5-7 of treatment or until follicle diameter was 13-14mm. 10,000 IU hCG was injected intramuscularly when the largest follicle diameter was 18 mm. Ultrasound guidance was used to transvaginally aspirate and collect oocytes after 36 hours from human chorionic gonadotropin (hCG) administration. Oocytes were cultured and mature oocytes were fertilized by ICSI procedure. Fertilization was controlled 18-20 hours after the ICSI procedure. Selected embryos were transferred by using a catheter. Pregnancy testing was performed 15 days after the embryo transfer (ET). Clinical pregnancy was determined by ultrasonographic screening of the fetal sac at seven weeks pregnancy.

Statistical evaluation

Data were given as mean ± standard deviation (mean ± sd) or percentage (%). Analysis of data was assessed by using NCSS 2007 software package program. One-way ANOVA and Chi-square tests were used in comparisons of data. A p < 0.05 was considered statistically significant.

Results

A total of 166 pregnancies occurred in 132 of all patients. Twenty-eight pregnancies were aborted clinically, 13 patients were biochemically pregnant, and the remaining 91 patients had live births. Seventy out of 91 patients delivered later than 37 weeks and the remaining 22 patients delivered before 37 weeks. Eighteen patients had twins and eight patients had triplets.

Table 1 shows the relationship of sperm morphology subgroups based on Kruger’s strict criteria. The average maternal age, duration of infertility, basal FSH, total dose of gonadotropin, endometrial thickness during ovulation, numbers of oocytes collected, number of grade 1 embryos on days 2 and 3, numbers of ET were similar in sperm morphology subgroups (Table 1, One-way ANOVA test, p > 0.05). There was no statistically significant difference between the groups in terms of fertilization rates and positive pregnancy (Table 1, Chi-square test, p > 0.05). There was no statistically significant difference between groups 1 and 2, when the causes of infertility were compared. However, tubal factor was lower in group 3 than groups 1 and 2, while male factor was higher in group 3 than in groups 1 and 2 (Table 2, Chi-square test, p < 0.05). The rates of implantation, clinical pregnancy, biochemical

<table>
<thead>
<tr>
<th>Table 1. — Relationship of sperm morphology subgroups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>NMS &lt; 4% (n = 247)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
</tr>
<tr>
<td>Basal FSH (IU)</td>
</tr>
<tr>
<td>Total dose of gonadotropin</td>
</tr>
<tr>
<td>Endometrial thickness during ovulation</td>
</tr>
<tr>
<td>Number of oocytes collected</td>
</tr>
<tr>
<td>Embryo number of grade 1 on day 2</td>
</tr>
<tr>
<td>number of grade 1 on day 3</td>
</tr>
<tr>
<td>Number of transferred embryos</td>
</tr>
</tbody>
</table>

NMS: Normal sperm morphology according to Kruger’s strict criteria

<table>
<thead>
<tr>
<th>Table 2. — The causes of infertility in the three groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Only male factor</td>
</tr>
<tr>
<td>Only tubal factor</td>
</tr>
<tr>
<td>Unexplained</td>
</tr>
<tr>
<td>Both male and tubal factor</td>
</tr>
</tbody>
</table>

NMS: Normal sperm morphology according to Kruger’s strict criteria

Chi-square test was used. p < 0.05 was considered statistically significant.
Does Kruger's strict criteria have prognostic value in predicting ICSI clinical results?

Table 3. — ICSI outcomes in the three groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 247)</th>
<th>Group 2 (n = 57)</th>
<th>Group 3 (n = 28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSM &lt; 4%</td>
<td>29.2</td>
<td>29.4</td>
<td>29.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>35.2</td>
<td>38.5</td>
<td>35.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Abortion rate</td>
<td>8.1</td>
<td>8.7</td>
<td>10.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Live birth rate</td>
<td>27.1</td>
<td>29.8</td>
<td>25.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

NMS: Normal sperm morphology according to Kruger's strict criteria. Chi-square test was used. p < 0.05 was considered as statistically significant.

Discussion

It is known that sperm morphology may affect fertilization rates in intrauterine insemination and IVF [3,4]. It is controversial whether the fertilization rate is associated with sperm morphology for ICSI. In contrast, it was shown that sperm morphology is an excellent indicator determining fertilization capacity for conventional IVF in the literature [5,6].

French and et al. [7] examined 1,074 ICSI cycles. They stratified patients into groups by using Kruger’s strict criteria and eight subgroups were created: 0%, 1%, 2%, 3%, 4%, 5%-7%, and > 7% according to normal sperm morphology. They found that Kruger’s strict morphology was not correlated to fertilization rates, clinical pregnancy rates, live birth rates, blastulation, or blastocyst quality, and selection of sperm with normal morphology under microscopic examination was a sufficient ideal for ICSI outcomes. At the same time, they showed that the rates of motile sperm were reduced with an increase of Kruger’s strict criteria abnormality, and the rates of fertilization, clinical pregnancy, and live births were not negatively affected. In the present study, the patients were divided into three subgroups based on Kruger’s strict criteria as < 4%, 4-14%, > 14% normal sperm morphology. Also, the authors did not find adverse effects in the rates of fertilization, clinical pregnancy, and live birth between the groups. In addition, the authors did not see a significant difference between the groups in terms of the mean number of grade 1 embryos on days 2 and 3. The rate of motile sperm was reduced with decreasing of normal sperm morphology according to Kruger’s strict criteria in the study population. As a result, ICSI outcomes were not affected from the rates of normal sperm morphology and motility in the present study.

Slavender et al. [8] evaluated 354 ICSI cycles. Semen samples were separated according to Kruger’s strict criteria (lower 4%, 4-14%, and over 14%). They found that the rates of fertilization were 61.9%, 66.8%, and 61.6%, respectively and the rates of pregnancy were 18.9%, 24.9%, and 28.3%, respectively. These differences were not statistically significant between the groups. In the present study, the rates of fertilization and pregnancy were 58.2% and 39.7% for group 1, 60.2% and 42.1% for group 2, 61.2% and 35.7% for group 3. There was no statistically significant difference between the groups. Furthermore, the mean number of oocytes collected and ET were similar in all groups as parallel to the literature [7-9].

Many researchers revealed that ICSI cycles with sperm morphology based on Kruger’s strict criteria did not affect fertilization and pregnancy rates [8,10,11]. This can be explained by the selection of the most normal looking sperm under microscope from even the most impaired specimens by embryologist during ICSI.

In conclusion, according to the results, in this study the authors consider that normal sperm morphology based on Kruger’s strict criteria had no prognostic value in predicting ICSI clinical outcomes. In ICSI procedure, selection of the most normal looking sperm cell under microscope may be provided for excellent results for infertile couples. Also, sperm motility does not seem to be sufficiently assisted in ICSI procedure. This subject should be elucidated by randomized prospective studies.

References


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Maternal adiponectin and visfatin concentrations in normal and complicated pregnancies

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Summary

Objective: To evaluate the role of adiponectin and visfatin in the pathophysiology of pre-eclampsia (PE) and how their concentrations correlate with the severity of the disease and neonatal outcomes. Study Design: A prospective case-control study was carried out in 52 preeclamptic and 28 healthy pregnant women during the third trimester. The maternal plasma concentrations of adiponectin and visfatin were determined. Neonatal outcomes were also recorded. Results: Mean maternal plasma adiponectin concentrations in healthy pregnant women did not differ significantly from those of mild PE and severe PE groups. The plasma adiponectin levels of PE patients with small gestational age (SGA) and those without SGA did not differ significantly, but the median plasma visfatin concentration of patients with SGA fetus was significantly higher if the patient was preeclamptic (p = 0.036). Conclusion: The severity of preeclampsia did not change the plasma levels of adiponectin and visfatin, but the median plasma visfatin concentration of patients with SGA fetuses were significantly higher if the patient was preeclamptic. Altered levels of adipocytokines strongly imply that the regulation of adipocytokines in PE is different and more complex compared to that in healthy pregnancy.

Key words: Pregnancy; Preeclampsia; Adiponectin; Visfatin; SGA fetuses.

Introduction

Changes in maternal metabolism occur during pregnancy in order to promote the increasing metabolic needs of the growing fetus and placenta. In normal pregnancy, alterations in the regulation of glucose metabolism lead to increased insulin resistance and changes in endothelial function [1]. The resistance to normal gestational insulin increases and also worsens during pregnancy complications that are associated with disturbed placental function (e.g., gestational diabetes mellitus (GDM), preeclampsia (PE), and intrauterine growth restriction (IUGR)) [2,3]. PE is a severe complication of pregnancy. It is characterised by endothelial dysfunction (one of the early stages of atherosclerosis) and in part, shares features of the metabolic syndrome including exaggeration of insulin resistance, obesity, low-grade systemic inflammation, and atherosclerosis [4,5]. The insulin resistance and low-grade systemic inflammation may contribute to the pathogenesis of endothelial dysfunction. However, the underlying mechanisms and the molecules involved in PE are not yet certain [3].

One of the facets of pathophysiology of PE is the altered expression of adipocytokines such as leptin, tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), adiponectin, resistin, and visfatin [5,6]. To regulate maternal energy metabolism and insulin sensitivity during normal pregnancy [6], adipocytokines are expressed and secreted by adipocytes and the placenta [7].

Adiponectin, the most abundant adipose-tissue-specific protein, has anti-inflammatory, antiatherogenic, and insulin-sensitizing properties [8]. During normal pregnancy, maternal adiponectin levels were found to be decreased or remained unchanged [9-11]. There is an ongoing debate as to whether the levels of adiponectin in preeclamptic women increase, decrease, or remain the same when compared to gestational age-matched normal pregnancies [11-17].

The adipocytokine visfatin is highly-expressed in visceral adipose tissue which promotes adipogenesis and exerts insulin-mimetic effects [18]. The association of visfatin with both low-grade inflammation and impaired endothelial function has already been studied [19]. In type-2 DM, obesity, GDM patients when compared with control subjects, fluctuations or no change in blood concentration of visfatin is detected [20-22]. Although maternal serum visfatin had been previously demonstrated to be increased in some studies, other studies showed that visfatin levels decreased significantly in PE irrespective of maternal body mass index (BMI) [23-25]. Thus, the relationship between visfatin and PE is not fully apparent.

Growth restriction is more common in infants born to preeclamptic women and is more pronounced with increasing severity [3, 4]. Deterioration of utero-placental perfusion predicts a high-risk for fetal growth restriction (IUGR). Maternal plasma adiponectin levels were studied in growth-restricted fetuses and were lower than normal fetuses, possibly reflecting the state of inflammation and chronic stress in IUGR mothers [26]. In contrast to this, other studies demonstrated no significant alterations in maternal plasma adiponectin concentrations in IUGR pregnancies [27]. In third-trimester patients with IUGR, visfatin was found to be increased nearly two-fold in comparison to healthy pregnant women, but this was not verified by other studies [28, 29].

The levels of adipocytokines are altered in pregnancies...
complicated with PE and growth-restricted fetuses. In the current study, the plasma concentrations of two adipocytokines, visfatin, and adiponectin are measured in normal and preeclamptic pregnancies. In addition, the effects of maternal plasma adiponectin and visfatin concentrations in neonatal outcomes, such as growth restriction and APGAR scores, are investigated.

Materials and Methods

For this prospective case control study, 80 third-trimester pregnant women were recruited among patients managed at the antenatal clinic. The study protocol was approved by the Research Ethics Committee of the Uludag University School of Medicine. All participants gave written informed consent before taking part in the study. The study population consisted of 52 preeclamptic and 28 BMI-matched healthy pregnant women (Group 1; the control group). The preeclamptic patients were divided into subgroups according to the severity of preeclampsia: mild preeclamptic group (Group 2; 23 patients) and severe preeclamptic group (Group 3; 29 patients). In the study, previously normotensive women after 20th gestational weeks of pregnancy were classified as having mild PE if on at least two different occasions that were more than six hours apart, the systemic blood pressure measurement was above 140 mm Hg and diastolic blood pressure reading was above 90 mm Hg, and in a 24-hour urine collection, the proteinuria was more than 300 mg/l. Patients were classified as having severe PE if any of the following conditions observed: 1) on two occasions that were at least six hours apart, the systolic blood pressure measurement was above 160 mm Hg or diastolic blood pressure measurement was above 110 mmHg; 2) in 24 hours, urinary protein excretion was more than five grams [9]; 3) 3+ and greater random urine dipstick testing; 4) in 24 hours, urinary discharge was less than 500 ml; and 5) cerebral or visual disturbances, pulmonary edema or cyanosis, or epigastric or right upper quadrant pain was observed. Gestational age was calculated by menstrual history and by ultrasound data obtained during the first or second trimester of pregnancy in case of presence of irregular cycles. Gestational age was calculated by the number of weeks. The BMI was calculated by dividing the weight by squared height (kg/m²). Women with chronic hypertension, diabetes, preexisting vascular and chronic renal diseases, and present or prior GDM were excluded from the study. Similarly, those women who had multiple gestations and were in active labor were excluded from the study. The diagnosis of SGA was based on ultrasonographic estimated fetal weight and confirmed by a birth weight below the 10th percentile of gestational age [29, 30]. The diagnosis of intrauterine growth restriction used for fetuses who were SGA and who showed other evidence of chronic hypoxia was based on Doppler assessment [31]. All patients with a fetus whose fetal weight was below 10th percentile underwent Doppler velocimetry measurements of the umbilical arteries. Umbilical artery doppler velocimetry was considered abnormal if either the pulsatility index (PI) was above the 95th percentile for gestational age or abnormal waveforms were present (i.e., absent or reversed end-diastolic velocities) [32]. The fetus of preeclamptic patient which was growth-restricted and showed abnormal Doppler values, was considered as intrauterine growth-restricted and such patients were classified into the severe preeclamptic group. If the fetus of preeclamptic women was SGA but had normal Doppler values, then these fetuses were grouped in SGA of mild PE group. The birth weight of all neonates was recorded and those below 10th percentile were considered as SGA. Moreover, APGAR scores at one and five minutes after birth and delta APGAR scores (the difference between the APGAR score of five minutes and that of one minute) of all neonates were recorded.

The blood samples, obtained from the antecubital area, were collected between 8 a.m. and 9 a.m. after a fasting period of 12 hours. A three ml venous blood sample from each patient was drawn into serum tubes. At the time of sampling, none of them was in labour. Serum was separated by centrifugation at 4,000 cycles/min for ten minutes and stored at ~80ºC until further analysis. Plasma adiponectin concentrations were measured by BOSTER Immunoleader Human ELISA Kit (BOSTER Biological Technology Co.) with standard sandwich enzyme-linked immunosorbent method and visfatin levels were (RayBiotech, Inc Norcross GA, USA) measured by competitive enzyme immunoassay method.

Statistical analyses were performed with SPSS, version 13 (SPSS, Chicago, IL). Normality of the data was tested by using the Shapiro-Wilk test. Maternal age was compared among groups by ANOVA test. Post-hoc comparisons were performed with Bonferroni test. For non-normality distributed data, Kruskal Wallis and Mann Whitney tests were performed for the comparisons of the groups, where appropriate. Maternal age was represented with mean value and standard deviations, while discrete and non-normality distributed variables were represented as median (minimum-maximum). Categorical variables between groups were compared by Pearson Chi square test with Yates correction. A p value smaller than 0.05 was considered statistically significant.

Results

The demographic and clinical characteristics of the study groups are presented in Table 1. The median gestational age at blood sampling was not different between the control group and the mild PE group, but differed significantly between the mild PE and severe PE groups (p = 0.005). The preeclamptic patients were younger than normal control patients (p = 0.003). The BMI values of patients did not differ between the preeclamptic groups and the control group (p = 0.442). When compared with control patients, the systolic and diastolic blood pressures were significantly higher in preeclamptic patients (p < 0.001). The creatinine and blood urea nitrogen (BUN) levels were higher in the severe PE group compared to the control group (p < 0.001, both groups). The babies of preeclamptic patients had a lower neonatal weight compared to the babies of normotensive pregnant group (p < 0.001). In mild preeclamptic patients, the incidence of SGA was 43.5% and it was not significantly different from the incidence of SGA in normal pregnancies (21.4%, p = 0.212). In severe preeclamptic group, 11 of 29 women had intrauterine growth-restricted neonate (37.9%). This was not significantly different between the mild and severe PE groups. The cesarean rate of the severe PE group was higher than those of the other two groups. This difference reached a statistically significance for control group (p = 0.002) (Table 2). However, that difference between the mild PE group and the control group was not statistically significant (p = 0.260). The APGAR score in one minute and the delta APGAR score of the control group were statistically significantly higher.
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than those of both preeclamptic groups (p < 0.001, both groups). Fetal complication rates (e.g., admission to the neonatal intensive care unit, need for neonatal resuscitation, etc.) were higher (p < 0.001) and the APGAR scores of neonates were significantly lower than those of the other groups (p < 0.001) in the severe PE group (Table 2).

Table 1. — The clinical characteristics of control, mild, and severe preeclampsia groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 28)</th>
<th>Group 2 (n = 23)</th>
<th>Group 3 (n = 29)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.71 (21.83-45.17)</td>
<td>28.84 (16.65-44.44)</td>
<td>28.76 (22.76-41.45)</td>
<td>0.442</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.00 (1.00-5.00)</td>
<td>1.00 (1.00-4.00)</td>
<td>1.00 (1.00-5.00)</td>
<td>0.035</td>
</tr>
<tr>
<td>Parity</td>
<td>0.50 (0.00-2.00)</td>
<td>0.00 (0.00-3.00)</td>
<td>0.00 (0.00-4.00)</td>
<td>0.032</td>
</tr>
<tr>
<td>Gestational age at sampling (wks)</td>
<td>33.00 (28.00-39.00)</td>
<td>34.00 (28.00-38.00)</td>
<td>30.00 (28.00-39.00)</td>
<td>0.009</td>
</tr>
<tr>
<td>Gestational age at delivery (wks)</td>
<td>39.00 (36.00-41.00)</td>
<td>36.00 (28.00-38.00)</td>
<td>31.00 (28.00-40.00)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110.00 (85.00-130.00)</td>
<td>150.00 (130.00-190.00)</td>
<td>170.00 (140.00-200.00)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70.00 (60.00-90.00)</td>
<td>100.00 (90.00-120.00)</td>
<td>100.00 (90.00-120.00)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>97.00 (77.00-113.00)</td>
<td>133.00 (120.00-166.00)</td>
<td>146.00 (123.00-166.00)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BUN</td>
<td>15.50 (9.50-31.00)</td>
<td>23.00 (12.50-36.00)</td>
<td>27.00 (13.00-102.60)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.60 (0.44-0.90)</td>
<td>0.70 (0.55-1.10)</td>
<td>0.78 (0.60-3.21)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Pairwise Comparisons:

<table>
<thead>
<tr>
<th></th>
<th>Group 1 - Group 2</th>
<th>Group 1 - Group 3</th>
<th>Group 2 - Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>0.001</td>
<td>0.027</td>
<td>0.206</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.111</td>
<td>0.012</td>
<td>0.367</td>
</tr>
<tr>
<td>Parity</td>
<td>0.263</td>
<td>0.009</td>
<td>0.154</td>
</tr>
<tr>
<td>Gestational age at sampling (wks)</td>
<td>0.361</td>
<td>0.025</td>
<td>0.005</td>
</tr>
<tr>
<td>Gestational age at delivery (wks)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.013</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>BUN</td>
<td>0.008</td>
<td>&lt; 0.001</td>
<td>0.016</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.019</td>
<td>&lt; 0.001</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Group 1: normotensive pregnant, Group 2: mild preeclampsia, Group 3: severe preeclampsia.

