Iron availability is increased in individual human ovarian follicles in close proximity to an endometrioma compared with distal ones

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STUDY QUESTION: Does the iron content of an endometrioma represent a potential source of toxicity for the adjacent follicles?

SUMMARY ANSWER: The presence of an endometrioma increases iron and H/L ferritin levels, and transferrin receptor (TfR1) mRNA in individual follicles proximal to the endometrioma and is accompanied by reduced oocyte retrieval.

WHAT IS KNOWN ALREADY: Levels of free iron in endometriotic ovarian cysts are much higher than those in normal serum or in non-endometriotic ovarian cysts. The presence of an endometrioma exerts a detrimental effect on the surrounding healthy ovarian tissue as reflected by a reduced number of developing follicles and oocytes retrieved in IVF cycles.

STUDY DESIGN, SIZE, DURATION: This is a research study with prospective collection and evaluation of individual follicles (follicular fluid and luteinized granulosa cells) from the affected and the healthy ovaries of 13 women with unilateral endometrioma.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Individual follicular samples (145) were obtained from 13 women with endometriosis-related infertility undergoing IVF-ICSI procedures from May 2012 to March 2013. All women had unilateral endometrioma not previously treated with surgery; the contralateral ovary was free of endometriomas and previous surgery. The average ± SEM age was 35.36 ± 2.5 years with anti-Mullerian hormone levels of 2.03 ± 0.55 ng/ml. Follicles were classified as: (i) proximal follicles, in physical contact with the endometrioma; (ii) distal follicles, present in the affected ovary but not in close contact with the endometrioma and (iii) contralateral follicles, in the contralateral healthy ovary. Iron content was measured by the FerroZine method. H/L ferritin subunits were evaluated by specific enzyme-linked immunosorbant assays. Expression of H ferritin and TfR1 was examined by semi-quantitative RT–PCR. Oocyte retrieval rates and Day 3 embryo quality were analyzed.

MAIN RESULTS AND THE ROLE OF CHANCE: Total iron levels were higher in endometrioma-proximal follicles compared with endometrioma-distal ones (P = 0.009) and to follicles in the healthy ovary (P = 0.02). L ferritin was higher in proximal versus distal follicles (P = 0.044) or follicles from the healthy ovary (P = 0.027). H ferritin was higher in the proximal and distal follicles compared with follicles in the healthy ovary (P = 0.042 and P = 0.0067, respectively). H ferritin transcript levels in granulosa cells were higher in proximal follicles versus follicles from healthy ovary (P = 0.02). TfR1 transcript levels were higher in proximal versus distal follicles (P = 0.03) and versus follicles from the healthy ovary (P = 0.04). The oocyte retrieval rate was lower in proximal and distal follicles than in follicles from the healthy ovary (P = 0.001 and P = 0.04, respectively).

LIMITATIONS, REASONS FOR CAUTION: This is a study on a relatively small sample size and confirmation in a larger group of patients may be required. The method used to purify luteinized granulosa cells offers the best combination of purity, viability and total number of cells recovered. However, a minor contamination by CD45⁺ cells (<5%) cannot be excluded.
**Introduction**

Ovarian endometriosis is considered one of the most common forms of endometriosis, present in up to 30–40% of women affected. The disease is characterized by the presence of one or more cysts lined by endometrial tissue that is histologically and functionally similar to eutopic endometrium. The cyst contains non-resorbed blood derived from repeated hemorrhages of ectopic endometrial cells lining the cyst wall during menstrual cycles. The cyst fluid contains cellular damage-mediating factors, proteolytic enzymes and inflammatory molecules in concentrations from tens to hundreds of times higher than those present in serum or in non-endometriotic cysts (Sanchez et al., 2013).

