Endometriosis Affects Oocyte Morphology in Intracytoplasmic Sperm Injection Cycles

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ABSTRACT
Objective: To identify associations between presence of endometriosis and oocyte defects, embryo developmental potential, and cycle outcomes.
Methods: This study looked into the impact of endometriosis on oocyte and embryo quality, and blastocyst formation probability. Endometriosis was also correlated with cycle characteristics. In order to avoid age-related bias, in the first analysis only patients aged 36 years or younger were included, and the cycles were split into endometriosis infertility cycles (n=431; 3172 oocytes) and other cycles (n=2510; 24480 oocytes).
Results: The number of retrieved oocytes (10.6±21.2 vs. 14.6±21.1, P<0.001), oocyte yield (68.1±20.0% vs. 70.6±19.6%, P=0.015), and embryos obtained (6.1±4.43 vs. 7.8±5.12, P<0.0001) were lower among patients with endometriosis. Implantation rates (28.1%±39.9% vs. 33.9±42.7, P<0.001) were lower among patients with endometriosis, but fertilization, pregnancy, miscarriage and cycle cancelation rates were not different. There was a significant increase in the incidence of extra-cytoplasmic, but not intra-cytoplasmic, oocyte defects among patients with endometriosis. The quality of embryos (45.3% vs. 47.3%, P=0.037) collected from patients with endometriosis was lower, but blastocyst formation rates were unaltered.
Conclusions: A possible explanation for the lower implantation rates seen in patients with endometriosis is the poorer quality of the oocytes and embryos observed in this group of patients.
Keywords: Endometriosis, ICSI, Oocytes, Embryos, Implantation

INTRODUCTION
It has been suggested that endometriosis is a heterogeneous disease characterized by the presence of endometrial-type cells outside the uterine cavity (Vercellini et al. 2013). The pathogenesis of endometriosis is still unclear, but retrograde menstruation (Giudice & Kao, 2004), altered immunity (Steele et al., 1984), coelomic metaplasia, metaplastic spread (Macer & Taylor, 2012), stem cell and genetic origins have been listed as possible causes (Chan et al., 2004; Du & Taylor, 2007; Zanatta et al., 2010).
Endometriosis patients may be asymptomatic or present a wide variety of symptoms, ranging from pelvic pain to infertility (Burns & Schenken, 1999). Infertility may be present in 30-50% of the individuals with endometriosis (Verkauf, 1987) for unclear reasons. Fecundity is reduced due to mechanical disruptions in cases of advanced pelvic endometriosis with extensive peritoneal adhesions and consequent pelvic anatomy distortions (Holoch & Lessey, 2010).
Substances produced by the implant or adjacent tissues such as prostaglandins, cytokines and growth factors have been listed as possible etiological factors of endometriosis-associated infertility (Olive & Pritts, 2001). Assisted reproductive technologies (ART) constitute a valid alternative for patients with endometriosis-associated infertility (Opoien et al., 2012; Dong et al., 2013).
More than a third of the women offered ART reportedly have endometriosis (Verkauf, 1987); however, studies on the outcome of ART for endometriosis-associated infertility have described conflicting results. Although some studies have reported decreased success rates in minimal/mild endometriosis when compared with non-endometriosis groups (Wardle et al., 1985; Matson & Yovich, 1986; Simon et al., 1994; Arici et al., 1996), other authors have failed to report differences between these groups (Inoue et al., 1992; Oliennes et al., 1995; Tanbo et al., 1995; Meden-Vrtovec et al., 2000). For advanced-stage endometriosis, the results are also contradictory with some reports indicating worse (Meden-Vrtovec et al., 2000; Aboulghar et al., 2003; Kuivasari et al., 2005; Harb et al., 2013) and others describing comparable outcomes after ART (Al-Fadhli et al., 2006; Gupta et al., 2006; Matalliotakis et al., 2007).
Studies supporting the poor outcome theory suggest that endometriosis-associated infertility patients offered ART present poor ovarian response (Al-Azemi et al., 2000; Matalliotakis et al., 2007; Lin et al., 2010), lower fertilization rates (Gupta et al., 2006; Lin et al., 2010), decreased endometrial receptivity (Giudice & Kao, 2004; Holoch & Lessey, 2010), and poor implantation rates (Harb et al., 2013). It has also been suggested that oocyte and embryo quality (Brizek et al., 1995; Pellicer et al., 1995; Garrido et al., 2000; Ota et al., 2000; Ota et al., 2002; Goud et al., 2014) may be compromised in patients with endometriosis-associated infertility. It has also been reported that the incidence of aneuploidy is significantly higher in patients with endometriosis (Gianaroli et al., 2010).
To our knowledge, the effect of endometriosis-associated infertility on oocyte quality, specifically related to intra- and extra-cytoplasmic defects, has not been investigated yet. Therefore, the goal of this study was to identify associations between the presence of endometriosis and oocyte defects, embryo developmental potential, and cycle outcomes.