Cell values were represented as mean ± standard deviation and median (minimum-maximum).

Table 2. — The neonatal characteristics of control, mild, and severe preeclampsia groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 28)</th>
<th>Group 2 (n = 23)</th>
<th>Group 3 (n = 29)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>3300 (1920-4400)</td>
<td>2150 (780-3950)</td>
<td>1280 (670-4850)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Apgar Score (one minute)</td>
<td>10.00 (7.00-10.00)</td>
<td>8.00 (3.00-10.00)</td>
<td>4.00 (0.00-10.00)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Delta APGAR score*</td>
<td>0.00 (0.00-2.00)</td>
<td>1.00 (0.00-4.00)</td>
<td>2.00 (-1.00-6.00)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SGA</td>
<td>6 (21.40)</td>
<td>10 (43.50)</td>
<td>11 (37.90)</td>
<td>0.212</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>14.00 (50.00)</td>
<td>16 (69.60)</td>
<td>26 (89.70)</td>
<td>0.005</td>
</tr>
<tr>
<td>Fetal complication</td>
<td>1 (3.60)</td>
<td>9 (39.10)</td>
<td>25 (86.20)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Pairwise Comparisons:

<table>
<thead>
<tr>
<th></th>
<th>Group 1 - Group 2</th>
<th>Group 1 - Group 3</th>
<th>Group 2 - Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>Apgar Score (one minute)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Delta APGAR score*</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.179</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>0.260</td>
<td>0.002</td>
<td>0.087</td>
</tr>
<tr>
<td>Fetal complication</td>
<td>&lt; 0.001</td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Group 1: normotensive pregnant, Group 2: mild preeclampsia, Group 3: severe preeclampsia.

Cell values represented as median (minimum-maximum) and n (%).

*Delta APGAR Score: APGAR of five minute – APGAR of one minute.

Table 3. — Visfatin and adiponectin levels in control, mild, and severe preeclampsia groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 28)</th>
<th>Group 2 (n = 23)</th>
<th>Group 3 (n = 29)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma adiponectin</td>
<td>34.66 (4.95-49.81)</td>
<td>29.66 (3.04-44.96)</td>
<td>25.70 (3.04-49.94)</td>
<td>0.140</td>
</tr>
<tr>
<td>Plasma visfatin</td>
<td>37.11 (5.59-119.94)</td>
<td>47.49 (1.20-107.18)</td>
<td>57.38 (3.76-119.94)</td>
<td>0.168</td>
</tr>
</tbody>
</table>

Group 1: normotensive pregnant, Group 2: mild preeclampsia, Group 3: severe preeclampsia.

Cell values represented as median (minimum-maximum) and n (%).
The maternal visfatin and adiponectin levels and association with SGA.

<table>
<thead>
<tr>
<th></th>
<th>Normotensive group</th>
<th>Preeclampsia group</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA no</td>
<td>n = 21</td>
<td>n = 22</td>
</tr>
<tr>
<td>Visfatin p value</td>
<td>0.036</td>
<td>0.091</td>
</tr>
<tr>
<td>Adiponectin p value</td>
<td>0.512</td>
<td>0.091</td>
</tr>
<tr>
<td>SGA yes</td>
<td>n = 6</td>
<td>n = 22</td>
</tr>
<tr>
<td>Visfatin p value</td>
<td>0.036</td>
<td>0.091</td>
</tr>
<tr>
<td>Adiponectin p value</td>
<td>0.512</td>
<td>0.091</td>
</tr>
</tbody>
</table>

Discussion

The results of the current investigation revealed no difference in plasma concentrations of adiponectin and visfatin between preeclamptic and healthy pregnant women. Between the preeclamptic patients with and without a SGA neonate, no significant difference was observed in both the maternal plasma adiponectin concentration and the median visfatin concentration level, but the plasma visfatin levels were higher in patients with SGA who had PE concomitantly than normotensive group.

It was found that circulating adiponectin concentrations, in contrast to other adipokines, were decreased in insulin-resistant states and obesity-related metabolic disorders including type 2 DM and coronary vascular disease [6]. Previous studies concerning adiponectin plasma levels in pregnant women revealed incompatible results. During normal pregnancy, maternal adiponectin levels were decreased [9] or remained unchanged [10, 11]. Although insulin resistance was shown to be increased dramatically during gestation [10], no significant alteration in adiponectin levels was observed throughout pregnancy.

Some studies showed that adiponectin plasma levels were lower in preeclamptic women than normotensive women [11, 12]. The study by Fasshauer [23] reported an association between hypoadiponectinemia in early pregnancy and subsequent development of preeclampsia. The decrease in plasma adiponectin levels was associated with the inhibition of adiponectin synthesis by an increase in glucocorticoids, sympathetic activity, and pro-inflammatory cytokines phenomena. These three characteristics of normal pregnancy were shown to be further increased in PE [12]. The increase in serum levels of adiponectin in preeclamptic women in the third trimester was first shown by Ramsay [16]. However the increased adiponectin levels in plasma of PE women could not be verified in adipose tissue mRNA levels of adiponectin [13]. Ramsay argued that adiponectin released during pregnancy could be a physiologic response representing a regulatory feedback to minimize fat accumulation in tissues of PE women, and inhibiting inflammatory processes that lead to endothelial damage and dysfunction in PE [13, 16].

Adiponectin resistance [15], alteration of renal functions in PE women [13], a difference in the rate of high-to low-molecular-weight oligomers of adiponectin in PE women [9] may possibly explain the increased levels of adiponectin in PE women. Those different findings might be due to the differences between study populations in terms of body fat, insulin sensitivity, and gestational weeks in which the blood sample was withdrawn. The results of previous studies suggested that fat tissue and obesity-related insulin resistance may play a role in the development of PE in obese pregnant women and probably mask the effects of compensatory raise in adiponectin levels at established PE [17].

On the other hand, some studies found no change in maternal adiponectin levels in PE [14, 17]. Even during early weeks of pregnancy, adiponectin levels did not change before the onset of clinical signs of PE [17]. In the presented study, the authors did not find any difference in plasma adiponectin levels of PE patients compared to normal controls. It was shown that adiponectin expression and secretion tend to the regulation of numerous factors. Whether or not placental adiponectin production contributes to maternal or fetal adiponectin levels...
remains to be determined. Adiponectin receptors were found to be abundantly expressed in human placenta [7]. However, adiponectin expression by the placenta during pregnancy is debatable and the production of placental adiponectin may not be strong enough to influence maternal adiponectin levels [6]. During pregnancy, adiponectin gene expression has been proved to be regulated in specific tissues [30]. No significant difference of adiponectin mRNA expression was observed in adipose tissue between PE women and normal controls [13], but placental adiponectin expression has been recently found to be decreased in severe PE [33].

Adiponectin is known to have a number of isoforms. These include low-, middle- and high-molecular-weight (HMW) forms [34]. The HMW form of adiponectin is the most active in mediating insulin sensitivity and glucose tolerance [35, 36]. Since most studies of clinical material use ELISA or RIA kits, none of them adequately distinguishes between various complexes of adiponectin. Thus, future research should focus on the role of different forms of adiponectin in PE.

In non-pregnant state, plasma adiponectin concentrations, unlike other adipocytokines, are inversely correlated with adiposity [9]. There is no consensus about the BMI effect on adiponectin levels in pregnant women. Although there exists studies reporting no correlation between adiponectin levels and gestational BMI [10], Hendler [14] stated that PE women with normal BMI had higher adiponectin levels than normotensive women and that adiponectin levels were decreased in women with a BMI greater than 25 kg/m². Because BMI correlation with adiponectin concentration is debatable, the groups in the present study were well-matched with respect to this variable (p = 0.442).

PE increases the fetal risk of being born SGA particularly in cases of early and recurrent disease [3, 4], but only one-third of fetuses of preeclamptic women are growth-restricted despite low placental perfusion [4]. Adiponectin can be assumed to play a regulatory role in fetal growth considering its importance in insulin metabolism and the fact that fetal growth is controlled by glucose and insulin to a great extent [37]. A positive correlation between cord blood adiponectin levels and birth weight has been shown in many studies [37, 38]. Therefore, it was reasonably speculated that augmented insulin sensitivity by adiponectin might be the cause of positive association of birth weight and adiponectin concentrations [39]. Contrarily, some studies demonstrated the lack of significant differences in fetal adiponectin concentrations between SGA cases and AGA controls. This is probably due to the lack of insulin resistance present in early life [20, 23, 40]. A previous study showed lower maternal adiponectin levels in the growth restricted state. This might be explained by the fact that the state of inflammation and chronic stress in IUGR mothers predisposes them to develop insulin resistance [23]. In this presented study, no significant correlations between maternal plasma adiponectin levels of women and neonatal birthweights or gestational ages were observed. In addition, the authors did not find any significant difference in adiponectin plasma levels of PE patients with or without SGA. In the SGA group, plasma adiponectin levels remained unchanged even in the presence of PE. Maternal serum adiponectin levels seem to be only a marker for maternal fat mass but do not play a major role in fetal growth [37].

Hyperinsulinemia, glucose intolerance, and lipid abnormalities are characteristics of physiological insulin resistance in the second half of pregnancies [1, 2]. Visfatin may play a role in PE since it promotes adipogenesis and exerts insulin-mimetic effects. In PE women, plasma visfatin levels were found to be markedly increased compared to normal pregnant women [23, 24]. However, in contrast to those studies, some studies showed decreased plasma visfatin levels in PE patients with comparable BMI [25]. Although increased synthesis or decreased degradation of visfatin in pregnancies was speculated to contribute in the pathogenesis of PE, this however has not yet been verified [24]. In contrast to these findings, the authors found no significant differences in maternal circulating visfatin concentrations between pregnant women with and without PE. In agreement with these findings, a study on third-trimester women demonstrated that the median maternal plasma visfatin concentration did not differ significantly between patients with PE and those with normal pregnancy [28]. Hu argued that a down-regulation of visfatin expression in adipose tissue may be responsible for decreased visfatin levels and pointed out the involvement of visfatin in excessive insulin resistance in the clinical state of PE [25], but in this study, circulating visfatin in the severe PE group was not significantly different from that in the moderate PE group. These apparent discrepancies between studies might be due to the differences in study population, sample size, or research design.

Fasshauer et al. reported higher visfatin concentrations in IUGR neonates compared to AGA counterparts in third-trimester pregnancies and suggested visfatin as a novel marker up-regulated in women with IUGR [27]. This is in agreement with another study where circulating maternal visfatin levels were found to be higher in IUGR women than those in the AGA group [29]. In that study, altered maternal endocrine environment in pregnancies that are complicated with IUGR were argued to be the reason for higher visfatin levels, and higher visfatin concentrations were hypothesized to serve as an early marker with prognostic value for later development of the metabolic syndrome in growth-restricted fetuses [29]. The population of the aforementioned study included patients with GDM and extreme obesity which might have affected the results. In the present study group, plasma concentrations of visfatin with SGA fetuses who were preeclamptic were higher than those of normotensive patients. Altered fetal development of adipose tissue in SGA fetuses may interfere with plasma visfatin concentrations. In a study where plasma visfatin concentrations of preeclamptic women with and without SGA were compared, it was reported that the presence of
a SGA neonate in PE patients was not accompanied by significant alterations in maternal circulating visfatin concentrations. This suggested that PE overwhelmed the effects of the presence of a SGA neonate on maternal plasma visfatin concentration [28]. This was compatible with the results in the present study where similar plasma visfatin levels were observed in preeclamptic women with and without a SGA neonate.

The mechanisms leading to increased visfatin levels in women with IUGR are yet unclear. In a previous study, visfatin was speculated to play a role in the glucose transfer from maternal to fetal circulation based on histological examination of the placenta [41]. In a recent study, visfatin mRNA expression has been shown to be significantly related to TNF-α and IL-6 mRNA expression in placental tissues [42]. Since TNF-α and IL-6, negative regulators of visfatin expression in fat [43], increase in pregnancy-related disease, they unlikely intervene up-regulation of visfatin plasma levels by induction in fat. However, in addition to fat, a wide range of other tissues can produce visfatin. Thus, the modulation of visfatin expression by insulin, TNF-α, and IL-6 might be tissue-specific [44]. The presence of PE in SGA patients is known to increase the levels of plasma visfatin concentrations and this might be related to placental functional deterioration in PE.

Altered levels of adipocytokines strongly imply that the regulation of adipocytokines in PE is different and more complex compared to that in normal pregnancy. Despite paradoxical and controversial reports of adiponectin levels in PE, adiponectin is attracting considerable interest as a potential biochemical indicator.

The reported study has several limitations. The cross-sectional nature of the study and relatively small number of participants included did not enable the authors to come up with a line of reasoning for the association between pregnancy and regulation of adiponectin levels during pregnancy. In order to overcome the inherent limitations of the study, the authors carefully examined the participants for possible maternal parameters that may affect adiponectin levels (i.e., BMI, GDM, age, and more). In conclusion, the authors recognized that further research is necessary to elucidate the pathophysiological role of adipocytokines in normal and complicated pregnancies, particularly defining the risk of future development of maternal insulin resistance.

Acknowledgement

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References


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Platelet count as a predictive factor of neonatal outcome in twin pregnancy with fetal demise

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Summary

Purpose: During the last decade, the rate of twin pregnancies has increased and reached 3% of all pregnancies. Materials and Methods: This study enrolled 36 twin pregnancies that were followed and delivered at the Clinic for Gynecology and Obstetrics, Clinical Center of Serbia over a five-year period. Results: The first group included 15 patients with a monochorionic twin pregnancy, and the second group consisted of 21 patients with a dichorionic twin pregnancy. The platelet count was significantly lower in patients with APGAR scores of more than 8, with an average of 185,000/ml, and in patients with a score of less than 4, the average count was 221,000/ml. The perinatal mortality rate of the surviving twin was 35% in the monochorionic group and 0.4% in the dichorionic group. Conclusion: An increase in the maternal platelet count can be used as a predictor for a negative neonatal outcome of the surviving twin.

Key words: Fetal demise; Twins; Platelet number.

Introduction

During the last decade, the rate of twin pregnancies has increased and reached 3% of all pregnancies. As mothers wait longer to have children and more of them use reproductive techniques, multiple pregnancies (mostly twins and triplets) have become more common. These pregnancies carry a higher risk for the mother and fetus, and obstetricians have developed more comprehensive regimens of check-ups and tests to minimize these risks [1]. The mortality and major morbidity rate that is associated with the death of one twin is reported to be 46%. When fetal demise occurs after midgestation, there is a 17% chance that the surviving twin in a monochorionic gestation will either die or suffer major morbidity. Morbidity and mortality approach 50% when twin-to-twin transfusion is present. Major morbidity is unlikely to occur in the surviving twin of dichorionic gestations.

Major maternal problems are the result of coagulation disorders. Maternal coagulopathy following twin demise appears to be uncommon; however, coagulopathy has been reported to occur up to three weeks following fetal demise [2].

It is recommended that all twin pregnancies that result in a dead fetus be managed in tertiary referral centers with sufficient neonatal support. A management plan should be personalized to the patient. Intensive fetal surveillance is required and the determination of chorionicity, particularly in the first trimester, is crucial. Prompt delivery of the surviving twin following the death of a co-twin has not been shown to prevent the pre-existing neurological injury or other injury that occurred at the time of the death of the co-twin. Therefore, a delivery for the purpose of preventing injury should be weighed against the risks of premature delivery [3].

Materials and Methods

The aim of this study was to analyze maternal coagulation status in twin pregnancies complicated by a single intrauterine death. Key maternal coagulation tests and their significance in predicting the neonatal outcome of the surviving twin were carefully studied.

This study enrolled 36 twin pregnancies that were followed and treated at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia, over a five-year period. The study was a retrospective (2004-2009) analysis, and it was approved by the Ethical Board of the Clinical Center of Serbia. Patients were divided into two groups that were similar in age, parity, method of conception, and termination of pregnancy. The first group included 15 patients with monochorionic twin pregnancies, and the second group consisted of 21 patients with dichorionic twin pregnancies. The antenatal records of the mothers, neonatal charts, and neonatal discharge summaries were reviewed. Autopsy and histology reports were also obtained. The approximate time interval between the death of the first twin and the delivery of the surviving co-twin was calculated from the information recorded in the case notes. Ultrasound scan data related to chorionicity, fetal growth, liquor volume, Doppler measurements, and a diagnosis of twin-to-twin transfusion syndrome (TTTS) were obtained.

The follow-up of the patients was conducted on a monthly basis at the clinic. Ultrasound follow-up included the biometry and Doppler flow measurements in the maternal and fetal circulation on an ACCUVIX ultrasound machine. Laboratory testing was performed in a biochemical laboratory on a Sysmex CA 1500 machine. The laboratory tests included prothrombin time (PT), partial thromboplastin time (PTT), platelet count, D-dimer, fibrinogen, and antithrombin (AT). After a pregnancy ended, an autopsy of the dead fetus and placenta was performed by an experienced clinical fetal pathologist.

The statistical analysis was performed using a chi-squared test, Student’s t-test, Spearman’s test, and Fisher’s test (Statistical base, SPSS version 17).
Platelet count as a predictive factor of neonatal outcome in twin pregnancy with fetal demise

Results

Multiple pregnancies are frequently the result of assisted reproduction procedures. In this study, multiple pregnancies represented 11 of all of the pregnancies. There was a significant difference between the two groups ($p = 0.35$). The majority of the monochorionic twins were conceived naturally (two in vitro fertilization (IVF) vs 13 natural), whereas dichorionic twins more frequently resulted from IVF (9 IVF vs 12 natural).

