21

Embryo Quality and Pregnancy Outcome in Infertile Patients with Endometriosis

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1. Introduction

Investigation of one complex pathological condition is definitively a challenging task, but trying to find the connection(s) between two is even more difficult. This is very true in the case of infertility and endometriosis. Both conditions have numerous symptoms, very diverse clinical pictures and multifactorial etiologies. The first step toward understanding the connection between the two is to prove the correlation between them. The next task is to try to understand the mechanisms by which they affect each other, which involves examination and comparison of numerous variables specific for each condition. The results are still the subject of many controversies.

1.1 Endometriosis and infertility

At present, there is little debate that endometriosis and infertility are actually associated. For example, early retrospective studies (Hasson, 1976; Drake & Grunet, 1980; Strathy et al., 1982) and one more recent prospective study (Mahmood & Templeton, 1991) performed in women who underwent laparoscopy (for various reasons) showed that endometriotic lesions were significantly more frequent in women who were treated for infertility than in those who requested laparoscopy for tubal sterilization. Prevalence of endometriosis in infertile women ranged from 21 - 48% which was in clear contrast to the prevalence of 1.3 – 5% in fertile women (Hasson, 1976; Drake & Grunet, 1980; Strathy et al., 1982). Another line of evidence of the existence of a link between endometriosis and infertility came from studies in women who underwent donor insemination because of severe male infertility. In these studies, women with endometriosis had significantly fewer conceptions per procedure than women without this condition (Hammond et al., 1986; Yeh & Seibel, 1987). In studies where peritoneal endometriosis was induced in rabbits (Schenken & Asch, 1980), primates (Schenken et al., 1984) and rodents (Vernon & Wilson 1985; Barragan et al., 1992) it was clearly demonstrated that endometriosis was strongly associated with infertility regardless of localization and/or extension of the lesions.

While it is relatively easy to document the link between the two conditions, defining precise pathophysiologic mechanisms and proving a causal relationship between endometriosis and infertility is much more difficult.

In severe cases of endometriosis, the seriously distorted anatomy of pelvic organs is the obvious cause of impaired fertility. In the absence of pelvic adhesions and scarring, when only mild to moderate endometriotic lesions are present, finding the cause and the consequence is anything but an easy task. Many confounding factors make these studies both controversial and difficult to interpret. One of the main problems is the choice of the appropriate control group. The common practice has been to choose women with tubal factor infertility or those with unexplained infertility as controls. The problem with this choice is our inability to identify women with inherently reduced potential for conception (fertilization and implantation) and to exclude them from the control group. Also, the common practice is to make observations on the patients with endometriosis who are treated by one of the techniques of assisted reproductive technologies (ART). This practice is problematic because the use of ART creates a non-physiologic environment and many subtle but important *in vivo* effects of endometriosis on the process of conception may be hidden in *in vitro* conditions.

Hypotheses on mechanisms by which mild to moderate endometriosis could impair fertility potential are numerous and will be mentioned here only briefly. One group of investigators tested hypotheses that endocrine abnormalities in women with mild and moderate endometriosis might be the cause of reduced fertility. As was already conveniently summarized in the literature (Garrido et al., 2000; Hunter et al., 2004), proposed mechanisms were hypersecretion of prolactin in patients with endometriosis (Muse et al., 1982), impaired folliculogenesis (Tummon et al., 1988), altered ovulation (Dmowski et al., 1986) and luteal phase defects (Grant, 1966). The other line of investigation was directed towards immune dysfunctions as potential causes of infertility in patients with endometriosis. Proposed mechanisms were chronic inflammatory reaction and altered immune responses induced by endometriosis (Harada et al., 2001), including increased production of cytokines and other soluble immunomodulators in the peritoneal fluid. These altered immune responses could further affect motility and velocity of the sperm, lead to sperm phagocytosis (Soldati et al., 1989), accelerate ovum transport (Croxato et al., 1978), impair the process of fertilization (Mahadevan et al., 1983), display direct embryo toxicity (Damewood et al., 1990) or adversely affect the process of implantation (Yovich et al., 1985; Matson & Yovich, 1986). Unfortunately, results of these studies were usually contradicting and no definitive conclusion could be made so far. It is likely that there is more than one answer to this complex problem.

2. Endometriosis and embryo quality

Among investigators who reported poorer results of IVF-ET outcome in patients with endometriosis, there is a general agreement on few final consequences in contrast to numerous possible pathophysiologic mechanisms leading to it.