MATERIALS AND METHODS
Study Design
This study included oocytes obtained from patients undergoing Intracytoplasmic sperm injection (ICSI) cycles at a private assisted fertilization center between January 2005 and May 2014. The patients were distributed into groups according to the cause of infertility. In order to avoid age bias, only females aged 36 years or younger were included and the cycles were split into endometriosis infertility cycles (n=431; 3172 oocytes) and other cycles (n=2510 cycles; 24480 oocytes).
The endometriosis group included individuals with moderate and severe endometriosis (ASRM III–IV).

The oocytes were evaluated immediately before sperm injection, and the embryos were evaluated 16-18 hours after ICSI and on days two, three and five of development. The influence of the presence of endometriosis on oocyte quality, embryo quality at the cleavage stage (day three), and the rate of blastocyst formation were assessed. Moreover, the presence of endometriosis correlated with cycle characteristics and clinical outcomes, such as:

1. Number of aspirated follicles;
2. Number of obtained oocytes;
3. Oocyte yield;
4. Mature (MII) oocyte rate;
5. Fertilization rate;
6. Clinical pregnancy rate;
7. Miscarriage rate; and
8. Implantation rate.

Patients provided written consent and agreed to share the outcomes of their cycles for research purposes. The local institutional review board approved the study.

**Controlled ovarian stimulation**

Controlled ovarian stimulation (COS) was achieved by pituitary blockage using a GnRH antagonist (Cetrotide; Serono, Geneva, Switzerland); ovarian stimulation was performed using recombinant FSH (Gonal-F; Serono, Geneva, Switzerland).

Follicular growth was monitored using transvaginal ultrasound examination starting on day four of gonadotropin administration. When adequate follicular growth and serum E2 levels were observed, recombinant hCG (Ovidrel; Serono, Geneva, Switzerland) was administered to trigger final follicular maturation. The oocytes were collected 35 hours after hCG administration through transvaginal ultrasound ovum pick-up.

**Preparation of oocytes**

The retrieved oocytes were maintained in culture medium (Global® for fertilization, LifeGlobal, Connecticut, USA) with 10% protein supplement (LGPS, LifeGlobal, Connecticut, USA) and covered with paraffin oil (Paraffin oil P.G., LifeGlobal, Connecticut, USA) for two to three hours before the removal of cumulus cells. The surrounding cumulus cells were removed after exposure to a HEPES-buffered medium containing hyaluronidase (80 IU/mL, LifeGlobal, Connecticut, USA). The remaining cumulus cells were mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlotte, USA).

Oocyte morphology was assessed using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon, Tokyo, Japan) with a Hoffmann modulation contrast system under 400X magnification.

**Statistical analyses**

The categorical and continuous variables of cycle characteristics, clinical outcomes, and laboratory outcomes were compared between the groups using the Chi-square and Student’s t-test, respectively. Continuous variables were expressed as the mean value ± the standard deviation, and percentages were used for categorical variables. Statistical significance was attributed to P-values of less than five percent (P < 0.05). Data analysis was carried out using the Minitab (version 14) Statistical Program.

**RESULTS**

Endometriosis patients had fewer aspirated follicles, retrieved oocytes, and obtained embryos, and reduced oocyte yield, and higher total doses of FSH used for COS and greater numbers of transferred embryos; however, the MII oocyte rate did not differ between groups (Table 1).