The average duration of pregnancy until fetal death was $4.8 \pm 3$ weeks in the first group and $6.3 \pm 2$ weeks in the second group. In all of the cases, the duration until fetal death was less than five weeks in 20, less than ten weeks in five, less than 15 weeks in five, and more than 16 weeks in six cases. There was no significant difference in the duration until fetal death between the groups ($t = 0.326$). Multiple significant correlations were observed between the duration of weeks until fetal death and the D-dimer levels, PT levels, week of delivery, and the birth weight of the dead twin. The D-dimer levels in the maternal blood increased with a longer duration until fetal demise ($p = 0.758$). The average D-dimer levels in the group of patients with less than five weeks until fetal demise occurred was $197 \pm 8 \mu g/l$, and in the group with more than 16 weeks, it was $214 \pm 7 \mu g/l$ ($p = 0.375$). The average week of delivery was $33.1 \pm 2$ weeks in patients with less than five weeks until fetal death and $37.2 \pm 1$ weeks in patients with more than 16 weeks until fetal demise. Prolonged PT levels were present in three weeks in patients with more than 16 weeks until fetal demise. Prolonged PT levels were present in three weeks in patients with more than 16 weeks until fetal demise and 37.2 ± 1 weeks in patients with more than 16 weeks until fetal demise.

The average D-dimer levels in patients with less than five weeks until fetal demise was 197 ± 8 µg/l ($p = 0.758$). The average D-dimer levels in the first group ($\chi^2 = 6.5$), AT ($\chi^2 = 6.35$) and the platelet counts ($\chi^2 = 8$).

The laboratory tests performed to examine the maternal coagulation status were PT, PTT, platelet count, D-dimer, fibrinogen, and AT. The average PT value was $112 \pm 7\%$ in the first group and $108 \pm 10\%$ in the second group ($p = 0.617$). The average PTT was $28.76 \pm 2$ seconds in the first group and $29.8 \pm 1.8$ seconds in the second group ($p = 0.851$). The fibrinogen level was $4.59 \pm 0.5 g/l$ in the first group and $5.2 \pm 0.3 g/l$ in the second group. The D-dimer level was $257 \pm 12 \mu g/l$ in the first group and $206 \pm 5 \mu g/l$ in the second group. The AT level was $90 \pm 2\%$ in the first group and $89\%$ in the second group ($p = 0.25$). The platelet counts were normal in all of the patients: $225,000 \pm 1,500/l$ in the first group and $215,000 \pm 2,000/l$ in the second group ($p = 0.47$). There was no significant difference between the study groups in any of the examined maternal coagulations parameters.

The analysis of the APGAR scores of the surviving twins indicated that three newborns died during Cesarean section delivery. These newborns were all monochorionic twins. One death was the result of a severe intrauterine infection and the other two deaths were the result of TTTS. There was a significant difference in the survivor mortality rates between the groups. In the monochorionic gestation group, the survivor mortality rate was 33%, and in the dichorionic gestation group, it was 0.4% ($p = 0.015$).

Severe perinatal asphyxia was detected in 11 newborns with APGAR scores of less than 4. Moderate perinatal asphyxia was detected in ten newborns with APGAR scores of less than 7. In total, only 12 babies were in good condition. There was no significant difference between the groups ($p = 0.08$). The degree of metabolic acidosis in the newborns correlated with the APGAR scores. A correlation was detected between the APGAR score and the time of delivery ($p = 0.568$), birth weight ($p = 0.566$), platelet count ($p = 0.501$), and duration until fetal death ($\chi^2 = 21.855$). The platelet count was significantly lower in patients with APGAR scores of more than 8, with an average of 185,000/ml, and in patients with a score of less than 4, the average count was 221,000/ml. No correlation was observed between other coagulation parameters and the APGAR scores of the newborns (D-dimer, $p = 0.4$; PTT, $p = 0.4$; and PT, $p = 0.35$).

Discussion

Krajenba et al. found an increased incidence of premature births in twin pregnancies with intrauterine fetal demise of one twin and an increased incidence of cesarean sections. He observed that the most frequent causes of death were monochorionicity and TTTS [4]. Sato et al. found an increased incidence of vascular thrombosis in the placentas of monochorionic twins with intrauterine growth restriction (UGR) or intrauterine fetal demise [5]. Aslan et al. found that in a study of twin pregnancies with intrauterine fetal demise, 78% of the cases resulted in preterm delivery and 56% resulted in a Cesarean section, and the most common cause of death was TTTS [6]. In a study of twin pregnancies with a fetal anomaly in one of the twins, Chang et al. found that the incidence of fetal congenital anomaly was 2.4%; however, 56% of the healthy twins suffered an intrauterine death [7]. Malinowski et al. also analyzed the chorionicity of twins with the death of one fetus [8]. Johnson et al. observed that a favorable outcome of a pregnancy with the fetal death of one twin depends on the week of gestation when the death occurred and that twin pregnancies with different sexes have a better chance for survival [9]. Axt et al. showed that 71% of these pregnancies ended prematurely and had the same percentage of cesarean sections as in monofetal pregnancies. The expectative management of pregnancy was proposed [10]. Petersen et al. showed that the increasing incidence of prematurity in these pregnancies is more significant than the death of one fetus [11]. Vial et al. suggested that only a monochorionic twin pregnancy should be terminated as soon as possible, whereas in other cases, the pregnancy should be followed to term [12].

After delivery, the placenta should be examined macroscopically and histologically to determine placenta. Santema et al. have argued that other than chorionicity, histology is not informative because of the extensive secondary changes caused by the dead fetus. This low rate
of observation could be due to the collapse of the chorionic vessels of the dead fetus, which may make the recognition of the vascular communications difficult [13].

Conclusion

The antenatal and neonatal outcomes of twin pregnancies with the fetal demise of one twin are strongly dependent on the number of placentas. Monochorionic gestations are at an increased risk for fetal anomalies, TTTS, and the death of the surviving twin. Morbidity of the survivors was rare.

The incidence of maternal coagulation disorders was very low. Nevertheless, regular monitoring of the clotting system in maternal circulation is strongly recommended, and the authors suggest that D-dimer and PT testing are the most accurate methods for monitoring. An increase in the maternal platelet count can be used as a predictor for a negative neonatal outcome of the surviving twin. This can lead to consider aspirin or even anticoagulation therapy in these cases with platelet number more then 200,000 / ml. There is a place for further research in this area considering the increase of twin pregnancies.

References


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Effects of flavonoids from semen cuscutae on the hippocampal-hypothalamic-pituitary-ovarian sex hormone receptors in female rats exposed to psychological stress

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Summary

Objective: To investigate the effects of flavonoids from semen cuscutae (FSCs) on the hippocampal-hypothalamic-pituitary-ovarian sex hormone receptors in female rats exposed to psychological stress and to explore the related mechanism. Materials and Methods: Flavonoids were obtained from semen cuscutae using solvent extraction and polyamide column chromatography. Sound, light, and electricity were combined into psychological stress for endocrine dysfunction model establishment in female rats. The effects of FSCs on estrogen receptor (ER) in the hippocampus, hypothalamus, and pituitaries, as well as on follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) in the ovaries of the psychologically stressed rats were quantitative analyzed using immunohistochemistry and image analysis. Results: FSCs increased ER expression in the hippocampus, hypothalamus, and pituitaries, as well as LHR expression in the ovaries, but had no effect on FSHR expression in the ovaries. Conclusion: FSCs are an effective medicine in the treatment of ovarian endocrine dysfunction in psychologically stressed rats.

Key words: Flavonoids from semen cuscutae; Psychological stress; Hippocampus; Hypothalamic-pituitary-ovarian (HPO) axis; Sex hormone receptors.

Introduction

The reproductive endocrine system is a system which is susceptible to stress-induced injuries. Psychological stress can induce reproductive endocrine disorders, which lead to reproductive endocrine disease. To date, an effective regulatory measure for this type of disease in Western medicine has not been found, and hormone replacement therapy is the primarily-adopted current treatment method in Western medicine practice. The administration of hormones however, has only an uncertain curative effect, and even worse, long-term use of them can result in obvious adverse effects. Chinese medicine has the characteristics of an integrative adjustment and multi-target and manner regulation. These characteristics give it advantages in the prevention and treatment of female ovarian functional disorders. Modern integrated traditional Chinese and Western medicine studies indicate that kidneys are correlated with the hypothalamic-pituitary-ovarian (HPO) axis and reproductive endocrine system. The occurrence of ovarian dysfunction is closely correlated with kidneys, liver, spleen, and hemostasis, among which kidney deficiency performs a basic role; kidney deficiency is caused by abnormal gene expression of neuroendocrine-immune network balance function-mediating related substances, which breaks the functional balance of the neuroendocrine-immune system [1]. Therefore, in the treatment of reproductive endocrine disorders, attention should be placed on kidney reinforcement, considering kidneys being the foundation of prenatal life, which store congenital essence, and govern reproduction. Kidney-reinforcing Chinese medicine has pluralistic and bi-directional regulatory effects on hypothalamus, pituitaries, and ovaries.

Semen cuscutae are the mature seeds of the convolvulaceae plant Cuscuta chinensis Lam. They are widely applied in Chinese medicine practice, especially in the treatment of reproductive endocrine disorders [2-4]. The authors of the current study have performed some studies of the regulatory effect of semen cuscutae on the reproductive endocrine system, and have discovered that semen cuscutae have multiple effects on the hypothalamic-pituitary-gonadal (HPG) axis: They improve the promotive effect of hypothalamus and pituitaries on gonads, enhance the reactivity of pituitaries to gonadotropin-releasing hormone (GnRH), promote follicular development, and increase the numbers of human chorionic gonadotropin (hCG)/luteinizing hormone receptors (LHR), and reinforce their functions, but without obvious influence on the LH level in plasma [5]; semen cuscutae, as well as the flavonoids from them (FSCs), obviously increase the testicular and epididymal weights of young male rats [6] and have estrogen-like activity.

Semen cuscutae are a kind of Chinese medicine which can reinforce and benefit liver and kidneys; FSCs, the efficacious component of semen cuscutae, have an obvious regulatory effect on ovarian endocrine disorders caused by psychological stress. It significantly increases estradiol (E2) and progesterone (P) levels, pituitary LH level, and hypothalamic beta-estradiol progesterone (β-EP) content, but does not affect the follicle-stimulating hormone (FSH) content in psychologically stressed rats...
Materials and Methods

Investigational drug

Flavonoid extracts were obtained from semen custutae using solvent extraction and polyamide column chromatography. The semen custutae were identified as the mature seeds of Cuscuta chinensis Lam by the Chinese Traditional Medicine Identification Division of the Children’s Hospital of Jiangxi province. Thin-layer chromatography demonstrated that four major components were contained in the extracts, including quercetin, hyperoside, astragulin, and quercetin-3-O-β-galactosyl-7-O-β-glucose, which was consistent to that reported in literature [9]. Colorimetry showed that the flavonoid content was between 40% and 50%, taking rutin as the standard reference. Suspensions at concentrations of 10 mg/ml and 5 mg/ml were prepared during the experiment, which were equivalent to and one gram of drug substance, respectively.

Animals

Female Sprague-Dawley (SD) rats, weighing 180-220 g, were supplied by the Laboratory Animal Center of Jiangxi. Experimental procedures were performed strictly following the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and the animal use protocol had been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Children’s Hospital of Jiangxi province.

These animals were bred under natural light exposure at room temperature (20 ± 2°C) and had liberal access to food and water. After acclimation for 2 d, 50 animals were subjected to a daily vaginal smearing examination for possible changes in estrous cycle. They were then divided randomly into the normal control model, high-dose FSC, low-dose FSC, and positive control (given menstruation-regulating and pregnancy-promoting pills) groups. Of the animals, those with two continuous normal estrous cycles were selected for experimental model establishment [10] while others were discarded.

Drug administration

The abovementioned five groups were given physiological saline (0.9%), physiological saline (0.9%), high-dose FSC (10 mg/ml), low-dose FSC (5 mg/ml), and menstruation-regulating and pregnancy-promoting pills (90 mg/ml; Beijing Tongrentang, China), respectively, according to 1.0 ml/100 g after lavage. In the meantime, they, except for the normal control group, underwent psychological stress for 20-d model establishment. After modelling, all groups received five more days of drug administration (once/d).

ER, FSHR, and LHR determination

Hippocampus, hypothalamus, pituitaries, and ovaries were taken rapidly, fixed in 10% formalin and embedded with paraffin. Pathological sections were made for immunohistochemistry. ER was observed after nickel ammonium sulfate-diaminobenzidin (N-DAB) coloration (positive substances are blue-black rather than buffy stained, which are mainly located in neural nuclei). FSHR was observed after DAB coloration (positive substances are buffy-stained, which are mainly located on the membranes of ovarian granular cells). LHR content was determined using an indirect method, based on the reactions between anti-LH antibody and LH-combing sites (LHR content and the reactions are in a positive correlation) [11, 12]. Then, LHR was observed after DAB coloration (positive substances are buffy-stained and are mainly located on the membranes of follicular endomembraneus, interstitial, and luteal granular cells). ER, FSHR, and LHR were analyzed quantitatively by a DVP image analyzer (Nanjing Great Wall, China). Ten sections were taken from each type of tissue, and ten visual fields (× 40) were randomly selected for each section for integral optical density (IOD) determination. The obtained IOD values were used to represent the relative intensity of the expression of ER, FSHR, and LHR in the corresponding tissues.

Statistical analysis

Data were presented as means ± standard error (x ± s) and analyzed by the SPSS10.0 software. Paired t-test was also performed.

Results

ER in the hippocampus

As shown in Table 1, the ER content in the model group significantly decreased, compared with the normal control group (p < 0.05); ER in the high-dose FSC, low-dose FSC, and positive control groups was significantly higher than the model group (p < 0.01); and ER in the high-dose and low-dose FSC groups was significantly lower than that in the positive control group (p < 0.05 and p < 0.01). These results indicate that high-dose FSCs have a better effect on ER than low-dose FSCs, though neither of them can achieve an effect as good as menstruation regulating pills.

ER in the hypothalamus

As shown in Table 2, the ER content in the model group was significantly lower than that in the normal control group (p < 0.05); ER in the high-dose FSC, low-dose FSC, and positive control groups was significantly higher than that in the model group (p < 0.01, p < 0.05, and p < 0.01, respectively); the high-dose FSC and positive control groups did not show a significant difference as compared with the positive control group (p < 0.05). These results indicate that high-dose FSCs have a better effect on ER in the hypothalamus, which is very close to that achieved by menstruation regulating pills.

ER in the pituitaries

As show in Table 3, the ER content in the model group was significantly lower than that in the normal control group (p < 0.01); ER in the high-dose FSC and positive control groups was significantly higher when compared with the model group (p < 0.01), but the low-dose FSC...
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Table 1. — Changes in hippocampal ER content in different groups (x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal number (n)</th>
<th>ER (IOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>9</td>
<td>22.33 ± 6.97</td>
</tr>
<tr>
<td>Model</td>
<td>8</td>
<td>13.45 ± 2.87*</td>
</tr>
<tr>
<td>Low dose</td>
<td>8</td>
<td>27.03 ± 2.63**</td>
</tr>
<tr>
<td>High dose</td>
<td>9</td>
<td>37.84 ± 6.99**</td>
</tr>
<tr>
<td>Positive control</td>
<td>8</td>
<td>49.70 ± 8.47**</td>
</tr>
</tbody>
</table>

* p < 0.05, compared with the normal control group; ** p < 0.01, compared with the model group; and *** p < 0.001, compared with the positive control group.

Table 2. — Changes in hypothalamic ER content in different groups (x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal number (n)</th>
<th>ER (IOD)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>9</td>
<td>32.65 ± 4.9</td>
</tr>
<tr>
<td>Model</td>
<td>8</td>
<td>18.19 ± 1.12*</td>
</tr>
<tr>
<td>Low dose</td>
<td>8</td>
<td>34.18 ± 6.79**</td>
</tr>
<tr>
<td>High dose</td>
<td>9</td>
<td>47.65 ± 15.95*</td>
</tr>
<tr>
<td>Positive control</td>
<td>8</td>
<td>56.48 ± 19.60**</td>
</tr>
</tbody>
</table>

* p < 0.05, compared with the normal control group; ** p < 0.01, compared with the model group; and *** p < 0.001, compared with the positive control group.

Table 3. — Changes in pituitary ER content in different groups (x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal number (n)</th>
<th>ER (IOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>9</td>
<td>42.25 ± 7.86</td>
</tr>
<tr>
<td>Model</td>
<td>8</td>
<td>24.44 ± 6.86**</td>
</tr>
<tr>
<td>Low dose</td>
<td>8</td>
<td>38.43 ± 1.66**</td>
</tr>
<tr>
<td>High dose</td>
<td>9</td>
<td>50.24 ± 2.5**</td>
</tr>
<tr>
<td>Positive control</td>
<td>8</td>
<td>45.42 ± 4.64**</td>
</tr>
</tbody>
</table>

** p < 0.01, compared with the normal control group; * p < 0.05 and ** p < 0.01, compared with the model group; and *** p < 0.001, compared with the positive control group.

Table 4. — Changes in the ovarian content of FSHR and LHR in different groups (x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal number (n)</th>
<th>FSHR (IOD)</th>
<th>LHR (IOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>9</td>
<td>68.97 ± 16.21</td>
<td>99.16 ± 12.93</td>
</tr>
<tr>
<td>Model</td>
<td>9</td>
<td>102.44 ± 8.28**</td>
<td>72.03 ± 10.47**</td>
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<tr>
<td>Low dose</td>
<td>8</td>
<td>101.90 ± 13.6**</td>
<td>84.74 ± 15.07**</td>
</tr>
<tr>
<td>High dose</td>
<td>9</td>
<td>97.89 ± 15.98</td>
<td>104.31 ± 16.7**</td>
</tr>
<tr>
<td>Positive control</td>
<td>8</td>
<td>86.68 ± 16.71*</td>
<td>101.60 ± 15.40**</td>
</tr>
</tbody>
</table>

** p < 0.01, compared with the normal control group; * p < 0.05 and ** p < 0.01, compared with the model group; and *** p < 0.001, compared with the positive control group; IOD = integrated optical density.

Discussion

The present study aimed to solve the questions of whether there are changes in hippocampal-HPO sex hormone receptors when rats are exposed to psychological stress and whether FSCs have a regulatory effect on these changes. The results in this study gave affirmative answers: hippocampal-HPO sex hormone receptors do change when rats are exposed to psychological stress and FSCs have a regulatory effect on these changes.

The hippocampus is a structure which contains different messenger receptors; when there is psychological stress, it does not only serve as a high-position center of accommodation, but becomes the most sensitive region to the stress [13, 14]. Evidence has shown that the hippocampus contains alpha and beta types of estrogen receptors (ERs and ERF). ER protein in the rat brain is basically located in neuronal nuclei, and it is not positively expressed in cytoplasm or process; its high expression was observed in the hippocampal fissures, lateral amygdaloid nuclei, and the horizontal part of the diagonal band; it is extensively expressed in the basal forebrain in rats, suggesting that estrogens may participate in the regulation of the neural structure and function in this area; estrogens can up-regulate ER expression, which, in turn, regulates reproductive endocrine [15]. The present study showed that decreased hippocampal ER expression in psychological stressed rats influenced the reproductive endocrine, but the study failed to demonstrate how this decrease influenced the hypothalamus. Presumably, this process is mediated by ER, through which the synthesis of the neurotransmitters in neuronal cytoplasm is influenced first and this condition further influences the hypothalamus through neurotransmitter pathways (i.e., nerve fiber bundles, like the prefrontal cortex region of noradrenergic nerves); or, psychological stress causing changes in the content and proportion of neurotransmitters first and then such a condition is mediated by ER to influence the hypothalamus (i.e., through the feedback action of ER). Nevertheless, how a change in ER influences the hypothalamus still needs to be explored in the future.