The presence of the cyst exerts a detrimental effect on the surrounding healthy ovarian tissue as reflected by (i) a lower follicular density in the ovarian cortex adjacent to the endometriotic cyst, (ii) a reduced rate of spontaneous ovulation in affected ovaries and (iii) a reduced number of developing follicles and oocytes retrieved in IVF cycles in women with bilateral endometriomas (Benaglia et al., 2013). Among the different cellular damage-mediating factors present in the cyst fluid, iron has attracted much attention due to its role in the pathogenesis of endometriosis and as a potential cause of carcinogenesis of the cyst (Redwine, 1999; Vercellini et al., 2011). Levels of iron in endometriotic cysts are much higher than those in normal serum or in non-endometriotic ovarian cysts (Yamuguchi et al., 2008). Uncomplexed iron together with superoxide, which reduces Fe(III) and hydrogen peroxide, which is decomposed by the Fenton reaction, provides a lethal mixture containing hydroxyl radicals that can directly damage DNA, lipids and proteins (Jonova and Valko, 2011). One of the proteins that plays an important role in iron balance is ferritin, which captures and buffers the intracellular labile iron pool as a main function (Picard et al., 1998). Ferritin levels were found to be higher in the peritoneal fluid of women with endometriosis compared with control women (Van Lengendonckt et al., 2002). Ferritin is a heteropolymer that contains two different subunits, the H- and L-chains which are similarly regulated by iron, although they have distinctive functional activities: the H-chain has ferroxidase activity and readily sequesters the excess cytosolic iron, while the L-chain, with a more efficient iron nucleation activity, assists the H-chain in the functionality of the hybrid molecules (Cozzi et al., 2003; Arosio and Levi, 2010). Also essential to iron homeostasis is the transport of iron by the protein transferrin (Tf). Iron-binding Tf binds tightly to its specific transferrin receptor (TfR), a homodimeric transmembrane protein. Efficient delivery of iron is critically dependent on Tf/TfR interactions. In pelvic endometriosis, peritoneal macrophages were found to express higher levels of TfR1 and transferrin levels in the peritoneal fluid were also increased (Martinez-Roman et al., 1997).

The presence of iron-related compounds that are potentially toxic to developing ovarian follicles adjacent to the endometrioma during IVF procedures has been poorly investigated (Sanchez et al., 2013). The purpose of this study was to evaluate the impact of the endometrioma on iron homeostasis in the follicular fluid and luteinized granulosa cells of individual ovarian follicles derived from women undergoing IVF. To better characterize the potential deleterious effect of the endometrioma, we studied individual follicles proximal and distal to the cyst and follicles from the contralateral healthy ovary. Levels of H and L ferritin, and TfR1 were also correlated with the number of oocytes retrieved and embryo quality.

**Materials and Methods**

Patients and follicular fluid samples

Follicular samples were obtained from 13 infertile women with unilateral endometrioma undergoing controlled ovarian stimulation and IVF-ICSI procedures at the Infertility Center of the San Raffaele Scientific Institute from January to July 2013 [mean ± SEM age 35.36 ± 2.5 years and anti-Mullerian hormone (AMH) levels 2.03 ± 0.55 ng/ml]. Written informed consent was obtained, and the local institutional review board approved the study (BC-GINEOS). The cysts, <4 cm in diameter, were present on an ovary that had not been treated previously with surgery; the contralateral ovary was free of endometriomas and also never treated previously with surgery. All women underwent a standard long protocol for ovulation induction. Starting dose of recombinant FSH (r-FSH) was based on the ovarian reserve test (Basal FSH and AMH) according to our standard clinical protocols. Treatment with r-FSH was modified according to individual responses. When at least one follicle reached 18 mm in diameter, triggering with 10 000 IU hCG was performed followed by oocyte retrieval up 34–36 h later. Before ovarian puncture, follicles were classified according to distance from the endometrioma as (i) proximal follicles, in physical contact with the endometrioma; (ii) distal follicles, present in the affected ovary but not in close contact with the endometrioma and (iii) contralateral follicles, in the contralateral healthy ovary (not affected by an endometrioma). Follicular aspirates were retrieved from 145 follicles by the same operator via the transvaginal route under ultrasound guidance. The fluid was aspirated until the follicle wall collapsed. A total of 42 follicular fluid samples were either contaminated with blood or in insufficient amount, and were therefore excluded from the analysis. The follicle was then flushed with 4.5 ml heparinized saline (3 × 1.5 ml automated flushes) and aspirated again. Three further flushes were carried out in case no oocyte was collected but these flushes were not added to follicular samples. Follicular fluids were centrifuged at 1000g for 10 min to remove cellular components; the clear supernatant was divided into aliquots and frozen at −80°C until further use. Prior to processing, the follicular fluids were centrifuged and filtered on Millipore filter to remove precipitates.

**WIDER IMPLICATIONS OF THE FINDINGS:** This study represents a further in-depth analysis of the toxic influence of the endometrioma content on the surrounding follicles. We demonstrate the presence of iron-related compounds that are potentially toxic to developing ovarian follicles adjacent to the endometrioma during IVF. Our findings provide novel information that suggests that when surgical removal of the endometrioma might increase the probability of retrieving oocytes.