These include:

1. Impairment of the quality of oocytes (resulting in lower fertilization rates) and/or
2. Decrease in the implantation capacity of embryos (Pellicer et al., 2001).

An indirect marker of oocyte quality and a possible predictor of embryo's implantation capacity is the quality of the developing embryo. As such, this parameter could be used for assessment of the effects of endometriosis on fertility.

2.1 Measures of embryo quality

Quality of the embryo may be described using many direct and indirect measures. For example, parameters which could be used for indirect assessment of embryo quality are number of embryos on Day 2, total number of blastocysts, number of frozen blastocysts, but also the implantation rate and early pregnancy loss rate. The only direct measure of embryo quality is embryo quality score based on morphological characteristics of a developing embryo. However, this parameter is quite difficult to use in practice. One reason for this difficulty is the absence of uniformity of scoring systems used by different laboratories. Another is a consequence of two facts: (1) quality score is a categorical variable and (2) it is (still) a common practice to transfer more than one embryo (blastocyst) at a time. In other words, if two or more embryos of different qualities have been transferred, it is not possible to calculate "the mean embryo quality score" or to determine exactly which one of transferred embryos has eventually implanted.

Researchers who would come across this issue tried to overcome it in various ways. The simplest was to exclude from the study those patients in whom embryos with different quality scores were transferred (La Sala et al., 2005). The main drawback of this approach was a significant reduction of observed cycles, i.e. of the sample size. Also, this tactic prevented incorporation of some other important variables (namely the number of transferred embryos) in the final analysis. On the other hand, some of the authors (Lambers et al, 2007) used a cumulative embryo score, previously introduced by Steer (Steer et al., 1992). Cumulative embryo score was defined as an additive parameter (i.e. following the transfer of two embryos with scores of 1 and 3, the total score of embryos transferred was 4). Other investigators (Winter et al., 2002) assessed embryo quality with relation to the number of embryos transferred and the possibility of elective transfer. According to this system, embryos were scored 1 in the case of an elective transfer of one or two embryos (highest score); elective transfer of 3 embryos yielded a score of 2; if two or three embryos had been transferred non-electively, the score was 3, and if only a single embryo was transferred non-electively, it was scored 4 (worst score).

In our research, we adopted yet another approach. If multiple embryos of different quality were transferred, we assumed that (1) implanting embryo was the best quality embryo (the so-called leading embryo) and (2) the leading embryo determined embryo quality score of the entire group of transferred embryos. The first assumption was well documented in the literature (Hourvitz et al., 2006) and the second one was additionally tested in our sample.

2.2 Relevant studies encompassing the measures of embryo quality

Despite the fact that important hypothesis blames defective early embryo development for poorer IVF-ET outcome in patients with endometriosis, relatively few studies have analyzed the association between quality of transferred embryo(s) and endometriosis. We will briefly present several important studies on the subject.

A group of Spanish investigators conducted three separate retrospective analyses of the success of their IVF-ET and oocyte donation program in patients and donors with and

without endometriosis (Pellicer at al., 1995). In the segment of the study on early embryonic development, which was performed on 36 women with endometriosis and on 34 with tubal infertility used as a control group, they explored the embryos grown in vitro for 72 hours before embryo transfer. Embryo quality assessment system included the number of blastomeres and the degree of fragmentation after 48 and 72 hours in culture. If embryos presented only one or two blastomeres 72 hours after oocyte retrieval, it was considered that an embryo arrest had occurred. After 72 hours in culture, there was a significant decrease in the number of blastomeres in endometriosis compared to tubal infertility patients (5.4 ± 0.1 versus 6.1 ± 0.3 blastomeres, respectively, $p<0.04$) and a significant increase in the percentage of arrested embryos (57.4 ± 2.3 in patients with endometriosis versus 45.2 ± 5.8 in control group, $p<0.05$). In order to control for the influence of semen parameter on embryo quality, researchers further subdivided groups of patients taking into account the quality of semen. If abnormal semen was used for in vitro fertilization, higher degree of embryonic arrest was observed in comparison to the group with normal semen parameters (55.6 ± 6.4 vs. 20.3 ± 7.9 ($p<0.01$), respectively, in the group with tubal infertility; 61.8 ± 2.6 vs. 47.5 ± 2.8 ($p<0.003$), respectively, in the group with endometriosis). Also, if the semen used had normal characteristics, significantly more arrested embryos were noted in patients with endometriosis compared to patients with tubal infertility ($p<0.001$). Further insight into the problem was attained when the same researchers analyzed pregnancy outcome of oocyte donation with regards to the origin of donated oocytes. This segment of the research incorporated a total of 178 embryo transfers in 141 women. If oocytes were donated by donors without endometriosis, implantation and ongoing pregnancy rates were comparable in both recipients with and those without endometriosis. If oocytes were collected from donors with endometriosis, significantly lower implantation rates were reported in recipients ($p<0.05$). The authors of this study concluded that infertility in patients with endometriosis may be related to oocyte alterations which result in embryos of lower quality and reduced implantation ability, although the impact of hostile (anti-implantatory) environment on embryos of normal developmental potential cannot be ruled out.