ER and P receptor coexist in some hypothalamic regions [16]. The binding of estrogens and progesterone...
with their receptors can affect the secretion of GnRH through regulation on neurotransmitters; a decreased level of E2 causes reduced GnRH, which results in an inhibitory effect on the HPO axis [17, 18]. Therefore, psychological stress causes decreases in E2 and ER in rats, which affect the synthesis and secretion of hypothalamic neurotransmitters. This condition may have an inhibitory effect on the HPO axis, which further affects the reproductive endocrine.

Since the first application of immunohistochemistry in the observation of the pituitary ERα and ERβ distributions in rats [19], more and more studies have focused on pituitary ER. ERα mRNA expression in rats decreases after ovariectomy, but such a decrease can be reversed after estrogen replacement treatment [20]. Surgical stress can induce the appearance of noticeable particles and Golgi vesicles in rat anterior pituitary prolactin (PRL) cells, increase PRL, and E2 in plasma and decrease ER in hepatic cells [21]. The present study showed that a decrease in hippocampal-hypothalamic-pituitary ER as well as ovarian LHR, and an increase in ovarian FSHR in psychologically stressed rats affected the secretion of reproductive hormones and neurotransmitters by the pituitaries, which further influenced the reproductive endocrine function of the ovaries.

This study also showed that FSCs can up-regulate hippocampal-hypothalamic-pituitary ER, and ovarian LHR in psychologically stressed rats, but cannot bring about an effect on ovarian FSHR. Presumably, FSHR and LHR are distributed in different regions of the ovaries (e.g., FSHR is mainly distributed on the surface of granular cells while LH is mainly distributed on the surface of follicular endomembranous cells), which results in different sensitive degrees of FSHR and LHR to FSCs. In addition, this study showed that the effects of FSCs on hippocampal-hypothalamic-pituitary ER and ovarian LHR in psychologically stressed rats displayed a certain dose-dependent manner.

To draw a conclusion, FSCs can up-regulate hippocampal-hypothalamic-pituitary ER and ovarian LHR expression in psychologically stressed rats, which is, at least, the partial mechanism of the regulatory effects of FSCs on HPO functions, and FSCs can serve as effective medicine in the treatment of ovarian endocrine dysfunction in rats exposed to psychological stress.

References

Clinical features and treatment of lactational mastitis: the experience from a binational study

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Summary
The characteristics of 38 patients with mastitis are listed in this study, including nationality, age, parity, history of mastitis, clinical and laboratory findings, and medical treatment. Differential diagnosis was mainly correlated to breast engorgement. Mastitis was primarily related to *Staphylococcus aureus* and it was more common in primiparous patients.

Key words: Lactational mastitis; Staphylococcus aureus; Parity.

Introduction
Mastitis is a relatively common infectious disease during lactation, typically presenting as a localized and painful inflammation of the breast. The main etiological agents are *staphylococcus aureus*, and less frequently Group A streptococci, and/or corynebacteria [1-4]. These bacteria gain access to stagnant milk through the nipple [5]. Mastitis is often complicated by abscess formation in the affected breast if treatment is delayed [6]. Therefore, cultures of breast milk for bacteria could be useful [7, 8], assisting in differential diagnosis [8] and are generally recommended when the infection is severe, hospital-acquired, or unresponsive to appropriate antibiotics [9].

Diagnosis of mastitis is usually clinical and it typically presents as a hard, red, tender, and swollen area of one breast in a nursing mother. It is commonly accompanied by fever and malaise [9] and other systemic symptoms that may vary, and includes myalgia, chills, and flu-like symptoms [5]. Generally, mastitis in the early stages has a subtle presentation, while patients with advanced infection may present with a large area of breast swelling with overlying skin changes (eg, erythema). Reactive lymphadenopathy can also cause axillary pain and swelling [5]. In general, laboratory tests are not needed for the diagnosis of mastitis considering that septic shock rarely occurs [10] and blood cultures are of little value (unless the patient appears septic).

In a lactating woman, severe engorgement must be distinguished from mastitis which typically occurs on days two to four postpartum in women who are not nursing or at any time if breastfeeding is interrupted. On the contrary, mastitis usually occurs during the third or fourth week postpartum. This is due to the fact that breast infection is acquired from the neonate after breastfeeding and nipple cracking [11].

Materials and Methods
Two hundred questionnaires, related to mastitis diagnosis and treatment during lactation, were distributed to 20 obstetricians-gynecologists and five midwives in five hospitals from two different countries (Greece and Romania). Fifteen questionnaires were incompletely answered and in two patients mastitis was not related to lactation. These 17 cases were excluded for further analysis. Similarly, cases with breast engorgement (bilateral with generalized involvement) were not included as “mastitis”. Finally, questionnaires from 38 patients were fully answered and were thus included in the study.

Results
It was estimated that patients included in the study constituted the three percent of patients that underwent vaginal delivery or cesarian section during the time of evaluation (> 1,200 women). Mastitis was mainly related to *Staphylococcus aureus* and it was more common in primiparous patients, although parity was not related to mastitis as a statistically significant factor (p > 0.2 with continuity correction). Nationality, age, parity of patients, and history of mastitis are shown in Table 1. Clinical and laboratory findings are shown in Tables 2 and 3, respectively. Medical treatment is shown in Table 4.

Discussion
Lactational mastitis in the present study was mainly related to *Staphylococcus aureus*, and less commonly to streptococci, or other bacteria. This finding is similar to the current bibliographic and investigational data [1, 4]. Although patients included in the study constituted three percent of women delivering during the time of evaluation, percentage of mastitis was actually higher, taking into account 15 more patients (total ~4.5%) with probable mastitis not included in the study due to incompletely returned questionnaires. Taking into account that mastitis has been estimated to occur in two to 16 percent of breastfeeding women, in the patients studied, this condition was near the lower limit of values reported from other studies [9, 12, 13]. The risk of mastitis requiring hospitalization is much lower (0.09%) [14]. As expected, mastitis was more common in the primiparous patients (> 50%) [4].

Risk factors for mastitis include an episode of mastitis in the past, and in our study this was the case for 17 women (44% primiparous, 14% multiparous) [4]. Mastitis is common in the primiparous patients (> 50%) [4]. Risk factors for mastitis include an episode of mastitis in the past, and in our study this was the case for 17 women (44% primiparous, 14% multiparous) [4].

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study, all belonging to the group of one previous term pregnancy with mastitis). Other main risk factors include severe prolonged unilateral engorgement, nipple excoriation or cracking, and poor milk drainage [15]. As far as the latter factor is concerned, it is obvious that breastfeeding should be encouraged in this situation, although that surprisingly a high percentage of health professionals advise patients to stop breastfeeding and do not recommend pumping. This is in contrast to all reported data from previous research and available data.

References

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Perinatal outcome of singleton pregnancies following in vitro fertilization

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Summary

Purpose of investigation: To determine whether in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) singleton pregnancies are at increased risk for maternal and fetal complications than spontaneous singleton conceptions. Materials and Methods: The pregnancy outcome of 634 singleton pregnancies after IVF/ICSI delivered at the Clinic for Gynecology and Obstetrics during the period January 2006 to January 2010 were compared to 634 matched singleton controls, matched one by one by age, parity, education, and body mass index (BMI). Differences in pregnancy outcomes between the groups were assessed using Student’s t-test with Yates correction for continuous variables and Chi-squared test for categorical variables. Results: The mean gestational age at delivery of the IVF group was 38.13 ± 1.72 weeks, slightly shorter than spontaneously conceived singletons at 38.65 ± 1.79 weeks. The diagnosis of gestational diabetes mellitus (GDM) was frequently made in the IVF group (11.82% vs 8.35%, t = 2.052, p < 0.05). Total preterm delivery rate of IVF pregnancies was 9.30%, significantly higher than the controls 5.85% (t = 2.33, p < 0.05), especially at the 30-32 weeks gestation period. The predominant mode of delivery after IVF pregnancy was cesarean section (80.75% vs 31.38% at spontaneously conceived, t = 17.71, p < 0.001), while vaginal route was the choice for naturally originated pregnancies 68.6% vs 19.24% (p < 0.01). No differences were found in the average birth weights, LBW, VLBW, SGA, and LGA regarding the pregnancy origin. Perinatal mortality rates were comparable among singletons with different pregnancy origin. Conclusions: Singleton from IVF/ICSI pregnancies have poorer perinatal outcome associated with higher rates of cesarean sections, preterm birth and prematurity, fetal malpresentation (breech presentation), and the occurrence of maternal GDM in pregnancy.

Key words: In vitro fertilization; Singletons; Pregnancy; Perinatal outcome; Cesarean section.

Introduction

Since the first child was born in 1978 after fertility in vitro treatment, the number of assisted pregnancies has increased steadily [1] and currently accounts for 1%-4% of all conceptions [2]. Much interest has been dedicated to the safety of assisted reproduction technology (ART) [3].

The use of in vitro fertilization (IVF) has shown to be associated with various pregnancy and neonatal problems, most significantly due to the high rate of multiple births. Multiple pregnancies after IVF account for 20%-25% [4].

Numerous studies at first indicated poor perinatal outcomes for assisted twin compared to spontaneous twin conceptions [5-7]. The increased risk for adverse perinatal outcome in IVF pregnancies cannot be entirely explained by the incidence of multiple births. The first indication that assisted singletons may also have poorer outcomes appeared in 1985 as reported by an Australian IVF collaborative group [8]. In numerous studies, it has been demonstrated that singletons, besides multiples, have a poorer outcome, compared to singletons in the general population [7, 9-11]. The cause of this risk is not fully understood, but for safety reasons it is important to elucidate this question.

The explanations include various categories such as the IVF laboratory procedures [9], differences in maternal characteristics as age, parity, smoking, obesity [7], type of infertility and duration, presence and type of ovarian stimulation, and presence of twin pregnancies spontaneously reduced to singletons (vanishing twins) [6, 12].

Unadjusted analysis suggested a two-fold increased risk of preeclampsia, placental abruption, cesarean section, and operative delivery, and five-fold increased risk of placenta previa in spontaneous singleton pregnancies in women with a history of infertility compared with the general population [13-16].

Several studies [17, 18] have indicated that the risk of perinatal mortality is increased in singleton pregnancies achieved after ART. Significantly higher rates of preterm delivery and low birth weight (LBW) were observed for IVF singletons [16], even after adjustment for maternal age, parity, duration of infertility, smoking, and body mass index (BMI).

Babies born after intracytoplasmatic sperm injection (ICSI) procedures have demonstrated to have a higher incidence of both autosomal and sex chromosome abnormalities, but surprisingly similar to those reported after classic IVF procedure [5].

The present authors investigated the influence of ART procedures on perinatal outcome by comparing the group of singleton ART pregnancies and the group of spontaneously-conceived singletons pregnancies.

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Materials and Methods

The obstetrical outcome of 634 consecutive singleton pregnancies after ART, delivered at the Clinic for Gynecology and Obstetrics, Clinical Center of Serbia, from the period January 2006 to January 2010 were compared to 634 matched controls (spontaneously conceived singletons), matching one to one by age, education, parity, and BMI. Only pregnancies with duration of more than 26 weeks were included. The Serbian definition of birth comprises all live born and stillbirths delivered after 26 weeks of gestation.

The study group was formed by 634 primiparous women delivered at the Clinic, with singleton ART pregnancy achieved with controlled ovarian hyperstimulation with a complete documentation of ART procedure (IVF or ICSI), and infertility history treatment. The control group was formed by 634 patients without infertility problems, that spontaneously conceived a singleton pregnancy and delivered at the same hospital within the study period. The controls and the cases were matched one to one for age, parity, education, body mass index (BMI), site, and time of delivery (within one month).

Those pregnancies resulting from an oocyte donation, cryopreserved cycles or conceived as twin but continued as singleton, were excluded from the study (26 pregnancies). Details on patient demographics, life style, and pregnancy outcome were collected by chart review and patients’ questionnaire. All pregnant patients with incomplete or insufficient data on the course of pregnancy and delivery were excluded from the study, as well as patients without appropriate control after IVF/ICSI procedure. The gestational age for the ART group was calculated as the duration between the date of embryo transfer and delivery plus fourteen days; for spontaneous conceptions the gestational age was based on the last menstrual period or estimated at the first trimester ultrasound scan.

Maternal complications’ pathology PIH (pregnancy-induced hypertension), gestational diabetes mellitus (GDM), preeclampsia, eclampsia, placenta previa, PROM, PPROM (preterm/premature rupture of membranes), need for cerclage application, and placental abruption were recorded. Data concerning fetal pathology as intrauterine fetal demise, chromosomopathy, presence of congenital malformations, IUGR (intrauterine growth restriction), and macrosomia were encountered. Complications during labor, mode of delivery, gestational age, birth weigh and Apgar scores, neonatal complications, admission to neonatal intensive care unit (NICU), and perinatal mortality were compared between the groups.

Low birth weight (LBW) and very low birth weight (VLBW) were defined as birth weight < 2,500 g and < 1,500 g, respectively. Preterm and very preterm deliveries were defined as such before 37 and 32 completed gestational weeks, respectively. Small for gestational age (SGA) and very small for gestational age were defined as < 10th and < 23rd percentile for gestational age.

In controlled ovarian hyperstimulation, a flare-up or down-regulation protocol was used with gonadotropin-releasing hormone (GnRH) agonists and recombinant follicle-stimulating hormone (rFSH) or highly purified-human menopausal gonadotropin (HP-HMG). When at least half of the dominant follicles reached 18 mm in average diameter, human chorionic gonadotropin (hCG) was administered and oocyte retrieval was performed after 34 to 36 hours. Conventional IVF and ICSI procedures were performed according to standard procedures. Embryo transfer was performed on the second or third day after oocyte retrieval. Luteal support consisted of micronized oral/vaginal progesterone 600 mg per day or muscular progesterone 250 mg on every second day.

The authors performed a univariable analysis, using Chi squared test for categorical outcome variables and Student’s t-test for continuous outcome variables. A p value of < 0.05 was considered significant. Linear regression was applied for the analysis of birth weight, with adjustment for prognostic factors. The Institutional review board approved the study.

Results

A total of 634 IVF/ICSI singletons and 634 consecutive spontaneously conceived singletons were compared during the 2006-2010 period. Baseline patients’ characteristics are presented in Table 1. The average age, education, parity, and BMI did not differ between studies and controls; however, regarding prior obstetric history, there was a higher risk of a spontaneous abortion (8.51% in the IVF group vs 4.258% in the controls) or ectopic pregnancy, if the pregnancy was of IVF origin. This differ-

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<th>Table 1. — Maternal characteristics.</th>
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BMI: body mass index; p: probability; NS: no significance.

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<th>Table 2. — Complications during pregnancy.</th>
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<td>Category</td>
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<td>Placental abruption</td>
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<td>Maternal blood transition</td>
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PIH: pregnancy-induced hypertension; GDM: gestational diabetes mellitus; PE: preeclampsia; PPROM: premature preterm rupture of membranes; PROM: premature rupture of membranes; p: probability; NS: no significance.
Perinatal outcome of singleton pregnancies following in vitro fertilization

The significant difference between groups in a number of pregnancies delivered through elective cesarean section is obvious ($p < 0.01$) and the most contributing category is on mother’s explicit request.

The causes of infertility in the IVF group were tubal pathology (28%), male factor (22%), ovarian etiology (15%), unknown origin (14%), and other causes (21% - combined, cervical, uterine, and immunological).

The management of ART pregnancies included singletons and seems to differ from that of spontaneously-conceived with singletons. The mode of delivery, indications, complications, and fetal presentation are listed in Table 3. Malpresentation or malposition of the fetus (excluding cephalic vertex occiput anterior presentation) was more common to occur in the IVF group, with a statistically significant difference (80 cases (12.6%) vs 56 cases (8.83%), $t = 2.508, p < 0.05$). Upon further analysis, malpresentation was divided into the categories breech presentation (9.46% of IVF vs 4.42% at controls), occipito-posterior position (1.26% vs 3.78%), and others (1.89% of IVF vs 0.63% of controls), included brow presentation, face presentation, vertex presentation, transverse lie). Among 634 singleton fetuses after ART, 60 (9.46%) were breech, compared with 28 (4.42%) among spontaneously-conceived pregnancies ($t = 3.533, p < 0.001$). Among ART pregnancies, there was no difference in the risk of breech presentation between the IVF group and the group conceived with ICSI procedure.

The rate of cesarean section were high in both groups (80.75% of IVF group vs 31.38% of controls), with almost two-thirds of pregnancies in the IVF group ending in an elective procedure (highly-significant difference, $t = 17.71, p < 0.001$). The large number of cesarean sections in both groups was elective, and the rate of emergency sections was 22.08% of IVF vs 11.58% of controls. Combined with the higher rate of malpresentation and breech in IVF group, the probable prominent contributing factors included the actual increase in complications predisposing operative delivery or a component of iatrogenic interventions. Most pregnancies of spontaneous origin ended up in vaginal delivery mode 68.6%, with only 19.24% of the IVF group delivered vaginally ($p < 0.01$). Also, the vaginal operative delivery by forceps, was almost exclusively performed in the controls (1.25% of controls vs 0.157% only one case of IVF group, $t = 2.34, p < 0.01$).

Since 80% of controls were delivered through vaginal route, the frequency of soft-tissue injuries expectancy was higher in terms of cervical and vaginal injuries and perineum ruptures than in the IVF group, with a high statistical significance.

Since the contribution of elective cesarean section rate is most evident in the high rate of pregnancies in the IVF group, the indications are listed in Figure 1. Similar data
is registered in categories of fetal indications 24.41% of the IVF group vs 14.39% of the controls, of maternal indications 6.10% vs 2.37%, of category maternal and fetal indications altogether 6.84% vs 3.06%, and mothers’ explicit request was only 21.48% in the IVF group. The indication that contributed mostly to the increased rate of elective cesarean sections in the IVF group was “explicit mothers’ request”, since the other indications presented with similar percentages.