Concerning cycle outcomes, endometriosis patients had lower fertilization and implantation rates, but their pregnancy, miscarriage, and cycle cancelation rates did not differ from those of healthy subjects (Table 2).

Lab tests showed that patients with endometriosis had a significantly higher incidence of extra-cytoplasmic oocyte defects. In contrast, the incidence of intra-cytoplasmic de-
effects did not differ between groups (Table 3).

Embryo quality on day three was decreased in the embryos derived from endometriosis patients; however, the presence of endometriosis did not affect the rate of blastocyst formation (Table 4).

**DISCUSSION**

The present study looked into the effects of endometriosis-associated infertility on ICSI outcomes. Significantly lower response to COS, lower oocyte quality, lower fertilization rates, lower cleavage stage embryo quality, and lower implantation rates were observed in individuals with endometriosis. Additionally, the pregnancy rates seen in subjects with endometriosis-associated infertility were comparable to the rates observed in cases of infertility for other causes.

These findings are in line with recent reports showing that women with endometriosis undergoing ART have a significantly lower oocyte yield (Singh et al., 2014) and lower fertilization rates (Opoien et al., 2012) in comparison with tubal factor infertility. In accordance with our findings, Opoien et al. (2012) also described that in addition to the lower fertilization rate, infertile women with endometriosis have comparable pregnancy rates and live birth rates to women with tubal factor infertility, even after adjusting for confounding factors. In a conflicting finding, Opoien’s study reported similar implantation rates between subjects with and without endometriosis.

A previous meta-analysis concluded that patients with endometriosis-associated infertility undergoing IVF respond with significantly lower levels of all markers of the reproductive process, resulting in a pregnancy rate that is almost half of that of women with other indications for IVF. The authors suggested that endometriosis affects not only the receptivity of the endometrium, but also the development of the oocyte and the embryo (Barnhart et al., 2002).

More recently, the presence of severe endometriosis was associated with poor implantation in women undergoing IVF (Harb et al., 2013).

A recently published trial by Filippi et al. (2014), investigated whether the presence of endometrioma affect-sed oocyte developmental competence. The study showed that the presence of ovarian endometrioma does not affect oocyte quality. In the study described above, although the authors reported that oocyte morphology was evaluated using an inverted microscope, these data were not described in the results and, apparently, oocyte developmental competence was measured based on the quality of the embryo at the cleavage stage. In our study, oocyte quality was measured based on the presence of individual intra- and extra-cytoplasmic defects. It was noted that when compared to other causes of infertility, endometriosis patients have a higher incidence of extracytoplasmic oocyte defects.

Mature oocytes with apparently normal cytoplasmic organization may exhibit extra-cytoplasmic defects, such as increased perivitelline space, perivitelline debris and/or fragmentation of the first polar body, which may impair the developmental competence of the oocyte (Xia, 1997).

In the present study, in addition to poor oocyte morphology, the fertilization rate was also significantly lower in endometriosis cases. This finding is in accordance with previous studies, showing that poor oocyte morphology is a major determinant of failed or failed fertilization (Xia, 1997; Javed & Michael, 2012). In addition, a recent meta-analysis published by our group (Setti et al., 2011) showed that the probability of an oocyte becoming fertilized is significantly reduced when extra-cytoplasmic defects such as a large polar body and large perivitelline space are present. Conversely, Balaban & Urman (2006) suggested that extra-cytoplasmic oocyte dysmorphisms should be considered merely as a phenotypic deviation resulting from the heterogeneity of the oocytes retrieved. Nevertheless, as described by Rienzi et al. (2010) in a
systematic review of the literature on the predictive value of oocyte morphology in IVF, published studies have produced contradicting results and do not entirely support the typical opinion about the features of 'good' and 'bad' oocyte quality and developmental competence.

Interestingly, even though oocyte quality and embryo morphology at the cleavage stage were decreased in the endometriosis group, the rate of blastocyst formation did not differ between groups. Previous studies failed to demonstrate (Graham et al., 2000) or demonstrated a poor correlation (Guerif et al., 2010) between early embryo morphology and blastocyst developmental competence. Conversely, we recently investigated the factors that may contribute to the rate of blastocyst formation and quality, and the data showed that good morphology on day three increased the likelihood by up to three-fold that a blastocyst would be of good quality (Braga et al., 2012).