Another study which reported negative impact of endometriosis on embryo quality was the prospective case control study in which researchers included 37 patients with "true" endometriomas and 56 patients without any complex ovarian cysts as controls (Yanushpolsky et al., 1998). All endometriomas were larger than 1 cm in diameter and would be classified as stage III endometriosis according to The ASRM-revised classification of endometriosis (The American Fertility Society, 1985). Only patients with complex "chocolate" cysts in which CA 125 levels in cyst fluid were >100.000 U/ml ("true" endometriomas) were included in the study. Embryo quality was expressed as the number of embryos reaching at least four-cell stage on the second day after oocyte retrieval. Quality of the embryos in the group of patients with endometriomas was significantly reduced compared to controls ($p=0.09$). Also, in patients with endometriomas, significantly fewer oocytes were retrieved ($p=0.06$) and early pregnancy loss rate (biochemical pregnancies and early clinical spontaneous miscarriages combined) was significantly higher ($p=0.04$). Interestingly, fertilization rate and implantation rate were not significantly different between the studied groups.

A group of Norwegian investigators also confirmed detrimental effect of endometriosis on embryo quality (Tanbo, 1995). They analyzed 215 women (385 cycles) whose main indication for IVF-ET was unexplained infertility (ovulatory women, with patent tubes and normal uterine cavity, normal laparoscopy and normal sperm characteristics), 143 women (285 cycles) with endometriosis as the only indication and 180 women (353 cycles) with tubal infertility (control group). Cleavage rate and failure of cleavage were used as criteria of embryo quality. Significantly lower cleavage rates were observed in both unexplained infertility and endometriosis groups compared with the tubal infertility group. Total failure of cleavage was 19.2% in unexplained infertility, 14.3% in endometriosis and 3.6% in tubal infertility group ($p<0.0001$). Since there were no differences in sperm characteristics between the groups, the authors speculated that lower cleavage rates could be a consequence of inferior oocyte quality in unexplained infertility and endometriosis group.

In an interesting study of authors from U.S.A. a total of 235 preimplantation embryos were retrospectively analyzed (Brizek et al., 1995). These embryos were obtained from 56 IVF-ET cycles performed in 30 women. Sixteen patients had endometriosis as the main indication for the procedure and the remaining 14 were controls without endometriosis who were chosen randomly from other diagnosis categories. The incidence of specific phenotypes ranging from normal 2PN zygote to different types of abnormal embryos was then recorded on days 1 and 2 following fertilization. An increased incidence of aberrant development of embryos derived from women with endometriosis was demonstrated. There were three abnormal phenotypes on day 1 and two abnormal phenotypes on day 2 which were significantly more prevalent in patients with endometriosis. However, there was no statistical difference in the number of normal embryos in patients with endometriosis compared to controls on day 1 or day 2. Despite the fact that the effect of endometriosis was observed in the developmental dynamics of the fertilized ovum, no gross endometriosis-specific morphological changes in oocytes recovered from endometriosis group could be seen.

In contrast to previously cited observations, several other studies failed to show negative influence of endometriosis on the parameters of embryo quality. A group of authors from the U.S.A. conducted a retrospective analysis of 284 IVF-ET cycles from patients with a sole diagnosis of endometriosis, or tubal factor, or unexplained infertility (Arici et al., 1996). All of the patients had laparoscopy prior to the IVF procedure. The criteria for the diagnosis of unexplained infertility were confirmed ovulatory cycles, normal tubal patency on hysterosalpingography, normal sperm analyses. The severity of endometriosis was graded as defined by the Revised American Fertility Society classification (The American Fertility Society, 1985) and patients were further divided into two subgroups as minimal to mild (stages I and II) and moderate to severe (stages III and IV). Quality of embryos was assessed on the day of the transfer in line with the system used by the authors' center according to their morphology as observed under the inverted microscope (morphological grades I to V). In the final analysis, the researchers used "the average embryo quality score" for the given subgroup of patients, which was probably calculated as the arithmetical average of all embryo quality scores expressed as grades I to V. No statistically significant difference in "average embryo score" among subgroups were noted (1.8 ± 0.5 in the minimal to mild