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Luteinized granulosa cell preparation and culture

Luteinized granulosa cells were isolated from follicular aspirates of women via transvaginal aspiration after a standard long protocol for ovulation induction. Cells from individual follicles were prepared by initial centrifugation followed by layering onto a 40% Percoll gradient (Sigma, UK). Following centrifugation, three layers could be distinguished: a top layer containing the follicular fluid, a bottom layer containing erythrocytes and, in the middle, a ring-like layer with the cellular component. The middle layer was collected, washed and centrifuged for 5 min at 600 x g, and resuspended in RPMI supplemented with 10% fetal bovine serum and 1% penicillin G100 IU/ml and 100 μg/ml streptomycin (complete medium) (Invitrogen, UK). In order to deplete the immune cells from the cell suspension, follicular fluid-derived cells obtained by the density gradient were collected from the interface, resuspended in 10 ml of complete medium and transferred to a petri dish (BD Biosciences, Oxford, UK). After incubation at 37°C for 15 min, white blood cells adhered to the plastic while luteinized granulosa cells did not, thus allowing the easy segregation of the two cell types according to their physical properties (Ferrero et al., 2012). The luteinized granulosa cells were recovered, plated at 0.5 x 10^5 cells/ml and incubated in complete medium for 24 h at 37°C in a CO2 incubator. No change in the normal morphology of luteinized granulosa cells was observed after a 24 h culture. Under these culture conditions, no phenotype shift is expected to occur, and an optimal balance of cell survival/proliferation and steroidogenesis was previously reported (Portela et al., 2010). At the end of the incubation, adherent cells were gently rinsed with phosphate-buffered saline, collected and mRNA was extracted as described below.

Total iron concentration

Measurement of total iron concentration in follicular fluid samples (undiluted) was carried out with the analytic FerroZine® method without deproteinization with an ADVIA® 2400 Clinical Chemistry System (Siemens, USA).

Semi-quantitative RT–PCR analysis

mRNA was extracted with RNesay Micro Kit (Qiagen, USA) and mRNA was reverse-transcribed using the High Capacity cDNA Reverse Transcript kit (Applied Biosciences, USA). RT–PCR amplification was performed with a SYBR Master Mix kit (Applied Biosciences, USA) on a AB 7700 Fast RT–PCR System and SDS software version 1.4 (Applied Biosciences, USA). The initial denaturation step was 95°C for 15 s followed by 40 cycles of amplification at 95°C for 10 s and 60°C for 30 s. Melting curves were evaluated for each gene. Three technical repetitions of each RT–PCR were performed, and the relative amount of the target was normalized with the 18S ribosomal RNA housekeeping gene. Reaction conditions included 10 μl of 2 x Sybr-Green Universal PCR Master Mix (Applied Biosystems), 1 μl of primers and probes mixture (0.4 μM), 50 ng of template cDNA and nuclease-free water in a 96-well reaction plate. The data were analyzed by using the comparative Ct method, where Ct is the cycle number at which fluorescence first exceeds the threshold. The Δ Ct values from each sample were obtained by subtracting the values for the 18S reference gene from the sample Ct. For each experimental sample the 2^{-Δ Ct} value has been calculated and data have been graphically presented as relative expression. The sequences of the primers forward/reverse were used for amplification of TFR1 and ferritin H chain (FT H) are the following:

**TFR1 FWS’ GGAACAGTTACCTGTAAGACTG3’; RWS’ AGCAATGGC ACGTTTACTGT3’.

**FTH FWS’ CCACTGTTACCTGTGACCTC3’; RWS’ TGCCTGTTTCT TGATGTTACTC3’.

**I8S FWS’ AAACAGGCTACGATCCTCCAAG3’; RWS’ CGCTCCCAAG ATCCAAACTC3’.

Ferritin levels

Ferritin levels in individual follicular fluids were measured by specific enzyme-linked immunosorbent assays (ELISA) for H-Ferritin (rH02) and L-Ferritin (LF03) calibrated on the corresponding recombinant homopolymers expressed in E. coli, as previously described. The total protein content in follicular fluid samples was determined with the Bio-Rad protein assay according to the Bradford method (Santambrogio et al., 2000).

Clinical outcomes

The following parameters of ovarian response were analyzed: (i) oocyte retrieval rate expressed as number of oocytes per punctured follicle and (ii) quality of the embryos derived from the retrieved oocytes. Evaluation of embryo quality was performed at the cleavage stage (Day 3) by two independent embryologists, according to published criteria (Steer et al., 1992; Veek, 1999).