It could be argued that although the presence of endometriosis may have a detrimental effect on oocyte quality and embryo morphology, after prolonged culture to the blastocyst stage, the embryonic genome has begun to be expressed (Tesarik, 2005). At this stage, sperm-derived genes that influence embryo viability have also been activated.

Our results also showed that implantation – but not pregnancy – rates were also decreased in women with endometriosis undergoing ICSI cycles. Whereas some studies (Bukulmez et al., 2001) fail to show decreased implantation rates in IVF patients with endometriosis, others (Kuivasari et al., 2005) demonstrate significantly lower implantation rates in individuals with early and late stages of the disease.

Embryo implantation depends on three critical events: proper embryo development, the acquisition of a receptive endometrium, and proper dialogue between them (Domínguez et al., 2003). Previous reports have discussed a possible role for endometrial receptivity accounting for lower implantation rates in these patients (Rajani et al., 2012), including decreased expression of implantation markers during the window of implantation (Donaghay & Lessey, 2007; Matsuzaki et al., 2010).

The reason why diminished implantation – but not pregnancy – rates were observed in the present trial is unclear. It may be suggested that the pregnancy rate does not consider the number of transferred embryos, which also differed between groups.

In conclusion, this study showed that in addition to a lower number of oocytes, endometriosis patients also had lower implantation rates. The poor implantation rates observed among endometriosis patients may be explained by the reduced quality of their oocytes and embryos, impaired endometrial receptivity, or perhaps both factors combined.

**CONFLICT OF INTERESTS**
No conflict of interest have been declared.

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**Table 3. Comparison of the presence of oocyte defects between the endometriosis and control groups**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Endometriosis (n=3172)</th>
<th>Other (n=24480)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-cytoplasmic defects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic granularity</td>
<td>14.35</td>
<td>14.25</td>
<td>0.875</td>
</tr>
<tr>
<td>Cytoplasmic color</td>
<td>14.15</td>
<td>13.54</td>
<td>0.336</td>
</tr>
<tr>
<td>Aggregates of smooth ERC</td>
<td>14.98</td>
<td>14.04</td>
<td>0.149</td>
</tr>
<tr>
<td>Vacuoles in the ooplasm</td>
<td>14.70</td>
<td>15.44</td>
<td>0.272</td>
</tr>
<tr>
<td>Retractile bodies</td>
<td>11.22</td>
<td>11.24</td>
<td>0.970</td>
</tr>
<tr>
<td>Extra-cytoplasmic defects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZP abnormalities</td>
<td>22.13</td>
<td>18.27</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PVS granularity</td>
<td>47.91</td>
<td>45.70</td>
<td>0.017</td>
</tr>
<tr>
<td>Large perivitelline space</td>
<td>28.80</td>
<td>24.28</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fragmented PB</td>
<td>38.15</td>
<td>35.40</td>
<td>0.002</td>
</tr>
<tr>
<td>Shape abnormalities</td>
<td>15.22</td>
<td>13.51</td>
<td>0.007</td>
</tr>
<tr>
<td>Increased membrane resistance</td>
<td>15.29</td>
<td>14.03</td>
<td>0.053</td>
</tr>
<tr>
<td>Non-resistant membrane</td>
<td>16.11</td>
<td>14.46</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**Table 4. Comparison of cleavage stage embryo quality and rate of blastocyst formation between the endometriosis and control groups**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Endometriosis (n=3172)</th>
<th>Other (n=24480)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High quality embryo on day 3</td>
<td>45.36</td>
<td>47.29</td>
<td>0.037</td>
</tr>
<tr>
<td>Rate of blastocyst formation</td>
<td>51.97</td>
<td>52.49</td>
<td>0.780</td>
</tr>
</tbody>
</table>

ERC: endoplasmic reticulum clusters, ZP: zona pellucida, PB: polar body. Chi-square and Student’s t-test. Values expressed as %.
REFERENCES


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