endometriosis group vs. 2.0 ± 0.6 in the moderate to severe endometriosis group vs. 1.9 ± 0.5 in the tubal infertility group vs. 1.8 ± 0.6 in the unexplained infertility group; p>0.05). Surprisingly, when the data were analyzed according to the stage of endometriosis, in the group with moderate to severe endometriosis (stages III and IV) a significantly higher fertilization rate was observed compared to the group with minimal to mild endometriosis (stages I and II) (78.4% vs. 66.8%, respectively; p=0.001). However, implantation rates were low and not significantly different between these subgroups (5.5% in the group with moderate to severe endometriosis vs. 2.8% in the group with minimal to mild endometriosis, p=0.46).

Another study which failed to show negative impact of endometriosis on embryo quality was conducted by Swedish researchers (Bergendal et al., 1998). The analysis included a total of 65 IVF-ET cycles in 48 patients with endometriosis as the only apparent cause of infertility and 98 cycles in 98 patients in whom tubal factor was the only apparent cause of infertility (controls). The embryos were graded according to criteria set by the authors' center (morphology and cleavage stage) with embryo quality scores raging from 1 to 3, with 3 being the best score. The average score of the whole subgroup (defined as arithmetical average of all scores) was used in the final analysis. Despite the fact that fertilization rate was significantly higher in patients with tubal infertility compared to patients with endometriosis (78.3 ± 18.3% vs. 60.1 ± 31.7%, respectively; p=0.00001), no difference was noted in cleavage rates (87.9 ± 19.1% in the tubal factor group vs. 85.2 ± 22.1% in the endometriosis group; p=0.43) or morphological score of embryo for ET (2.5 ±0.39 in the tubal infertility group vs. 2.4 ± 0.4 in the endometriosis group; p=0.45).

In yet another study which reported results on the impact of endometriosis on embryo quality (Dmowski et al., 1995), a retrospective analysis of 237 consecutive IVF-ET cycles in patients with and without endometriosis was conducted. In the group without endometriosis, indications for IVF-ET were tubal disease, pelvic adhesions, male factor, unexplained infertility, ovarian dysfunction ant other factors. In this study, the number of oocytes cleaved was taken as the indirect measure of embryo quality. The authors reported there was no difference between groups in the number of fertilized and cleaved oocytes, but no exact numerical values for these variables were included in the published report. The lack of properly defined control group (endometriosis vs. all other indications) and the absence of further details on development of transferred embryos warrant caution for interpretation, at least in the segment of the study pertaining to embryo quality.

2.3 Our study

We conducted a retrospective clinical study which encompassed 346 stimulated IVF or ICSI cycles with the transfer of one or two blastocysts performed at the Department of Reproductive Medicine and Gynecological Endocrinology at the University Medical Centre of Maribor, Slovenia.

The primary objective of our study was to examine possible differences in direct and indirect indicators of embryo quality between women with endometriosis as the only indication for the treatment and an adequate control group of women with tubal factor only. Possible differences in various other outcomes of IVF-ET cycles between these two groups

were also analyzed. The secondary goal was to examine the influence of embryo quality on various outcomes of IVF-ET cycles against all other important variables as controls in the group of women with endometriosis.

2.3.1 Materils and methods

Data used in this analysis were received from the centre's database on couples treated for infertility from 2003 to 2010. If there any data for any variable was missing from the database, the patient's documentation (paper records) was checked. If it was still impossible to find the missing data, the patient was excluded from further analysis. Patients included were under 43 years of age and prior to entering an IVF/ICSI treatment, underwent all tests prescribed by the protocol for clinical examination of infertile couples.

The observed cycles were divided into two groups: 173 cycles were performed in patients with endometriosis as the only indication for treatment and 173 cycles in women with tubal factor infertility (control group). The patients from tubal factor group were individually matched with women with endometriosis by age group (<30, 30-34, 35-39, >39 years), number of retrieved oocytes (<5, 5 or more) and number of transferred embryos (1, 2 or 3).