The data on neonatal i.e. perinatal outcome are presented in Table 4. The mean gestational age at delivery of the IVF group was 38.13 ± 1.72 weeks, and was slightly shorter than the spontaneously conceived singletons 38.65 ± 1.79 weeks, but with no significance. Total preterm delivery in the IVF group in 9.30% was substantially higher than the controls in 5.85%, with significance (t = 2.33, p < 0.05). Upon further analysis, when total preterm delivery rate was divided into categories of different gestational age (< 32 weeks of gestation, < 34 weeks of gestation, < 37 weeks of gestation), the difference between the groups became insignificant; however, if the time interval of the preterm pregnancy duration less than 32 weeks of gestation, it was divided into two periods, first from 30-32 weeks of gestation and the second less than 30 weeks of gestation, and the difference therefore became obvious and highly-significant 2.52% (16 cases) of the IVF group and 0.47% (three cases) in the controls (t = 3.00, p < 0.01).

The mean birth weight in the IVF group was 3,214 ± 579.4 g, lower than those of the controls 3,294 ± 549.2 g, but with no statistical significance. The rates of VLBW infants with less than 1,500 g (1.57% of IVF vs 0.79% of controls) and LBW less than 2,500 g (8.04% of IVF group vs 6.00% of controls), were comparable. Also, the prevalence of SGA, defined as birth weight less than 10th percentile for gestational age (23 cases; 3.62% of IVF group vs 18 cases; 2.84% of controls) and LGA with a birth weight more than 90th percentile for the appropriate gestational age were also similar between groups.

Neonatal admission to NICU accounted for 55 infants in the IVF group and 42 cases in the control group. There were six stillbirths in the IVF group including one sudden fetal death at 39 wg, weight 3,900 g, with umbilical knot accident; one with a weight 2,200 g, at 34 wg, with hydrothorax; one at 31 wg, weight 1,250 g for chorioamnionitis; one at 31 wg, weight 1,400 g, with placenta previa for abruption; one at 35 wg, weight 1,450 g, with severe IUGR; one at 38 wg, weight 2,700 g, for placental abruption. Two neonates died during the early neonatal period, the first delivered at 32 wg, weight 700 g with severe asphyxia, and the second delivered at 26 wg, weight 640 g with placental...
abruption. At controls, the three cases of stillbirth included infants delivered at 27 wg, weight 1,000 g, and two in term pregnancies at 39 wg weight 2,650 g for unknown reasons and at 37 wg, weight 2,400 g with IUGR and multiple anomalies. Two live born infants died after birth due to complications of acute asphyxia with instrumental forceps delivery and one case after prolonged pregnancy and prolonged delivery.

Only one case of chromosopathy included Klinefelter syndrome 47XXY, was recorded in the whole study in the IVF group and was detected prenatally through amniocentesis. Congenital malformations were present in six cases in the IVF group (two cases of intrabdominal cysts in females, heart anomaly (one case), hydrothorax (one case), right hand anomaly (one case), and isolated ventriculomegaly (one case); all except hydrothorax at stillbirth fetus, compatible with postnatal life. Of the spontaneously-conceived infants, five showed evidence of malformations including two cases of torticollis, one case of multiple anomalies, and two cases with heart malformations. Perinatal mortality accounted for eight cases (1.26%) at IVF group and five cases (0.788%) of controls, assuming the categories of intrauterine death after 26 wg and neonatal death within 28 days of birth.

Discussion

The first indication that assisted singleton pregnancies, besides multiples, have poorer pregnancy outcome compared to spontaneously conceived ones, appeared in 1985 by an Australian IVF collaborative group [8]. At that time, it was not clear how much the above reasons were related to assisted reproduction procedure or to confounders, such as maternal age and parity.

Several matched-cohort studies have since confirmed those findings regarding differences between assisted singletons and spontaneously conceived ones [19-22]. However, a number of studies, mainly formed one center, showed an opposite trend [23, 24], but the problem of most studies was the selection of controls, the heterogeneity of assisted reproductive group, small sample size, and different parameters for pregnancy outcome.

Singletons conceived after assisted fertilization are at higher risk of low birth weight, preterm delivery, and perinatal death than spontaneously-conceived singletons, suggesting that the technology and not the factors contributing to infertility might cause differences in risk [7, 9, 25, 26].

Increased risk of adverse pregnancy outcome after IVF or ICSI procedure, opposite to the previous theories, may be the result of treatment-related factors (in vitro culture conditions, or hormonal stimulation) as well as patient-related factors (type of infertility, duration of infertility, other characteristics of subfertile patients) [27].

Romundstad et al., in their recent study found no significant differences in birth weight and perinatal outcome parameters when compared to sibling singletons born in 2,500 women who had conceived one child spontaneously and another child with IVF. These results suggest that patients’ factors (contributing to infertility), rather than IVF technology, are responsible for the less favorable outcome after IVF [25].

The mean maternal age, grade of education, BMI, and parity were comparable between groups but there were almost twice as many women in the IVF/ICSI group with history of spontaneous abortion (8.51% IVF group vs 4.258% controls, p < 0.01) and ectopic pregnancy (6.15% IVF group vs 3.22% controls, p < 0.001). The difference is significant and expected, in favor of infertility patients, because these patients were in demand for IVF technology as the last option to solve their infertility problems.

IVF singletons do not have a greater risk of maternal complications in categories of pregnancy-induced hypertension (PIH), pre-eclampsia, placenta previa, PPROM, PROM, anemia, and the need for blood transfusion in the study, as in many studies [12, 28]. Interestingly, there is a significant difference in the term of GDM if the IVF/ICSI treatment is present (11.82% IVF group vs 8.35% controls, p < 0.05). The present authors found no studies reported with similar findings. These results need to be tested on larger samples in multicentre studies. Perhaps, pregnancy management was more meticulous in the IVF group than in the controls, with better diagnosis and more required oGTT performance, which resulted with an obvious difference.

The risk of developing pre-eclampsia and pregnancy-induced hypertension is increased in ART pregnancies reported by Shevell et al., contrary to the present study results [29]. Obstetric complications as pre-eclampsia, placental abruption, and placenta previa occur more frequently in IVF singleton pregnancies compared with spontaneous ones [16-18]. Confirmation of this finding has no support in the present research.

Fetal position/presentation was unfavorable in 12.61% of cases in the IVF group vs 8.83% in the controls with an obvious significant difference (p < 0.05) with a dominance of breech presentation. Among 634 singletons after ART, 9.46% were breech presentation compared with 4.42% among spontaneously conceived ones. (t = 3.533, p < 0.001), the most frequent malposition in both groups. The other fetal malposition/malpresentation are similarly distributed in both groups, but with no significance.

There is some evidence that breech presentation occurs more often in pregnancies following assisted fertilization in studies of Ombelet et al., and Romundstad et al., but it is unclear to which degree the excess risk is due to technology itself or to the other factors associated to assisted fertilization [30, 31]. The etiology of breech presentation still remains unclear, but it is linked to prematurity, a first pregnancy, advanced maternal age, placenta previa, uterine anomalies, and a previous breech presentation [32].

The obstetrics management of ART pregnancies seems to differ from that of spontaneously conceives ones, and there is a growing tendency to treat them as “special” pregnancies. ART deliveries are more likely to be
induced, and the rate of cesarean sections, especially elective, is considerably higher [33-36]. About 80% of ART pregnancies in the present study were delivered through cesarean section, opposite to 31.38% pregnancies of spontaneously-conceived group, with substantial difference (p < 0.001). The frequency of cesarean section in the current hospital is about 35% in the general population. More than two-thirds of all number of ART cesarean sections are elective, while at controls that percentage reached about half in all. The possible explanation of the difference may include the different management of IVF pregnancies and allowance of “mothers’ explicit request” as indication for elective cesarean section in IVF pregnancies. Along with relative indication “pregnancy after IVF”, these are the most contributing factors to a high rate of elective cesarean sections. For most couples with fertility problems, there is also a psychological burden, accompanied with the substantial economic burden for infertility treatment. These factors may also explain why their pregnancies are subjected to closer surveillance and different management by obstetricians than spontaneously conceived pregnancies.

The optimal mode of delivery for breech presentation is controversial. Indications for cesarean sections are typically diverse; some will be performed as an elective procedure, whereas others may be due to an acute obstetric situation. Romundstad et al., found that the rate of elective cesarean section for breech deliveries was considerably higher in ART group, and the rate of acute cesarean sections for cephalic deliveries was also higher [31]. These findings indicate that ART pregnancies are a target for more active obstetric management. This need for intervention is probably one of the contributing factors to a slightly shorter length of ART gestation.

The average duration of pregnancy is similar, regardless of origin and is 38.68 ± 1.79 weeks for spontaneously-conceived while a little shorter for IVF group, 38.13 ± 1.72, weeks, but when total deliveries are divided into the categories of term and preterm delivery, the difference becomes obvious. The event of preterm delivery is more likely to occur in IVF pregnancies (9.30% of IVF group vs 5.85% of controls, p < 0.05). Further analysis of preterm labor discovered that the significant difference between the groups is in those delivered in the interval from 30 to 32 weeks of gestation - 2.52% (16 cases) of IVF group and 0.47% (three cases) of controls (t = 3.00, p < 0.01).

Four meta-analyses of perinatal outcomes in singleton pregnancies found that compared with spontaneously conceived singletons in the general population, those born after IVF/ICSI are about twice as likely to be born preterm, are nearly three times more likely to weigh less than 1,500 g, and have 50% higher risk of being SGA [7, 9, 25, 37]. No differences were recorder in the frequency of LBW < 2,500 g and VLBW < 1,500 g in the present study. This is consistent with other studies referring data from single hospital centers [24, 34, 38]. Some previous studies found an increase risk for VLBW < 1,500 grams among the singletons conceived after IVF, but the present study does not uphold those findings [9, 10, 39]. Also, the incidence of SGA and large for gestation age (LGA) do not differ according to the pregnancy origin. The term singletons were more likely to have a low birth weight, consistent with previous reports of LBW in children conceived with ART [3, 36, 40, 41]. Wang et al., 1994 [42] suggested that ART pregnancies have increased risk for placental insufficiency and IUGR. On the contrary, the current study results, showed comparable rates of infants classified as SGA (< 10th percentile) in IVF and in spontaneously-conceived pregnancies, are similar to recent single center studies [43, 44].

In conclusion, singletons from IVF/ICSI pregnancies have poorer perinatal outcome associated with higher rates of cesarean sections, preterm birth and prematurity, fetal malpresentation (breech presentation), and the occurrence of maternal GDM in pregnancy, compared with singletons spontaneously conceived.

References

Perinatal outcome of singleton pregnancies following in vitro fertilization


The use of sympathomimetic amines for the treatment of severe constipation refractory to conventional therapy - case report

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Introduction
There have been several gastrointestinal (GI) pathologic conditions that proved to be refractory to standard therapy and yet responded quickly and quite effectively to treatment with the sympathomimetic amine dextroamphetamine sulfate. Several anecdotal reports have demonstrated that sympathomimetic amine therapy has successfully provided long-term relief from GI motility disorders of the esophagus, gastroparesis, and pseudo-intestinal obstruction that had been refractory to other therapies [1-3]. Similarly marked improvement of some inflammatory bowel disorders, e.g., ulcerative colitis and Crohn’s disease, following treatment with dextroamphetamine sulfate have been reported despite failing to respond to other therapies, including drugs that inhibit the cytokine tumor necrosis factor alpha [4, 5]. These GI conditions are all believed to be related to sympathetic nervous system hypofunction which can cause diminished function of the enteric nerves leading to decreased motility [6]. Alternatively, the sympathetic nervous system acts to diminish cellular permeability. Sympathetic fibers are known to innervate the mucosal epithelial cells [6]. Thus diminished sympathetic nervous system function may allow absorption of chemicals and toxins from the stool leading to inflammation or muscle paresis [7-9]. The purpose of this report is to describe another GI disorder that failed to respond to standard therapy but responded immediately to dextroamphetamine sulfate therapy.

Case Report
At the age of 16, a young woman developed marked constipation. Bowel movements would occur every two to three weeks and even that would require taking laxatives, e.g., magnesium citrate, metamucil, konsyl, and docusate which did not help very much. A gastroenterologist was consulted. Further evaluation including magnetic resonance imaging (MRI) of the abdomen, gastroscopy, and bowel motility studies all failed to detect any abnormalities.

She was prescribed lubiprostone 24 mcg daily. She did have a bowel movement in three days and then seemed to have one every two days. However after two weeks of therapy, her severe constipation of two to three week intervals (and sometimes five weeks) returned along with moderately severe abdominal pain when a two-week interval was approaching.

Her severe constipation problem persisted for two years. She consulted our group hoping to find a possible endocrine etiology that could be corrected. Her thyroid tests were normal with a free thyroid level of 1.2 ng/dl (nl 0.7 - 1.8) and her thyroid-stimulating hormone level was 2.76 micro IU/ml (nl 0.35 - 4.50). Considering our very positive experience in treating various GI problems with dextroamphetamine sulfate, she was started on the same with 15 mg extended release capsule once daily [1-5]. The first day she took the sympathomimetic amine, she had a bowel movement two hours after taking it and she has had one everyday for 1.5 years since she has been taking this...
medication. Interestingly, she ran out of medication for a week before she could return to our office for a prescription and her bowel movements ceased the day she stopped only to return the first day she started it again. She has had no side-effects.

Discussion

The hope of publishing these case reports is to generate sufficient interest in some physicians to initiate randomized controlled trials (RCT). If efficacy of this very safe well-tolerated drug which has no dependence or withdrawal effects in the dosages prescribed (usually 30 mg/day is the maximum dosage) and is confirmed by a larger RCT, then hopefully it will be found to be the most effective and safest drug for inflammatory bowel disease and GI motility disorders.

Recently, the importance of the sympathetic nervous system in the pathophysiology of GI motility disorders and inflammatory bowel disease has been established [1-9]. What is not well-known is the potential of quick and effective resolution of symptoms with the sympathomimetic amine dextroamphetamine sulfate, which is usually without immediate side-effects or risk of developing cancer or risk of severe infection.

Catecholamines are the classical neurotransmitters of the sympathetic nervous system and they increase intracellular cyclic adenosine monophosphate, which in turn, inhibit certain proinflammatory cytokines, e.g., tumor necrosis alpha or interferon gamma [10]. It is hoped that this case report will help elicit interest in a randomized controlled or randomized comparison study using dextroamphetamine sulfate for people with GI motility disorders, as in this case, or for cases of inflammatory bowel disease. It should be recalled that this type of therapy may be the treatment of choice for pelvic pain [11]. Thus this condition may be more familial to the gynecologist than most gastroenterologists. Thus it may be prudent for the gynecologist to prescribe this drug first if presented with the symptoms rather than subject the patient to extensive invasive testing and other potential therapies with less efficacy and more side-effects. Obviously if significant improvement does not occur, the gynecologist should then refer the woman to a gastroenterologist to exclude serious pathology, e.g., colon cancer.

References


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Vanishing twins in diamniotic dichorionic in vitro fertilization gestation in mid-second trimester

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Summary
The authors report a diamniotic dichorionic twin pregnancy after in vitro fertilization (IVF) in mid-second trimester. The dead fetuses were delivered by cesarean section at the 20th week of gestation. The authors discuss management aspects and review of the literature.

Key words: Vanishing twins; IFV gestation; Twin pregnancy; Intrauterine demise in second trimester.

Introduction
It is common knowledge that the incidence of abortion decreases as pregnancy progresses. The risk of perinatal morbidity and mortality in twins is higher than in singletons. In comparison to dichorionic twins, monochorionic twins are at increased risk for perinatal mortality and morbidity. In both types of twins growth disordance can occur. Complications (acardiac twins, acute and chronic twin to twin transfusion syndrome) are due to different combinations of vascular anastomoses. Monochorionic diamniotic twin pregnancies have a riskier pregnancy course than their dichorionic counterparts because of the vascular anastomoses between the two fetal circulations.

Case Report
A 51-year-old woman with a history of one induced abortion was referred at the antenatal clinic at 18 weeks of gestation after an in vitro fertilization (IVF) attempt.

Blood tests were normal at that time. Detailed ultrasonography showed an intrauterine diamniotic dichorionic gestation with two viable fetuses. The first fetus had an estimated fetal body weight (EFBW) of 212 g that sonographically corresponded to 17 + 5 weeks of gestation, whereas the second one had an EFBW of 165 g that corresponded to 16 + 6 weeks of gestation.

During the next visit at the clinic, negative embryo pulses of both twins were revealed while the infection and blood coagulation parameters remained within the normal range and no signs of chorioamnionitis were detected. Antibiotics were administered and an effort for evacuation of the uterus of the dead embryos was initiated. Finally, due to psychological distress of the woman, the two embryos were removed from the uterus via hysterotomy.

Results
The first dead embryo corresponded to 17th week of gestation whereas the second one to 16th week of gestation approximately. Laboratory blood findings of the mother after surgery remained unchanged, excluding any infection or coagulation disorder.

Polymerase chain reaction (PCR) testing was negative for cytomegalovirus (CMV), mycoplasma, chlamydia, and toxoplasma gondii infection. The placenta demonstrated multiple infarcts, ischemic necrosis at the umbilical cord’s insertion point, and signs of non-specific chorioamnionitis were detected. The male’s umbilical cord had a complete twist around its axis (Figure 1), which led to total obstruction of blood supply to both fetuses and eventually to their death (Figure 2).

Discussion
The uniqueness of this case report lies on the fact that both fetuses demised inside the uterus during mid-second trimester, whereas in most cases a healthy twin is delivered after demise and expulsion of the first twin [1].

In such cases, when the fetus dies inside the uterus and remains therein a few weeks prior to its abortion, it becomes usually dry and papery, resulting in what is widely-known as fetus papyraceous [2].

In terms of managing twin IVF pregnancies, it is highly recommended that transvaginal ultrasound should be performed between 11 and 14 weeks of gestation in search for structural and chromosomal anomalies [3].

Undoubtedly all patients with twin pregnancy – moreover when that results from assisted reproductive technologies like IVF intracytoplasmatic sperm injection (ICSI) – should have first-trimester ultrasound examination performed (for amnionicity, chorianicity, and gestational age estimation) [4].

Since monozygotic monochorionic twins seem to have a greater risk of a spontaneous abortion and congenital malformations compared to dizygotic dichorionic twins, most experts recommend serial ultrasound assessment every two to three weeks for the first group starting at 16th week and every three to four weeks starting at 20th week for the second group.

Pregnancies diagnosed with the vanishing twin syndrome after IVF carry a higher rate of adverse pregnancy outcome in terms of preterm deliveries and low birth weight compared to IVF singleton pregnancies [5]. Furthermore, significant similarities were observed in pregnancy outcome of vanishing twin pregnancies and twin
Vanishing twins in diamniotic dichorionic in vitro fertilization gestation in mid-second trimester

Pregnancies. Early demise of one twin may in turn affect the co-twin either due to infection or to disturbed placental circulation of blood shunting through inter-twin anastomoses, especially in monochorionic twins.