Patients were most frequently stimulated according to the protocol involving gonadotrophin-releasing hormone agonists (GnRH-a) (almost exclusively using the long protocol). In the few remaining patients, the protocol with gonadotrophin-releasing hormone antagonists (GnRH-ant) was applied. GnRH agonists used were triptorelin (Diphereline®, Ipsen Pharma Biotech, France), gosereline (Zoladex®, Zeneca Pharmaceuticals, England) or busereline (Suprefact®, Sanofi Aventis, France). Cetrorelix (3 mg) (Cetrotide®, Merck Serono, Switzerland) was used as a GnRH antagonist. Follicle growth was predominantly stimulated by recombinant FSH (Gonal F®, Merck Serono, Switzerland), while human menopausal gonadotrophin (HMG) (Menopur ®, Ferring Pharmaceuticals, Switzerland) was used occasionally. On the day when at least two follicles reached an average diameter of 18 mm, final maturation of the oocyte was stimulated by the urinary human HCG (Profasi®, Merck Serono, Switzerland, using a dose of 10,000 IU) or human recombinant HCG (Ovitrelle®, Merck Serono, Switzerland, 250 mg dose). A detailed description of the laboratory procedures can be found elsewhere (Kovacic et al., 2009). Approximately 36 hours (36 ± 1) following the administration of HCG, oocytes were recovered by ultrasound-guided trans-vaginal follicle aspiration. Fertilization was performed through IVF or ICSI. Medicult® media (MediCult, Denmark) were used for oocyte culturing. Pursuant to the protocol of our centre, only one or maximally two blastocysts were transferred on the fifth, exceptionally on the fourth day following follicle aspiration. Labotec® catheter (Labotec, Germany) was used for blastocyst transfer. In line with the legislation in force at the time of the study, the couple was allowed to decide on the number of embryos to be transferred. Embryos were transferred only after both partners signed the official consent form for the transfer of embryos. A day after the follicle aspiration, all patients started receiving didrogesterone (30 mg/day) (Dabroston®, Belupo, Croatia) or micronized progesterone (600 mg/day) (Utrogestan®, Laboratories Besins International, France) for luteal support.

Quality of transferred blastocysts was evaluated by a blastocyst classification system based on morphological criteria, developed by our centre (Kovacic et al., 2004). This classification

is a modification of the earlier, well established blastocyst evaluation system (Gardner & Schoolcraft, 1999). The classification used in our laboratory takes into consideration four parameters: blastocoel expansion, inner cellular mass (ICM) form, morphology and cohesion of the trophoectoderm (TE) as well as the degree of embryo fragmentation. There are 8 grades of quality of blastocysts (B1 to B8) in this system, with B1 being the best quality score and B8 the worst. The data on blastocyst quality expressed in this way had to be transformed before entering the statistical model. The transformation was performed in two steps. First, the blastocysts from B1 category were designated as optimal quality blastocysts, while those in categories B2–B8 were classified as being of suboptimal (non-optimal) quality. In the second step, in cases where blastocysts of different quality were transferred, the subgroup with blastocysts of different quality was merged with the subgroup in which all transferred blastocysts were of optimal quality. In this way, for the final statistical analyses we had a subgroup with blastocysts of suboptimal quality only and a subgroup with at least one blastocyst of optimal quality. All other possibilities for regrouping were also tested, but it was concluded that the chosen transformation of data showed the best fit with this model. This conclusion was expected, because it was in line with the assumption that in those cases in which multiple embryos of different quality were transferred and only one of implanted, the higher quality embryo (so-called leading embryo) had the highest probability of implantation.

In our analysis we made a distinction between premenstrual pregnancy loss (loss of conceptus prior to the first measurement of βhCG level 14 days after ovulation or embryo transfer), biochemical pregnancy (loss of conceptus after the first measurement of βhCG level but before the ultrasound (US) confirmation of implantation) and early clinical miscarriage (pregnancy loss after US confirmation of viable pregnancy but before the beginning of the second trimester) (Došen et al., 2011). Biochemical pregnancies and early clinical miscarriages are commonly identified together as early pregnancy losses (EPL).

Basic demographic and clinical characteristics are presented as mean ± standard deviation (SD) or median with 1st and 3rd quartile and analyzed by independent-samples t-test if normally distributed or by Mann-Whitney test if skewed. Categorical data are expressed as proportions and analyzed by chi-squared test. The results are presented as odds ratios and their 95% confidence intervals (CI). P value of under 0.05 was considered to be statistically significant. Statistical analysis was performed using STATISTICA® software, version 8.0 (StatSoft Inc., OK, USA).

2.3.2 Results

2.3.2.1 Differences in embryo quality indicators and other parameters between the groups

Average age of the patients was 32 years (in the group of women with endometriosis 32.6 ± 3.5, the youngest patient was 25 and the oldest 42 years old; in the group with tubal infertility 32.5 ± 3.9, the youngest patient was 22 and the oldest 43 years old).