Clinical studies [6] have shown an increased risk of perinatal mortality of the co-twin in case of intrauterine death of one fetus during the second and third trimesters in twin pregnancies. Recent review reported a risk of co-twin death in utero of 12% and 4% for monochorionic and dichorionic pregnancies, respectively.

Both maternal and perinatal outcomes in non-IVF dichorionic diamniotic twin pregnancies compared to those that originated from successful IVF efforts are similar in the two study groups [7].

IVF patients show an increased rate of cesarean section due to the obstetrician’s and woman’s anxiety for successful management of the ‘precious’ pregnancy. In IVF twin pregnancies, the authors anticipate a higher frequency of cervical incompetence and discordant growth of the twins when compared to dichorionic – diamniotic and monochorionic – diamniotic twin pregnancies conceived spontaneously [8].

Most guidelines recommend limiting the number of transferred embryos in order to obtain singleton IVF pregnancies with a more favourable pregnancy outcome. However, there are studies in the literature where it is noted that IVF twin pregnancies have a better potential for survival than singleton pregnancies [9]. In addition, the risk of abortion declined as gestational age progressed while an increased risk of fetal death with increasing maternal age was confirmed.

In twin gestations, the vast majority of spontaneous abortions occurred during or after the second trimester [10]. In the same study, it was suggested that placental blood flow imbalance was the cause of spontaneous reduction in second trimester, whereas implantation site crowding and genetic factors seem to be responsible for first trimester’s fetal loss.

Intrauterine death of a twin can severely affect the co-twin especially in monochorionic twins who are at a greater risk than dichorionic ones [11]. Fetal intracranial hemorrhage and periventricular leukomalacia can be revealed in a surviving co-twin via magnetic resonance imaging (MRI) and detailed neurosonography despite the fact that their diagnostic value is currently under debate.

The need to determine zygosity and chorionicity in all twins is fundamental for managing the pregnancy and evaluating the probability of any complications [12]. Monochorionic diamniotic twin pregnancies present a more adverse pregnancy course than their dichorionic counterparts because of the vascular anastomoses that connect the two fetal circulations [13]. Placental anastomoses are a major contributor to adverse outcome in these pregnancies [14]. Monochorionic twins often tend to have a significant body weight discordance and polyhydramnios due to shared fetal circulation [15].

References


Figure 1. — Photograph of the umbilical’s cord twist in the male fetus.
Figure 2. — Photograph of the two fetuses demonstrating the umbilical cord’s twist in the male (left side).

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Management of a late-presenting complex - an unclassified uterine anomaly in the presence of large leiomyomas

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Summary

This is a case report of a unique, late-presenting, Müllerian anomaly in an infertile patient. The authors discuss the diagnostic challenges of characterizing distorted gynecological anatomy by Müllerian anomalies in the presence of sizeable coexisting fibroids. This case report adds new insight to the already-existing understanding of Müllerian anomalies by demonstrating how a symptomatic and benign uterine pathology can complicate the diagnosis and management of patients with Müllerian defects.

Key words: Complex Müllerian anomaly; Leiomyomata; Surgical management of Müllerian anomalies; Diagnostic imaging of Müllerian anomalies.

Introduction

Müllerian anomalies are rare defects of the internal female reproductive system with an estimated prevalence of 6.7% in the general population and 7.3% in the infertile population [1]. The American Fertility Society anatomically classifies Müllerian anomalies into six different classes based on availability of treatment and fertility prognosis [2]. Cervical agenesis and dysgenesis have been organized into four categories: 1) cervical agenesis, 2) cervical fragmentation, 3) cervical fibrous cord, and 4) cervical obstruction [3]. Very rare and unclassified complex anomalies have been described [4-6]. The authors present a case of congenital absence of the cervix and a vertical uterine fusion defect. This as of yet described Müllerian anomaly has features of both class I (agenesis) and class III (non-fusion) defects.

Additionally, Müllerian anomalies are commonly associated with defects of the urinary system. Preoperative evaluation of the collecting system is imperative. The surgical management of Müllerian anomalies remains controversial given the numerous variants, their associated urologic anomalies, as well as their uncertain prognosis [4]. Because of its rare incidence, the management of cervical agenesis has been minimally discussed in the literature. Classically, patients have been managed by abdominal hysterectomy or by restoring the menstrual outflow tract via uterovaginal anastomosis [3-9].

The authors describe a complex, unclassified Müllerian anomaly with a large uterine leiomyoma in one uterine horn and duplication of ureters. A review of the pertinent literature, as well as the diagnostic challenges and logic supporting the present surgical management, are discussed in this case report.

Case Report

A 39-year-old G0 presented with intermittent abdominal pressure and pain for six months. Her history was significant for primary amenorrhea and a history of diagnostic laparoscopy 20 years ago.

Speculum exam revealed a seven cm vagina and absent cervix. Her external genitalia were normal, and she had Tanner stage 5 breasts and pubic hair. Pelvic exam revealed an irregularly-shaped, non-tender, mobile mass extending to the level of the umbilicus.

A second, small, non-tender left-sided mass was palpated, which was thought to be either an ovary or a pedunculated fibroid.

Transvaginal ultrasound (TVUS) demonstrated a midline pelvic mass suggesting multiple myomas, the largest of which measured 12 cm. A computed tomography (CT) scan revealed a 18 x 15 cm complex pelvic mass arising from a right pelvis, a second 6 x 6 cm mass arising from the left (Figure 1A), a duplex left kidney with duplicated left renal collecting system (Figure 1B), and a normal right kidney and ureter.

The surgical management of symptomatic, large pelvic mass was planned via exploratory laparotomy with pelvic mass removal. Due to the identification of a complex Müllerian anomaly along with a duplicated left collecting system, the patient underwent preoperative cystoscopy and bilateral ureteral stent placement.

Intraoperative findings at time of exploratory laparotomy were significant for a duplicated left ureter and uterine didelphys with cervical agenesis. The right uterus had multiple leiomyomas, the largest of which measured 18 cm (Figure 2A). Neither uterus had an identifiable endometrial cavity. No overt cervical tissue was palpable; however, a 0.5 cm fibrotic area was noted between the vagina and base of the uteri. Ovaries were bilaterally normal.

A hysterectomy of the didelphys uterus with cervical agenesis and associated multiple, large leiomyomas was performed (Figure 2B). The patient’s post operative course was uneventful. She was discharged home on post-operative day 2.
Discussion

The management of Müllerian anomalies should be tailored to the unique aspects of the defect and/or clinical presentation. Patients with cervical agenesis and hematometra or pain may be candidates for fertility-conserving surgical management. Surgical canalization has been described resulting in normal menstrual bleeding, resolution of cyclic pelvic pain, and some potential for fertility [3-6]. Definitive surgical management is a hysterectomy.

Cervical agenesis is an extremely rare anomaly with 116 cases of transverse cervical defects described in the literature since 1900 [6]. Patients often present in late adolescence with amenorrhea and cyclical abdominal pain. Patients without an endometrium, like the present patient, may not have associated pain. It is important to evaluate patients for the presence of an endometrium. If the endometrium is absent, patients do not necessarily require surgical intervention. However, they should have routine pelvic exams to evaluate other vaginal, uterine, or ovarian pathologies.

The standard imaging modality for Müllerian defects is magnetic resonance imaging (MRI) [10, 11]. MRI allows for evaluation of uterine cavities, communication between rudimentary uterine horns, and renal or collecting system anomalies [11]. The present patient had a prior CT scan demonstrating her pelvic mass and collecting system anomaly. She also had an ultrasound confirming cervical absence and the authors felt that there was no added benefit from obtaining further imaging. The most common collecting system anomaly found with Müllerian defects is a unilateral duplicated ureter that fuses into one ureteral orifice. If managed surgically, the authors recommend placement of ureteral stents, especially if a urinary anomaly is suspected or known. Stent placement allows for rapid identification of anatomy and intraoperative recognition of ureteral injury.

The question of fertility is common in these patients. Fortunately, the present patient had normal ovaries; thus biologic fertility would be an option via in vitro fertilization (IVF) with a gestational carrier.

In summary, the authors have demonstrated the characterization of a unique, late presenting, Müllerian anomaly. They have discussed the diagnostic challenges of characterizing distorted gynecological anatomy by Müllerian anomalies in the presence of sizeable coexisting fibroids along with a surgical management option. Thus, this case report adds new insight to the already-existing understanding of Müllerian anomalies by demonstrating how a symptomatic and benign uterine pathology can complicate the diagnosis and management of patients with Müllerian defects.

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Herlyn-Werner-Wunderlich syndrome - a case report

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Summary
This is a case report of Herlyn-Werner-Wunderlich syndrome in a 28-year-old patient. She was admitted to hospital for surgical treatment of the pelvic mass accompanied by painful menstruation periods. This syndrome was diagnosed by US and MRI and it was treated by hemi-hysterectomy with vaginectomy. After the surgery, the patient has had regular and painless menstruation.

Key words: Müllerian duct anomalies; Hemivagina; Hematocolpos; Ipsilateral renal agenesis.

Introduction
Herlyn-Werner-Wunderlich (HWW) syndrome is a rare congenital anomaly of the female genital tract characterized by obstructed hemivagina, hematocolpos, ipsilateral renal anomaly, and uterine anomaly, more commonly uterus didelphys than uterus septus. Recently, this syndrome has been referred to as OHVIRA syndrome [1-3]. We still cannot accurately define the frequency of HWW syndrome. The incidence of obstructive Müllerian duct anomalies is 0.1-3.8% [4], 2-3% [5]. Isolated anomaly, like uterus didelphys is present in 1/2,000 to 1/28,000 cases, and ipsilateral renal agenesis is present in approximately 43% cases [6]. Presence of a pelvic mass in association with pelvic pain after menarche points to this syndrome even in women with regular menstrual periods [7]. Ipsilateral renal agenesis with pelvic mass should imply that this syndrome is to be seriously considered. The age of women with this syndrome ranges from 10 to 29 years (mean age: 14) [2]. Magnetic resonance imaging (MRI) will help render an accurate diagnosis, whereby the early endoscopic resection of the septum can be the final treatment followed by good and long-term outcome and preserved fertility [8].

Case Report
We report the care of a 28-year-old patient with HWW syndrome who was admitted to hospital for surgery due to a pelvic mass and severely painful menstrual periods. Six months prior to hospitalization, the patient had undergone laparoscopic hysterectomy because of an endometriotic cyst on the left ovary. During the laparoscopic treatment, the uterus duplex and pelvic mass were visualized below the right uterus. The patient had menarche at the age of 14. Menstrual cycles were regular, every 30 ± 4 days and severely painful. Upon being admitted to hospital, she was examined rectally as she was virgo intacta. Soft tumefaction was palpable below the uterus on the right side, parallel to the vaginal wall, and ellipsoid in shape. The shape of the uterus was irregular, slightly enlarged. The adnexal findings aroused no suspicion. Blood and urine laboratory analyses were within the reference ranges. Transabdominal ultrasound revealed absence of the left kidney, the existence of two normal size uteri, with blood retention in the right one and with pelvic cyst 60 x80 mm filled with hyperechogen content. Each uterus had one adequately developed ovary of normal size. In order to confirm the ultrasound diagnosis, i.e., the existence of a congenital urinary tract anomaly, intravenous pyelography was performed. It revealed that the left kidney filtered and concentrated the contrast; the collecting system functioned properly, its morphology being normal; a proximal ureter of normal diameter; bladder contours not well demarked from the uterus. The right kidney did not filter the contrast. MRI was done and it showed that there were two uteri of normal size with blood retention in the right one. Each uterus had communication with the related hemivagina. There was a cyst formation (60 x 80 mm) between the bladder and rectum filled with fluid, while the signal intensity implied dilated blood-filled right hemivagina obstructed by the transverse septum. Each uterus had well-developed adnexa. Given the imaging findings and clinical history, the diagnosis was Herlyn-Werner-Wunderlich syndrome. Preoperative preparations being completed, the patient underwent surgery. Hemi-hysterectomy with vaginectomy was performed because of the position of two hemivaginas, since drainage of a blind vagina and ipsolateral uterus would not be possible by simple septum resection. The operative field was prepared and abdomen was opened by a low transverse Pfannenstiel incision. The genital organs appeared, two uteri and adnexa in each uterus (Figure 1). Once the Lig ovarii proprium and Lig rotundum were clamped, they were ligated and cut while the right adnexa was conserved. Peritoneum and plica vesicovaginalis were dissected first sharply, then bluntly and the bladder was retracted towards the vagina. The hemiuterus was cut by thermocautery after ligation of uterine arteries and the uterus had been clamped, ligated, and cut from the right side. The right hemivagina was opened and chocolate-colored fluid was drained from a 4 cm long blind vagina (Figure 2). The right hemivagina was sharply divided from the paracolpium and removed together with the hemiuterus (Figure 3). Hemostasis was checked and the right adnexa was fixed to the left hemiuterus (Figure 4). Postoperative treatment was uneventful. The patient was discharged from hospital five days after surgery in good condition. Her menstrual periods have been regular and painless.
Discussion

HWW syndrome is a rare anomaly manifested immediately after menarche. This syndrome is a result of developmental disorders of genital organs in the period from the 6th to 17th gestational week. The cause is unknown. It is difficult to precisely determine the incidence of the syndrome. The incidence of congenital anomalies of the Müllerian duct is about 2-3% [5]. Congenital Müllerian duct anomalies result from nondevelopment or nonfusion of Müllerian ducts. These abnormalities include double uterus, uterus didelphys, uterus bicornuate, and uterus septus. Uterus didelphys represents a complete duplication of the uterus in two separate horns, two cervixes and two vaginas. Uterus didelphys with obstructed hemivagina is one of the rarest congenital anomalies of the Müllerian ducts, occurring between the 12th and 16th gestational week. It is the result of lateral fusion defects of caudal Müllerian ducts, and includes abnormalities caused by failed septum resorption after fusion of the ducts. This anomaly is accompanied by renal agenesis on the side of the obstructed hemivagina. Renal agenesis occurs due to the failure of the ureteric bud to differentiate from the mesonephric duct. As two-thirds of the upper vagina develop from Müllerian ducts, defects in fusion of the ducts can lead to vagina duplex, and failed wall resorption between the ducts causes vagina septum. This condition is often associated with uterus didelphys or uterus septus [9]. Uterus didelphys is most frequently accompanied by transverse vaginal septum [10]. Transverse vaginal septum can be complete or incomplete and is not usually associated with other urologic and Müllerian anomalies [4]. We have reported a 28-year old patient with HWW syndrome admitted to hospital for surgery because of a pelvic mass and severely painful menstrual periods. Genital tract anomalies often occur in association with urinary tract anomalies. Abdominal ultrasound evaluation can easily reveal the absence of a kidney, accompanied by functional hypertrophy of the other kidney due to which laboratory analyses of renal functions remain within the reference ranges. It is important to make a timely diagnosis of the syndrome. This diagnosis must be suspected in girls complaining of dysmenorrhoea and recurrent pains in the lower abdomen between the periods and subsequent pelvic mass. Accurate and final diagnosis can be rendered not only by vaginal examination but must be accompanied by ultrasound and MRI, or by laparoscopy and hysteroscopy.
MRI is a very efficient, non-invasive diagnostic method that can show anatomic visualization of the organs at all levels and help differentiate myometrium from fibrous septum. MRI helps render an accurate diagnosis and determination of the follow-up treatment [11, 12]. HWW syndrome is to be suspected in young girls complaining of painful menstrual periods and having pelvic and paravaginal masses. Diagnosis is usually rendered after menarche. All the symptoms for this syndrome, like dysmenorrhea, pelvic mass, and hematocoles are even more obvious after menarche [13]. This syndrome is diagnosed in patients at the age of 20 to 30. HWW syndrome has been found in 12 patients (mean age: 13 years). Pelvic mass and abdominal pain were present in 11 of the girls out of 12, whereas four of them had dysmenorrhea. Paresthesia lasted for 0.5-12 months [1]. We have presented a case of uterus didelphys with obstructed hemivagina and pyocolpos and ipsilateral renal agenesis where the final diagnosis was postponed till pregnancy [12]. Symptoms of this syndrome have already been described in girls even prior to menarche. One patient with renal agenesis and microscopic hematuria had these symptoms before menarche [14]. All the characteristics of this syndrome were described in a four-year-old girl [8]. Ipsilateral renal genesis accompanied by a pelvic mass is always a step further in diagnosing this syndrome. Transvaginal ultrasound (TVUS), especially 3D TVUS is important in diagnosing this syndrome. There was a case of incomplete syndrome with asymmetric obstructed uterus didelphys diagnosed by 3D ultrasound [11]. HWW syndrome is not an anomaly resulting in female sterility. It is a syndrome found in women with primary infertility, although rarely [15]. Uterus didelphys, obstructed right hemivagina, pyocolpos and ipsilateral renal agenesis were found in a 25-year-old patient suffering from infertility with dysmenorrhea when examined by ultrasound and MRI. Vaginal septum excision and drainage were performed. The patient got pregnant spontaneously three months after surgery [3]. According to the literature, 23% of all the patients suffer spontaneous miscarriages, 15% of them undergo pre-term delivery, and 62% of them undergo full-term deliveries successfully [16]. If septum of the obstructed vagina is timely recognized and surgically removed and drained, it can contribute to the disappearance of symptoms and prevention of complications relating to chronic cryptomenorrhea, like endometriosis, pelvic adhesion, and infectious pyocolpos [1, 17]. The aim of the surgery is to preserve normal fertility. Treatment of these anomalies includes hematocolpos drainage and vagina septum excision which is traditionally done by scalpel or scissors. A less invasive procedure is hysteroscopic resection of the vagina septum which improves visualization [18]. Surgical removal of the septum of the obstructed vagina with drainage is the adequate treatment of the syndrome [1, 17] with good long-term results and preserved fertility [8]. Tracheobronchial stent is inserted in patients with bilateral hemivagina and uterus didelphys, hematometra, and hematocoles after vaginal septum excision to preserve communication. Six months later the stent was removed. Twelve months later the patient had regular menstrual periods which were painless [19]. Out of 12 patients with HWW syndrome undergoing vaginal septectomy, 11 had no more symptoms, and one patient had irregular menstrual periods [1]. There was one case of a minimally invasive combination of laparoscopy and vaginoscopy in a ten-year-old girl. After laparoscopic incision of the horn of the uterus through its cavity and cervical canal, a Maryland dissector was introduced – a hole was made on the septum of the obstructed hemivagina and the dissector placed in the vaginal canal; a Penrose drain was inserted from the vagina, through the cervical canal into the uterine cavity up to the horn. Incision was made on the ipsilateral Fallopian tube and the drain was inserted [20].

Conclusion

Combined laparoscopy and hysteroscopy represent the best approach leading to accurate diagnosis and adequate treatment of the syndrome.