Analysis of differences in quality score of transferred blastocysts between patients with endometriosis and tubal factor infertility showed marginal statistical significance, if all the scores (B1-B8) were analyzed together (chi-square=14.03, p=0.051). Further analysis was

undertaken to identify the subgroups of the same blastocyst quality score in which possible significant difference was present. The results are presented in Table 1.

Blastocyst quality score	Endometriosis group (N=173)	Tubal factor group (N=173)	P value
B1	195 (24.8)	201 (25.2)	0.873
B2	54 (6.9)	70 (8.8)	0.161
B3	106 (13.5)	114 (14.3)	0.653
B4	85 (10.8)	71 (8.9)	0.198
B5	47 (6.0)	46 (5.8)	0.851
B6	105 (13.4)	140 (17.5)	0.022
B7	41 (5.2)	25 (3.1)	0.038
B8	153 (19.5)	132 (16.5)	0.127
Total	786 (100.0)	799 (100.0)	

Table 1. Comparison of embryo quality scores between the studied groups

Since a significant difference was present only in the subgroups of transferred blastocysts of very low quality (scores B6 and B7), this finding doesn't provide any further insight into the problem.

Analysis of indirect embryo quality indicators showed no statistically significant differences between the groups. The results are presented in Table 2.

Variable[a]	Endometriosis group (N=173)	Tubal factor group (N=173)	P value
No. of embryos on Day 2	7.5 ± 0.30	7.5 ± 0.29	0.890
No. of blastocysts	3.7 ± 0.21	4.0 ± 0.20	0.317
No. of frozen blastocysts	2.0 ± 0.18	2.4 ± 0.20	0.210

[a] Data are expressed as mean ± standard error.

Table 2. Comparison of indirect indicators of embryo quality between the studied groups.

Analysis of outcomes of IVF-ET cycles also failed to show any statistically significant differences between the groups. Results are presented in Table 3.

The outcome	Endometriosis group (N=173)	Tubal factor group (N=173)	P value
Implantation rate (%)	40.6 (112/276)	47.1 (130/276)	0.123
Clinical pregnancy rate (%)	49.7 (86/173)	54.3 (94/173)	0.389
Clinical miscarriage rate (%)	4.6 (8/173)	6.4 (11/173)	0.479
Early pregnancy loss* rate (%)	8.7 (15/173)	11.6 (20/173)	0.373
Live birth rate (%)	40.0 (64/173)	45.1 (74/173)	0.351

*Biochemical pregnancies and clinical miscarriages combined

Table 3. Comparison of IVF-ET cycles outcomes between the studied groups

In the additional analysis of some of the other important parameters, no significant differences were noted between the groups.

Variable	Endometriosis group (N=173)	Tubal factor group (N=173)	P value
No. of oocytes retrieved (N)	11.2 ± 0.41	11.1 ± 0.39	0.773
No. of fertilized oocytes (N)	7.6 ± 0.31	7.5 ± 0.29	0.859
Fertilization rate (%)	69.4 ± 1.39	69.0 ± 1.38	0.847
Male factor present (%)	96 (55.5)	84 (48.6)	0.197

Table 4. Comparison of variables of IVF-ET cycles between the studied groups

2.3.2.2 Blastocyst quality and other possible predictors of various outcomes

The other objective of our investigation was to define the influence of blastocyst quality on various outcomes of IVF-ET cycles against all other important variables as controls in the group of women with endometriosis.

In our analysis, we incorporated 11 parameters as possible predictors of four main outcomes of stimulated IVF-ET cycles. The encompassed parameters were:

- patient's age,
- fertilization method (IVF or ICSI),
- type of gonadotrophin used for stimulation (human menopausal gonadotrophin (HMG) or recombinant follicle stimulation hormone (FSH)),
- number of retrieved oocytes,
- number of fertilized oocytes,
- fertilization rate,
- number of embryos on Day 2,
- number of blastocysts (embryos on Day 5),
- number of transferred blastocysts,
- number of frozen blastocysts and
- embryo quality score of transferred blastocysts.

Several examined predictors were transformed in categorical variables, as will be explained below. The six observed outcomes were:

- positive βhCG 14 days after ET,
- clinical pregnancy rate,
- live births rate,
- biochemical pregnancy rate,
- early clinical miscarriage rate, and
- early pregnancy loss rate (EPL - biochemical pregnancies and early clinical miscarriages taken together).

The relationship between continuous predictors and the number of implanted blastocysts was analyzed using Spearman correlation, while the effect of categorical predictors on the number of implanted blastocysts was tested by Mann-Whitney test or Kruskal-Wallis one-way analysis of variance. For all other outcome variables, the effect of possible predictors was analyzed using univariate logistic regression model. Before the incorporation of possible predictors in the multiple regression model, correlations among variables were tested to detect possible multicolinearity and to choose appropriate variables for the final

analysis. The impact of possible predictors on the number of live born infants was evaluated using analysis of variance or chi-squared test, as appropriate. For post-hoc comparison of continuous variables, Bonferroni correction of alpha was used, while Keppel modification of Bonferroni correction was used for categorical variables.

Because of the low number of events per variables (EPV) for early clinical miscarriages, biochemical pregnancies and EPLs included in the logistic regression (Vittinghoff & McCulloch, 2007), a multiple model for analyzing the relative contribution of each predictor was constructed only for these three outcomes: **positive** βhCG 14 days following embryo transfer, clinical pregnancy rate and live births rate.

After testing all parameters for multicolinearity, a problem was detected in these pairs of variables: the number of retrieved oocytes/the number of fertilized oocytes, the number of retrieved oocytes/the number of embryos on Day 2, the number of fertilized oocytes/the number of embryos on Day 2 and the number of blastocysts/the number of frozen blastocysts. Accordingly, these pairs of variables were not included in the multiple regression model.

2.3.2.2.1 *Positive βhCG*

A univariate logistic regression model suggested a statistically significant correlation between the positive βhCG 14 days after ET and these indirect indicators of embryo quality: the number of embryos on Day 2 (OR=1.091; 95% CI 1.004 - 1.185, P<0.039), the number of blastocysts (OR=1.303; 95% CI 1.138 - 1.491, P<0.001), the number of frozen blastocysts (OR=1.436; 95% CI 1.208 - 1.708, P<0.001) and the embryo quality score of transferred blastocyst (in the form of two subgroups: the one with blastocysts of suboptimal quality only and the other with at least one blastocyst of optimal quality) (OR=5.339; 95% CI 2.782 - 10.246, P<0.001). A statistically significant correlation in univariate model was also noted for the age of the woman (OR=0.857; 95% CI 0.781 - 0.940, P=0.001), the number of retrieved oocytes (OR=1.069; 95% CI 1.006 - 1.135, P=0.030) and the number of fertilized oocytes (OR=1.088; 95% CI 1.003 - 1.180, P=0.041).

After controlling for the all other independent possible predictors in the multiple logistic regression model, the predictors of positive βhCG 14 days after ET in patients with endometriosis identified as statistically significant were the embryo quality score of transferred blastocyst (OR=4.278; 95% CI 1.976 - 9.265, P<0.001) and the age of the woman (OR=0.848; 95% CI 0.757 - 0.950, P=0.005).

2.3.2.2.2 *Clinical pregnancy rate*

Application of a logistic regression in univariate model showed that there was a statistically significant correlation between the clinical pregnancy rate and these indirect measures of embryo quality: the number of blastocysts (OR=1.278; 95% CI 1.122 - 1.457, P<0.001), the number of frozen blastocysts (OR=1.376; 95% CI 1.170 - 1.618, P<0.001) and the embryo quality score of transferred blastocyst (in the form of two subgroups: the one with blastocysts of suboptimal quality only and the other with at least one blastocyst of optimal quality) (OR=4.708; 95% CI 2.466 - 8.986, P<0.001). A statistically significant correlation in univariate model was also noted for the age of the woman (OR=0.875; 95% CI 0.781 - 0.941, P=0.001) and the number of retrieved oocytes (OR=1.069; 95% CI 1.007 - 1.134, P=0.027).

After controlling for all other independent possible predictors in the multiple logistic regression model, the predictors of the clinical pregnancy rate in patients with endometriosis identified as statistically significant were the embryo quality score (OR=3.485; 95% CI 1.608 - 7.553, P=0.002) and the age of the woman (OR=0.861; 95% CI 0.770 - 0.963, P=0.009).