References


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Gonadotropinoma presenting as a case of pseudo-ovarian failure changing to macroprolactinoma

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Summary

Purpose: To present the first gonadotropinoma presenting as pseudo-menopause in a teenager. Methods: Human menopausal gonadotropins (hMG) were given to a 37-year-old woman whose hypergonadotropic amenorrhea with estrogen deficiency as a teenager was changed to hypogonadotropic amenorrhea by the growth and prolactin secretion of a macroprolactinoma. Results: The patient responded multiple times, and every time to stimulation with hMG and each time produced several dominant follicles. She delivered two babies including conception at age 40. Conclusions: The fact that this woman could respond consistently to hMG 20 years after the diagnosis of premature menopause, it is clear that initially the etiology of the extremely high LH and FSH levels in an estrogen-deficient 18-year-old was the presence of gonadotropinoma secreting inert LH and FSH. Since serum prolactin was measured the first time at age 37, it is not clear whether the endogenous biologically active gonadotropine were suppressed by replacement of the gonadotroph cells with tumor cells or suppression of endogenous gonadotropins by hyperprolactinoma.

Key words: Gonadotropinoma; Hyperprolactinoma; Pituitary macroadenoma; Ovarian failure; Hypergonadotropic amenorrhea.

Introduction

The presence of a pituitary tumor causing supranormal circulating levels of one or both gonadotropins is not common [1, 2]. Less than 200 cases were reported by 1993 and not that many have been reported since that time [2].

These tumors are difficult to detect in postmenopausal women since gonadotropins are normally elevated in that group. Most of the time the gonadotropin hormones have normal physiologic function and in younger patients there have been 20 cases of gonadotropinomas causing ovarian hyperstimulation, and these cases have been summarized by the case report of Castelo-Branco et al. [3].

A case is presented which we believe is the first one ever described where replacement of normal gonadotrope cells by an follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreting gonadotropinoma that secreted immunoreactive but biologically inactive gonadotropins led to the false diagnosis of premature ovarian failure.

Case Report

The patient had menarche at age 13 but had developed amenorrhea by age 18. She was estrogen-deficient as determined by failure to have menses with withdrawal of progesterone but a normal uterus and endometrium by having menses with oral estrogen followed by progesterone.

Evaluation found a serum FSH of 55 mIU/ml and LH of 42 mIU/ml. She was advised that having children with her own oocytes would not be possible, and she was continued on estrogen/progesterone replacement for ten years when she consulted a reproductive endocrinologist who confirmed the diagnosis of premature ovarian failure with a serum FSH of 78 mIU/ml and LH of 92 mIU/ml and placed her back on hormonal replacement.

At 35 years of age the woman consulted our group based on publications concerning methods of inducing ovulation in women with premature menopause [4]. Since the length of time from diagnosis to treatment correlates negatively with success in achieving ovulation in this group, the woman was advised about the poor prognosis [5]. However she still wanted to try the technique.

Off hormonal replacement her repeat gonadotropin levels were quite a surprise – her LH and FSH were < 1.0 mIU/ml and serum estradiol (E2) measured at 5 pg/ml. This prompted measuring serum prolactin which was markedly increased at 975 ng/ml.

Computerized axial tomography revealed a large pituitary mass with supracellular extension into both the sphenoid sinus and left middle fossa. At this stage the woman was considered inoperable and not a candidate for radiation therapy. She was then treated with bromocriptine and the tumor shrank to 50% of the previous size allowing transphenoidal hypophysectomy.

Though her prolactin level remained elevated even with 5 mg of bromocriptine per day being continued, there was no evidence of growth of the residual pituitary tumor.

The assumption was made that a woman with premature ovarian failure subsequently developed a macroprolactinoma which not only destroyed the gonadotrope cells but the high prolactin also contributed to bringing the elevated levels down to non-measurable levels. Nevertheless the woman wanted to try to conceive. The technique for reversing menopause involves lowering elevated FSH to restore FSH receptors in the paucity of the remaining follicles [6-8]. One possible explana-
tion was that her initial diagnosis of premature ovarian failure was incorrect and all along she had a gonadotropinoma secreting biologically inactive gonadotropins which eventually became replaced by a macroprolactinoma. This concept was supported by the fact that she ovulated every cycle with human menopausal gonadotropins leading to several pregnancies with miscarriages but also two live deliveries. The last successful pregnancy was at age 40. She always produced multiple follicles.

Discussion

When women are able to reverse menopause they rarely ovulate every cycle and even when they do they usually make just one follicle. This patient ovulated every time with human menopausal gonadotropins and made multiple follicles. Considering the 20 year length of her amenorrhea it is clear that this was not a case of premature ovarian failure but rather a case of pseudo-premature ovarian failure related to the secretion of biologically inert gonadotropins that eventually converted to a macroprolactinoma.

We believe this is the first case where a gonadotropinoma was found that secreted biologically inert gonadotropins leading to a false diagnosis of menopause. Perhaps it is more common than this one case since if asymptomatic gonadotropinomas exist in postmenopausal women (i.e., no mass effects, e.g., headaches or visual disturbances) these tumors would go undetected. Since serum prolactin levels were never measured until age 37 when the FSH and LH were found to be low, it is possible that her own gonadotropin cells were not entirely replaced by the tumor cells but she did secrete endogenous gonadotropins related to concomitant hyperprolactinemia. Many of the gonadotropinomas have been found to exist with hyperprolactinemia [3].

References


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Pregnancy with 15 live fetuses and severe ovarian hyperstimulation syndrome after ovulation induction and intrauterine insemination

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Summary
The empirical use of ovulation induction and intrauterine insemination for male factor infertility, unexplained infertility, and anovulatory infertility can be associated with multiple gestation and ovarian hyperstimulation syndrome (OHSS). A 30-year-old lady was referred for fetal reduction of very high-order pregnancy. She became pregnant after ovulation induction and artificial insemination. The stimulation protocol included clomiphene citrate and fixed-dose gonadotropins. Triggering of ovulation was done with 5,000 units of human choric gonadotropins (hCG). Cycle monitoring was done with ultrasonography only. The patient was admitted to the hospital due to severe OHSS. Physical examination revealed that the uterus size was equivalent to 28 weeks gestation. Transvaginal ultrasonography (TVUS) and pelvic magnetic resonance imaging (MRI) showed 15 intrauterine gestational sacs with viable eight-week fetuses and 7 cm x 4.5 cm fluid collection. Both ovaries were enlarged. The right ovary was 12 cm x 5 cm and the left ovary was 10 cm x 6.5 cm. The patient had a spontaneous miscarriage of the 15 fetuses.

Key words: Multiple gestation; OHSS; Ovulation induction.

Introduction
The aim of ovulation induction is to produce two to three mature follicles and ova for in vivo fertilization (IVF). Multiple gestation and ovarian hyperstimulation syndrome (OHSS) are known complications of ovulation induction with exogenous gonadotropins and intrauterine insemination. Severe OHSS can cause strokes, kidney failure, heart attack, and even death, although these consequences are extremely uncommon with adequate treatment. Guidelines and strategies for prevention of high-order pregnancy and OHSS after assisted reproductive technologies (ART) are well-established and include daily monitoring of estradiol level and transvaginal ultrasonography (TVUS) [2]. Inadequate cycle monitoring can result in medical catastrophic situations [3]. The aim of this case report was to document a case of very high-order multiple gestation and severe OHSS after ovulation induction and intrauterine insemination.

Case Report
A 30-year-old lady in her third pregnancy was referred for fetal reduction of very high-order pregnancy. She became pregnant after ovulation induction and artificial insemination for male factor infertility. The stimulation protocol included clomiphene citrate 100 mg orally from the second day of her menstrual cycle for five days and human menopausal gonadotropins (hMG) 150 IU daily from the third day of her period for ten days. Triggering of ovulation was done with 5,000 units of human chorionic gonadotropins (hCG). Cycle monitoring was done with ultrasonography only. She was admitted to the hospital for 16 days due to severe OHSS with right pleural effusion four weeks prior to the referral. She had two previous full-term normal deliveries. Physical examination revealed that the uterus size was equivalent to 28 weeks gestation. TVUS and pelvic magnetic resonance imaging (MRI) showed 15 intrauterine gestational sacs with viable eight-week fetuses (Figures 1 and 2) and 7 cm x 4.5 cm fluid collection (Figure 3). Both ovaries were enlarged (Figure 2). The right ovary was 12 cm x 5 cm and the left ovary was 10 cm x 6.5 cm. No selective reduction was done. She had a spontaneous miscarriage of the 15 fetuses.

Discussion
Infertility is estimated to affect 10% to 15% of couples. The incidence of multiple pregnancy has increased dramatically in the last two decades due to new technologies and advances in reproductive medicine that overcome infertility. The rate of spontaneous twin pregnancy has been estimated to range from 1% to 1.35% and that of a triplet pregnancy from 0.01% to 0.017%. The incidence of multiple gestations is more than ten to 20 times higher with ovulation induction and intrauterine insemination, ranging from 7.5% to 29% per couple [4]. Treatment of male factor infertility with ovulation induction and intrauterine insemination is empirical. It is thought to work by increasing the number of ovarian follicles and depositing the good quality selected sperms in the uterine cavity. The two major complications of ovulation induction and intrauterine insemination are multiple pregnancy especially high-order pregnancy (triplets and more) and OHSS. The maternal risks of multiple pregnancy are well-documented. These include hypertension, preterm
labor and delivery, postpartum hemorrhage, anemia, and emotional distress. There are many ovulation induction protocols. Clomiphene citrate alone, exogenous gonadotropins alone, or clomiphene citrate in combination with exogenous gonadotropins are commonly used for ovulation induction and intrauterine insemination. The risks of multiple pregnancy and OHSS increase with the use of exogenous gonadotropins. Maternal age < 32 years, high gonadotropins starting dose, longer days of stimulation, and number of follicles are important risk factors that increase the occurrence of multiple gestation. In series of more than 4,000 cycles or more than 1,800 pregnancies, twin pregnancies ranged from 15% to 20% and high-order pregnancies from 5.7% to 8.8% when the initial dose of gonadotropins was 150 IU or greater. In contrast, in series of more than 500 cycles using minimal stimulation initial doses of 37.5 - 75 IU of gonadotropins, twin implantations ranged from 6% to 15% and high-order pregnancies from 0 to 1.3% [5]. Similarly, with 150 IU of gonadotropins, high-order pregnancies increased from 4% when stimulation was less than nine days to 14% when stimulation lasted more than 12 days. Strategies to prevent multiple gestation include the use of mild stimulation protocol, the use of low-starting dose of gonadotropins, daily monitoring of ovarian response, and cancelation of the cycle when there are more than three follicles 15 mm or more according to the American College of Obstetrics and Gynecology [6]. The sequential use of clomiphene citrate followed by gonadotropins from cycle days seven to nine is thought to be “mild stimulation protocol” which would reduce the incidence of multiple gestation when compared to gonadotropins-only protocol. However, recent studies suggest that multiple gestation and cancellation rates are similar to gonadotropins-only protocol [5]. The stimulation protocol used in the current report is different than the typical sequential protocol of clomiphene citrate and gonadotropins. Fixed dose of gonadotropins were given daily from the third day of the menstrual cycle for ten days.

All ovarian stimulation protocols result in some degree of hyperstimulation, usually with no adverse conse-
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quences to the patient. In contrast, OHSS is an iatrogenic complication of ovulation induction and is characterized by ovarian enlargement, third spacing of fluid, hemoconcentration, and even systemic organ function impairment with the need for hospitalization, sometimes in intensive care units. Severe OHSS can be a life-threatening situation for a young previously healthy woman. The best strategy to deal with OHSS is prevention. Women at risk of OHSS include young age, a history of elevated response to gonadotropins, previous OHSS, polycystic ovary syndrome, high estradiol level, development of high number of follicles, and exposure to hCG. Complete prevention of OHSS is still not possible. Early identification of potential risk factors and careful daily monitoring with estradiol and TVUS can result in significant reduction in the incidence of OHSS [2]. In addition, coasting of the cycle, reduced dose of hCG, triggering of final oocyte maturation with gonadotropins releasing hormone agonist (instead of hCG) and cancellation of the cycle can be helpful to prevent OHSS. In the Western World, treatment is monitored by a specialist team because this is likely to improve the effectiveness and efficiency of treatment and is known to improve patient satisfaction [7]. However, in the developing world, due to many factors including limited resources and inadequate cycle monitoring can result in medical catastrophic situations as illustrated in the current case and a published case series [3].

References

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The significance of 3D power Doppler in prenatal diagnosis and the evaluation of the anatomical structure of vein of Galen aneurysmatic malformation: case report

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Summary

Prenatal diagnosis of vein of Galen aneurysmatic malformation (VGAM) is made with the use of color Doppler, while in B-mode it is seen as a centrally placed supratentorial cystic structure. 3D-power Doppler (3D-PD) is a method that enables precise visualization of the vascular anatomy of this complex malformation. In our case, VGAM was detected in the 33rd week of gestation with power Doppler, and the use of 3D-PD enabled better visualization of the angioarchitecture and detection of feeding and drainage vessels of aneurysmatic widening. The diagnosis was confirmed postnatally with the use of MRI. A prenatal study of the angioarchitecture could have prognostic significance as well as being important in the therapeutic approach during the postnatal period.

Key words: Intracranial cystic structure; Vein of Galen aneurysmatic malformation; Prenatal diagnosis; 3D-power Doppler.

Introduction

One of the severe causes of mortality and morbidity in the early neonatal period and during childhood is vein of Galen aneurysmatic malformation (VGAM), a rare congenital disorder, forming up to 1% of total cerebral vascular disorders [1]. VGAM is viewed by ultrasonography as an anechoic tubular intracranial lesion in the midline, superiorly located in relation to the cerebellum, and with high turbulence flow detected by color Doppler [2]. Due to the possibility of the three-dimensional power Doppler angiography (3D-PD) to provide views comparable to those formed by traditional neonatal angiography, additional valuable information can be obtained in the evaluation of VGAM [3, 4].

We present a case of aneurysmatic malformation of the vein of Galen, prenatally diagnosed by power Doppler with particular attention to the significance of 3D-PD in terms of evaluation of anatomical structure of this vascular malformation, and accordingly the potential prognostic role of this method.

Case Report

A 26-year-old woman became pregnant after a two-year history of infertility. Ultrasound (US) was performed with a Voluson 730 (GE Medical Systems, Kretz, Austria) which is equipped with an abdominal transducer of 4-8 MHz. Previous US screening examinations performed in the 9th, 12th, and 22nd gestational weeks revealed a vital singleton fetus without apparent structural abnormalities in the uterus bicornis (Figure 1). A cystic structure 22 x 13.9 mm in diameter, located dorsal to the tectum was visualized on B-mode imaging at the 33rd gestational week. Blood flow in this mass was demonstrated using power Doppler (Figure 2). To understand what sort of vascular lesion was involved and also to study its angioarchitecture, 3D-PD was used. Using the 3D-PD made possible not only a confirmation of the diagnosis, but also a more precise orientation of the area, meaning a visualization of the anatomy of the earlier noticed vascular anomalies (Figures 3 and 4). This technology clearly displayed the existence of arteriovenous communication between the arterial circle of Willis, more precisely the a. cerebri posterior, and aneurysmatic widening of the vein of Galen (Figures 4 and 5), as well as a widening of the straight sinus opening into which blood drains from the lesion (Figures 3, 4, and 5). The morphology of the fetus as a whole was normal, without any signs of hemodynamic disorders. The fetal heart was also morphologically normal with normal size.

The birth was completed at term by cesarean section and resulted in a vital female baby weighing 2,900 g. The Apgar score was 8/9 at one and five minutes after birth, respectively. The prenatal diagnosis was confirmed postnatally by echosonography and magnetic resonance imaging (MRI) of the central nervous system (Figure 6). Echocardiography revealed the existence of pronounced hypertrophy of the myocardium of both ventricles, particularly the right one. At that moment, the shunt was not large, so there were no elements to cause congestive heart insufficiency. Echo-sonographic findings in the abdomen were regular with no signs of ascites. All the laboratory findings were within the normal parameters. Bearing in mind that the newborn was hemodynamically and neurologically normal, the medical team that was following the progress of the newborn took an expectative position. At this time the child is two years old, without any negative implications and is under regular pediatric and neurological supervision. The child is developing well with regular neurological development and with no aneurysm enlargement. The cardiac state is stable with no signs of heart function decrease. The left ventricle is slightly smaller in size than it was at previous examination with discrete remodeling, first of all, of the myocardial mass, with normal ranges of the heart insufficiency biomarkers.

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Figure 1. — Three-dimensional rendering technique. The scan shows uterus bicornis in the ninth week of gestation with an embryo (E) embedded in the left horn (LC) of the uterus. (RC) the right horn of the uterus.

Figure 2. — Doppler analysis showing pulsatile venous blood flow pattern through an aneurysm of the vein of Galen.

Figure 3. — A 3D power Doppler analysis of the angioarchitecture of a VGAM in body glass B-mode. Scan showing the aneurysm (a), dural sinuses (sinus rectus (b)) and the circle of Willis (C) as well as shunts between the circle of Willis and the aneurysm.

Figure 4. — Scan showing the aneurysm of the vein of Galen (a) which directly drains in the sinus rectus (b) by 3D-PD mode. There is also the circle of Willis (c) as well as feeding arteries (e) which mainly come from a.cerebri posterior (d).

Figure 5. — By rotating Figure 3, the relation between the aneurysm and the circle of Willis is shown better. The feeding arteries (arrows) mainly come from a.cerebri posterior (ACP).

Figure 6. — Postnatal magnetic resonance imaging showing aneurysmal malformation of the vein of Galen.
Discussion

VGAM, which makes up 1% of all intracranial malformations, is a complex arteriovenous malformation which appears in different forms, from a considerable widening of the vein itself up to multiple communications between the vein of Galen system and the cerebral arteries (carotid and/or vertebral basin) [3, 5]. The etiological origin of this disorder is controversial but it is thought that it appears between the 6th and 11th week of embryonic development. The assumption is that reduced capillary resistance, probably combined with stenosis of the dural sinus leads to progressive enlargement of the anterior segment of the medial prosencephalic vein (vein of Markowski), which is considered to be the precursor of the vein of Galen and which normally regresses, and to the formation of an aneurysmatic component typical of the arteriovenous malformation of the vein of Galen [6].

Several types of VGAM have been recorded. Arteriovenous communications create an intracranial shunt (L-R shunt) which can cause cardiomegaly with cardiac insufficiency and non-immunological hydrops fetalis as a result of cardiac decompensation. Low resistance in utero circulation can reduce the flow through the fistula and minimize cardiac decompensation, but a sudden increase in systematic vascular resistance after birth results in a considerably increased flow through arterio-venous communication, resulting in cardiac weakness. Changing the course of cerebral circulation can lead to cerebral infarction and the resulting porencephalia. This is often accompanied by hydrocephalus and it is thought that it occurs because of aneurysm pressure on the Sylvian aqueduct or because of increased intracranial venous pressure [7].