2.3.2.2.3 *Live births*

A univariate logistic regression model indicated that there was a statistically significant correlation between the live births rate and these indirect measures of embryo quality: the number of blastocysts (OR=1.313; 95% CI 1.139 - 1.513, P<0.001), the number of frozen blastocysts (OR=1.402; 95% CI 1.183 - 1.661, P<0.001) and the embryo quality score of transferred blastocyst (in the form of two subgroups: the one with blastocysts of suboptimal quality only and the other with at least one blastocyst of optimal quality) (OR=3.316; 95% CI 1.693 - 6.496, P<0.001). A statistically significant correlation in univariate model was also noted for the age of the woman (OR=0.861; 95% CI 0.780 - 0.950, P=0.03).

After controlling for all other independent possible predictors in the multiple logistic regression model, the predictors of the live births rate in patients with endometriosis identified as statistically significant were the age of the woman (OR=0.851; 95% CI 0.756 - 0.958, P=0.07) and the number of frozen blastocysts (OR=1.319; 95% CI 1.034 - 1.683, P=0.026).

3. Conclusion

It is generally accepted that endometriosis and infertility are associated. However, the mechanisms connecting these complex conditions are still elusive. Results of different studies on virtually every aspect of this subject are controversial. Despite controversy, there is general agreement on relatively few final consequences of these pathophysiologic processes - endometriosis impairs the quality of oocytes with resulting lower fertilization rates and/or decreases implantation capacity of embryos (Pellicer et al., 2001).

One of indirect markers of oocyte quality and a possible predictor of embryo's implantation capacity is the quality of the developing embryo. As such, this parameter could be valuable for the assessment of influence of endometriosis on fertility of affected individuals. There are several indicators of embryo quality. Some of them are indirect measures of quality (the number of embryos on Day 2, the number of blastocysts, the number of frozen blastocysts, but also the implantation rate), while the only direct measure is the embryo quality score based on morphological characteristics of a developing embryo.

Our study showed no statistically significant difference of quality score of transferred blastocysts, indirect measures of embryo quality, common outcomes of IVF-ET cycles (implantation rate, clinical pregnancy rate, clinical miscarriage rate, early pregnancy loss rate and live births rate) or other analyzed parameters (male factor present, number of oocytes retrieved, number of fertilized oocytes, fertilization rate method of fertilization) between the group of infertile patients with endometriosis and the group with tubal factor infertility only.

In the further analysis of our data, we also showed that in infertile patients suffering only from endometriosis, embryo quality was a statistically significant positive predictor of positive βhCG measurement (if embryo quality was expressed in the form of embryo quality score, OR=4.278; 95% CI 1.976 - 9.265, P<0.001), clinical pregnancy rate (if embryo quality was expressed in the form of embryo quality score, OR=3.485; 95% CI 1.608 - 7.553, P=0.002) and live births rate (if embryo quality was expressed in the form of number of frozen blastocysts, OR=1.319; 95% CI 1.034 - 1.683, P=0.026). As expected, the patient's age was a statistically significant negative predictor of the success of IVF-ET cycles (positive βhCG measurement, clinical pregnancy rate and live births rate) in the observed group of patients.

Endometriosis is still an insufficiently explained condition. Numerous controversies still surrounding this complex disease indicate an obvious need for further clinical studies, meta-analyses and explanation of its pathophysiologic mechanisms. Should a consensus be reached on a precise methodology, future studies would definitely be more informative and results easier to use in clinical practice.

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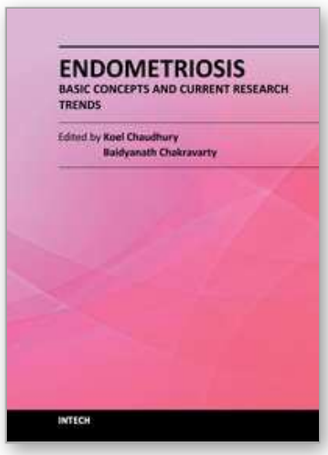

Endometriosis - Basic Concepts and Current Research Trends

Edited by Prof. Koel Chaudhury

ISBN 978-953-51-0524-4
Hard cover, 490 pages
Publisher InTech
Published online 09, May, 2012
Published in print edition May, 2012

This book provides an insight into the emerging trends in pathogenesis, diagnosis and management of endometriosis. Key features of the book include overviews of endometriosis; endometrial angiogenesis, stem cells involvement, immunological and hormonal aspects related to the disease pathogenesis; recent research reports on infertility, endometrial receptivity, ovarian cancer and altered gene expression associated with endometriosis; various predictive markers, and imaging modalities including MRI and ultrasound for efficient diagnosis; as well as current non-hormonal and hormonal treatment strategies This book is expected to be a valuable resource for clinicians, scientists and students who would like to have an improved understanding of endometriosis and also appreciate recent research trends associated with this disease.

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