While the malformation of vein of Galen may be diagnosed by pulse and color Doppler, it is still hard to accurately localize feeding arteries and venous drainage, and yield satisfactory prognosis. Using MRI antenatal has turned out to be useful in the final diagnosis [8]. This paper showed that it is possible to perform not only diagnosis, but also the accurate localization, as well as detailed identification of elements forming VGAM by using 3D-PD. The advantage of 3D-PD is the possibility to memorize volume and additional processing, i.e. rotation and visualization of scans from various angles, which enables identification of structures unavailable to 2D technology.

Several factors may have an effect on the prognosis of this anomaly. Yuval et al. illustrated for the first time prenatal sonographic indices that may predict fetal outcome. In their report, anatomic brain changes, hydrops, dilated drainage tract, multiple (five or more) feeding vessels, dilated jugular vein and/or inferior vena cava, retrograde aortic flow, and cardiomegaly were associated with adverse outcome [9]. The volume of the aneurysm is also a factor that may influence outcome [3, 10]. Sepulveda et al. reported ventriculomegaly, cardiomegaly, and dilated neck vessels being a common associated finding [1]. On the other side, Pilu et al. reported changes of the cerebral structure in cases with vein of Galen aneurysm being a negative sign [11].

The true therapeutic approach has not yet been established. Overall, what is needed is a detailed anatomic picture of the changes in order to evaluate a method that could be suitable in each individual case. As many studies have shown, the neurological outcome of subjects with VGAM [6, 12, 13] is highly improved by endovascular embolization [6, 12, 13]. Transcatheter embolization of Galen’s aneurysm through the confluence of sinuses after delivery is possible owing to identifying straight sinus drainage [14].

The age of the newborn infant is a critical factor when talking about the success of endovascular therapy. As long as the patient shows no signs of heart or kidney weakness and is free of neurological disorders, meaning if the patient is clinically stable, it is better to delay treatment until the fifth or sixth month [6, 13]. There is evidence of rare cases of spontaneous thrombosis or regression of arteriovenous malformation. Patients without any symptoms may be treated with adequate medical therapy, and with repeating angiography with the possibility of occurrence of spontaneous thrombosis [6, 15].

Even though angiography represents the gold standard in the precise evaluation of the angioarchitecture of this malformation, including the detailed anatomy of the supply arterial blood vessels, as well as the venous drainage which allows precise endovascular access and successful therapy, the pictures obtained in the case described are comparable with postnatal diagnostic methods. It has been demonstrated that the use of 3D-PD enables detailed identification of all elements of VGAM such as feeding arteries and venous drainage or the localization of the same. These findings suggest that 3D-PD is one more additional prenatal diagnostic method which, as well as MRI, can also be very informative and significant not only in the context of the diagnosis of VGAM, but also in the verification of the seriousness of the illness.

References


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Cesarean section scar pregnancy treatment - case report

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Summary
This is a case report of a 36-year-old patient with an ectopic pregnancy located in the previous cesarean section scar following in vitro fertilization (IVF). The patient was treated by 50 mg of intrasacular methotrexate locally under ultrasound guidance. Transvaginal ultrasound (TVUS) confirmed that the pregnancy was no longer vital within 24 hours, dilatation and aspiration of the ovular tissue were performed after seven days and it was sent for pathohistological analysis. Eight hours after the procedure, the patient began bleeding abundantly and was consequently treated locally by 1 ml of Beriplast® P Combi set, human fibrinogen, and human thrombin set (CSL Behring). After the treatment, the patient was discharged in good health, with normal laboratory values. Her menstrual period resumed 35 days after the procedure.

Key words: Ectopic pregnancy; Cesarean section scar; Methotrexate.

Introduction
Cesarean section scar pregnancy is a very rare and life-threatening condition, which can also lead to uterine rupture, bleeding, and fatal outcome [1, 2]. Before the XIX century, the mortality rate due to ectopic pregnancies was over 50%, while during the XX century it declined to 1% [3, 4]. Today, ectopic pregnancy mortality rate in the USA is one in every 56,730, in Japan one in every 978 and in Austria one in every 4,500 [5]. The survival rate has increased despite the obvious increase in the number of ectopic pregnancies.

At its early stage, this kind of pregnancy is usually accompanied by pain during amenorrhea followed by abundant bleeding which is difficult to control conservatively. In the past, these pregnancies were usually terminated by removal of the uterus. Today, there is a trend, since it is a serious condition affecting even younger women where fertility preservation is of vital importance, toward conservative treatment, particularly now that the cesarean sections rates continue to increase [6, 7].

Various methods of conservative treatments of cervical pregnancies have been developed. The most frequently used ones include: uterine artery embolization, blood vessel ligation, balloon tamponade, medicinal administration, and local application of chemicals with or without iodoform gauze, and coagulation medications [8]. Severe hemorrhage, caused by syncytiotrophoblast invasion without enough muscle tissue to compress and control the bleeding by contractions, can be prevented by the obliteration of the local blood vessels.

Case Report
A pregnant 36-year-old woman was hospitalized in the Clinic of Gynecology and Obstetrics "Narodni Front" in Belgrade, after she had been diagnosed with cesarean section scar ectopic pregnancy (by the use of ultrasound and color Doppler). The pregnancy was the result of in vitro fertilization (IVF). The patient reported laparoscopic surgery of the right ovary endometrioma and uterine myoma three years prior and cesarean section delivery two years prior. The previous pregnancy was also the result of IVF.

When admitted to the hospital, her biochemical and laboratory values were within reference ranges, as well as urinary values, except beta human chorionic gonadotropin (hCG) levels which showed an increase corresponding to the pregnancy and the period of amenorrhea.

Vaginal and cervical microbiological smears showed normal physiological flora. Transvaginal ultrasound (TVUS) imaging showed: yolk sac 3.4 mm, crown-rump length (CRL) 2.2 mm, and the gestational sac was located at the previous cesarean section scar with anechoic shadow 7 x 2.5 mm. Beta hCG level was 24,577.5 IU. Three days later TVUS showed: CRI 7 mm, fetal cardiac activity present, and beta hCG level 27,347.8 IU. As the patient was pregnant for the second time via IVF and whose fertility preservation was of vital importance, the authors decided to combine chemical conservative and surgical treatment of the ectopic pregnancy.

After obtaining a substantial amount of her blood group, guided by ultrasound and using 22G needle, 50 mg of methotrexate were instilled into the sacus vitelinus through the front abdominal wall. Ultrasound confirmed that the pregnancy was no longer vital after 24 hours, and then waited for seven days for blood vessels to obliterate. Afterwards, the authors surgically removed the devitalized ovular tissue from the scar area. Stitches were made paracervically at the three and nine o’clock positions and the ovular tissue was removed from the scar area. Dilatation up to Hegar 8 was performed with subsequent ultrasound-guided aspiration, and the tissue was sent for pathohistological analysis. The procedure was performed in the operating room and all the necessary precautionary measures were undertaken in case of severe hemorrhage or need to change the method (Figure 1).

The procedure was not accompanied by any complication. Dur-
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Following menstrual period. Her menstrual period, painless and similar to the previous periods, resumed 35 days after the procedure. The patient was in good health and her next beta hCG was negative.

Discussion

Cesarean section scar pregnancy is a rare form of ectopic pregnancy first reported by Larsen and Solomon in 1978, with an incidence of 6.1% [9, 10]. As there is a trend toward increasing the number of cesarean sections, we can expect to see the increase, as well, in the number of these pregnancies [6, 7].

The estimated prevalence of cesarean section scar pregnancies within the local population is between one in 1,800 and one in 2,200 [10, 11]. When diagnosed, the gestational age of cesarean section scar pregnancy ranges from four to 23 weeks, while TVUS examination has confirmed fetal cardiac activity in 61% of all pregnancies [12]. This fetal cardiac activity was confirmed in the patient, as well, by TVUS.

The gestational sac can be implanted and continue to develop in the scar area and the increase in beta hCG levels can mimic normal pregnancy. Therefore, hCG surveillance is not the method of choice. There was a corresponding increase in beta hCG titre in the patient presented.

This kind of implantation demands intensive pregnancy surveillance, so the patient had to be frequently and regularly hospitalized. Early diagnosis is usually established by ultrasound, but it is difficult to establish differential diagnosis between cesarean section scar pregnancy and cervical pregnancy or lower uterine segment pregnancy [13, 14]. The implantation of a pregnancy within cesarean section scar in the patient was diagnosed by TVUS at 6.1 gestational weeks.

A timely and accurate diagnosis is of vital importance, otherwise, there is a high risk of uterine rupture and massive hemorrhage [15, 16].

There are various approaches for the treatment of this condition. However, as only individual cases, small series, and individual experiences have been reported so far, and there is no generally-accepted approach to the treatment of this condition which would preserve fertility and avoid surgery [16-18].

Methotrexate application is the most commonly used procedure, since it is very convenient for early pregnancies and can be administered both locally and systemically, as a single or multiple-dose methotrexate [9, 19-21]. Methotrexate application can be combined with uterine embolization followed by curettage if necessary [22]. The authors administered 50 mg of methotrexate transabdominally directly into the sacus vitellinus [19, 20]. Vaginal bleeding might follow this procedure, although the authors did not experience this complication. The treatment of advanced pregnancies is more specific as there is a risk of uterine rupture. Additional surgery is sometimes needed. Spontaneous rupture is possible due to Cesarean scar tissue invasion [9].

Surgery implies opening the abdomen and uterus resection – hysterotomy or hysterectomy accompanied by adnexal conservation [9, 10].

Curettage is considered to be inappropriate because it can cause bleeding. Nevertheless, it can be combined with medication therapy in early pregnancies. Being prepared to change from vaginal to abdominal approach if necessary, the authors successfully performed dilatation and aspiration and removed the devitalized oval tissue. This procedure is not recommended for advanced pregnancies since it can cause scar perforation and massive bleeding. If ectopic pregnancy is diagnosed in its early stage, and the patient is hemodynamically stable, it is possible to perform laparoscopic excision with minimal morbidity rate and fertility preservation [9].

Blood loss amounting in 500-1,000 ml requires blood transfusion. As this patient only lost 200 ml of blood, a transfusion was not necessary [13].

The patients with heterotopic pregnancies i.e. intrauterine cesarean section scar pregnancies can receive potassium chloride injection in the cesarean section area. Some studies report that intrauterine pregnancies can continue to develop normally within heterotopic pregnancies [18].

The prevalence of first trimester cesarean section scar pregnancies is much higher in patients than reported by some studies. This is explained by TVUS use in diagnosing abnormal uterine implantation. Ultrasound examination is performed routinely in all the patients who have undergone previous cesarean section and special attention should be paid to the new pregnancy location [14].

Figure 1. — Devitalized trophoblast tissue.
TVUS is the diagnostic methods used to confirm the condition. Laparoscopic approach, where implantation site can be determined by single port technique, is also possible [9, 14].

Methods of conservative treatment of this kind of pregnancy vary. After removing the ovular tissue, selective embolization of uterine blood vessels is usually performed in order to control hemostasis [8, 22, 23]. Nowadays, local coagulation medications are used frequently in order to keep the haemostasis under control after surgical treatment, and that is how massive bleeding is managed.

In addition to blood vessels embolization, there have been trials to ligate the uterine blood vessels prior to any other intervention. Stitches are made paracervically at the nine and three o’clock positions before any other action. The authors followed this procedure and afterwards used iodoform gauze tamponade for the cervix. Surgical amputation of the cervix is another method for hemostasis control following surgery [9].

Local and systemic methotrexate instillation was the method the authors decided to apply since it causes the disappearance of fetal vitality and gradual reduction of vascular network, enabling the growth of the fertilized egg cell. Methotrexate application is accompanied by folic acid. Recommended methotrexate doses are 50 mg per m2 of skin, while folic acid doses are ten times smaller. It is possible to apply it under US guidance directly into the sacus vitelminus [16, 19].

With the absence of fetal vitality confirmed, embolization of blood vessels affected by syncytiotrophoblast invasion should be performed, and followed by dilatation, aspiration and removal of the devitalized ovular tissue. This procedure is performed in the operating room preceded by adequate preparation. Sufficient amounts of the same blood type should be obtained in case of severe bleeding and in case some inner organs (e.g. uterus) are to be removed.

Conclusion

Conservative treatment by locally-administered methotrexate via direct instillation into the sacus vitelminus under the guidance of US, and consequently followed by dilatation and aspiration of devitalized ovular tissue, has proved successful in the treatment of cesarean section scar ectopic pregnancies. In case of uncontrollable bleeding, it is possible to locally administer coagulation drugs which will improve hemostasis. TVUS can confirm suspicions of abnormal gestational tissue. The patients with previous cesarean section delivery deserve special attention. Late detection of ectopic implantation in the area of cesarean section scar causes increase in morbidity rate.

References


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Successful pregnancy after pulmonary embolism and heparin-induced thrombocytopenia - case report

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Summary
The authors present the case of a nulliparous 34-year-old patient. At the tenth week of gestation, she developed phlebothrombosis of veins of the right leg and massive pulmonary embolism. After thrombolytic and heparin therapy she developed rethrombosis and heparin-induced thrombocytopenia type II. Lepirudin was introduced in therapy and in the 12th week of gestation acenocumarol was added. After the 34th week, she received danaparoid sodium. After a week, by cesarean section, a healthy and mature female was delivered.

Key words: Pregnancy; Heparin-induced thrombocytopenia; Pulmonary embolism.

Introduction
Pulmonary embolism is the leading cause of maternal mortality during pregnancy and labor. Incidence is one per 1,000 - 3,000 deliveries. Heparin-induced thrombocytopenia is a life-threatening condition with mortality rate of 29%. In 0.5%-3% of cases, it is caused by venous thromboembolism in pregnancy. The question the authors had to answer was: what to do and how to help this patient who developed heparin-induced thrombocytopenia during therapy of massive pulmonary embolism at ten weeks gestation?

Materials and Methods
The authors present the case of a 34-year-old patient, primipara. At the tenth week of pregnancy, she developed phlebothrombosis of femoral popliteal and crural veins of the right leg and massive pulmonary embolism. She received thrombolytic therapy with tissue plasminogen activator and intravenous heparin therapy; at the beginning she showed clinical signs of improvement, but also developed rethrombosis, proved by Doppler scan of veins of lower extremities, and heparin-induced thrombocytopenia type II (platelet count was 46,000 per ml) which was proved by laboratory tests. Immediately lepirudin was added to the therapy by intravenous infusion and it resulted in clinical and hematological improvement. In her 12th week of pregnancy, oral anticoagulant therapy was started with acenocumarol, controlled by international normalized ratio (INR) (between 1.5 and 2.5). Careful monitoring of fetus was applied, with ultrasound and Doppler assessments. Complete biochemical fetal noninvasive screening testing was performed as well nuchal translucency (NT), double and triple screen) with normal results. Resistance indexes in umbilical and cerebral fetal circulation were within normal values. After the 34th week, danaparoid sodium 750 IU subcutaneously every 12 hours was introduced in therapy with control of anti-Xa levels in maternal blood. At the end of the 35th week, cesarean section was performed. The last dose of danaparoid was administered six hours before surgery and continued six hours after. The newborn was healthy with birth weight of 2,700 g and Apgar score of 9. Intensive neonatal care unit was not necessary. For two weeks after delivery the mother received danaparoid sodium twice a day; four days after surgery acenocumarol was introduced in therapy again. After 13 days, therapy with danaparoid was terminated and continuous oral anticoagulant therapy maintained. Lactation was regular. The postoperative course was carefully monitored and was normal. The patient was discharged after 14 days from hospital with ultrasound and laboratory results within the normal range. Follow up of the child in following three years showed normal mental and physical development.

This review shows how important proper and prompt diagnose of phlebothrombosis is during pregnancy. Otherwise pulmonary embolism is frequently a consequence. This severe and life-threatening situation needs anticoagulation therapy with heparin. Some patients are at risk of developing heparin-induced thrombocytopenia, which additionally complicates an already difficult situation. Danaparoid is the medication of choice in these cases. It is subcutaneously administrated, with low side-effects. During second-trimester pregnancy, oral anticoagulant therapy is a safe option. From available literature the authors see similar experiences, although the number of patients treated in this manner is not large, number of complications is greater, but the present authors had no maternal and fetal complications. This is certainly a result of cooperative team work. Lindhoff-Last et al. reviewed the use of danaparoid in 51 pregnancies of 49 patients identified in literature between 1981 and 2004. All patients had developed heparin-induced thrombocytopenia, 19 mainly due to heparin-induced skin rashes), and had a current and/or past history of thromboembolic complications. The initial danaparoid dose regimens ranged from 1,000 to 7,500 IU subcutaneously every 12 hours.
U/day administered subcutaneously or intravenously. The median duration of danaparoid use was ten weeks. Danaparoid was used until delivery of a healthy infant in 37 pregnancies. In the remaining 14 pregnancies, it was stopped earlier, because anticoagulant treatment was no longer required (3/14) or an adverse event led to a treatment discontinuation (11/14). Four maternal bleeding events were recorded during pregnancy, delivery or postpartum, two of them were fatal due to placental problems. Three fetal deaths were recorded, all associated with maternal complications antedating danaparoid use [1]. Schindewolf et al. found a suitable alternative anticoagulant when heparin-induced thrombocytopenia type II (HIT II) or allergic skin reactions occur. Their results showed that 40/59 pregnancies were carried to term under use of danaparoid and resulted in the delivery of a healthy infant. In 16/19 pregnancies, danaparoid was stopped due to a major adverse event. Five patients showed bleeding complications, seven fetal losses were documented, but there was no association with the use of danaparoid. In 31/59 (52.5%) pregnancies, adverse events were documented, 14/31 (45.2%) could be attributed to danaparoid. Anti-X activity was not detected in five fetal cord blood samples and in four maternal breastmilk samples [2]. Myers et al. described a case where danaparoid was used prophylactically in a high-risk twin pregnancy following the development of heparin-allergy while on prophylactic dalteparin. Danaparoid was substituted for dalteparin at 20 weeks of pregnancy following the development of a severe skin reaction while on low molecular weight heparin. Although there was no significant fall in platelet count, an aggregation assay for heparin-induced thrombocytopenia was positive. The skin lesions rapidly resolved following the change to subcutaneous danaparoid. Delivery was through emergency cesarian section at 35 weeks under a general anesthesia, as a dose of danaparoid had been given six hours prior to delivery. A sample of breast milk showed no anti-X activated factor activity. Danaparoid was continued post-delivery until the patient was fully warfarinized [3].

Danaparoid, which has low cross-reactivity for heparin-dependent antibodies and no known teratogenic effects, was used successfully to treat the patient, who developed heparin-induced thrombocytopenia during pregnancy [4, 5]. Danaparoid may be the treatment of choice for this difficult clinical situation in which there are limited therapeutic options.

References


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