Statistical analysis

Values are reported as mean ± SEM. Statistical analysis was performed using one-way analysis of variance followed by the Mann–Whitney test to determine the statistical significance of the data. P < 0.05 was considered statistically significant. Statistical calculations were performed with the Prism software 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Iron and H/L ferritin levels in follicular fluid samples

In order to evaluate the potential toxic effect exerted by an endometrioma on proximal follicles, we analyzed iron and H/L ferritin levels in follicular fluid aspirates from a total of 103 single follicles divided into three different groups: 35 follicles proximal to the endometrioma, 28 from the same ovary but distal to the endometrioma and 40 from the contralateral healthy ovary. Total iron levels were found to be significantly increased in the endometrioma-proximal follicles (74.87 ± 5.27 μg/dl) compared with the endometrioma-distal follicles (56.15 ± 1.91 μg/dl) and contralateral follicles (55.83 ± 4.39 μg/dl) (Fig. 1A). We found that the levels of H ferritin were significantly higher than those of L ferritin (1.02 ± 0.19 and 0.29 ± 0.04 ng/mg of total proteins, respectively) (P = 0.002) (Fig. 1B). The levels of the L ferritin, which represents the major iron storage protein, were higher in the endometrioma-proximal follicles compared with those isolated from the healthy ovary (0.35 ± 0.07 versus 0.11 ± 0.02; P = 0.027). Moreover, in the endometrioma-affected ovary, L ferritin levels were significantly higher in the proximal follicles compared with the distal ones (0.35 ± 0.07 versus 0.19 ± 0.03; P = 0.044) (Fig. 1C). Similar results were found for H ferritin, whose content was significantly higher in the endometrioma-proximal follicles than in those isolated from the contralateral healthy ovary (1.05 ± 0.15 versus 0.48 ± 0.10; P = 0.042). The H ferritin levels were also significantly higher in the endometrioma-distal follicles compared with those isolated from the contralateral healthy ovary (1.02 ± 0.14 versus 0.48 ± 0.10; P = 0.0067). However, we did not observe any difference between the H ferritin levels in endometrioma-proximal versus distal follicles (P = 0.87) (Fig. 1D).
Transcript levels of H ferritin and TfR1 in luteinized granulosa cells from individual follicles

We found that the H ferritin mRNA levels in luteinized granulosa cells increased depending on the proximity to the endometrioma, with significantly higher levels in proximal follicles ($n = 18; 0.034 \pm 0.005$ relative units) compared with follicles in the contralateral healthy ovary ($n = 11; 0.014 \pm 0.002$ relative units) ($P = 0.02$) (Fig. 2A). We found that TfR1 transcripts were higher in endometrioma-proximal follicles compared with -distal ($n = 8$) and to contralateral follicles ($P = 0.03$ and $P = 0.04$, respectively) (Fig. 2B).

The presence of an endometrioma affects oocyte retrieval rate but not embryo quality

A total of 95 oocytes were retrieved from 145 follicles. The follicles were classified as proximal ($n = 60$), distal ($n = 30$) and contralateral ($n = 55$). We found that the oocyte retrieval rate was significantly lower in the endometrioma-proximal follicles compared with those isolated from the contralateral healthy ovary ($P = 0.001$). Moreover, the oocyte retrieval rate was significantly lower in the endometrioma-distal follicles compared with those isolated from the contralateral healthy ovary ($P = 0.04$). In the endometrioma affected ovary, follicles that were in close contact with the cyst appeared to show a lower oocyte retrieval rate than distal follicles, although the difference was not statistically significant (Fig. 3A).

We also analyzed the quality of Day 3 embryos obtained upon IVF. As shown in Figure 3B, embryo quality was unaffected by the presence of the endometrioma. We did not find any significant correlation between H and L ferritin levels in the follicular fluid and embryo quality (Table I). In addition, no correlation was detected between TfR1 and H ferritin mRNA levels in luteinized granulosa cells from single follicles and Day 3 embryo quality (Table I).

Discussion

The results of this study indicate that the presence of an endometrioma affects iron levels primarily in the adjacent follicles and to a minor degree in the distal ones when compared with the follicles in the contralateral healthy ovary. It has been reported that levels of free iron in the endometriotic cyst are much higher compared with the normal serum levels or the content of non-endometriotic ovarian cysts (Yamuguchi et al., 2008). Iron in the cyst can bind to haptoglobin released by erythrocytes. Indeed, hemosiderin-laden macrophages that are thought to contribute to removal of the iron-haptoglobin represent a frequent histologic finding in the endometriotic cyst (Canet et al., 2012). While most of the iron present in living organisms is tightly complexed in proteins, in heme or in iron-sulphur clusters, uncomplexed iron could mediate the production...
of reactive oxygen species (ROS) via the Fenton reaction (Jonova and Valko, 2011). ROS are extremely reactive causing lipid peroxidation, DNA strand breaks and degradation of other biomolecules (Harrison and Arosio, 1996). Indeed, specific oxidative stress-related factors, including lactose dehydrogenase, lipid peroxide and 8-hydroxyguanosine are present in the cyst fluid and represent significant inducers of oxidative stress in the surrounding healthy ovarian tissue (Sanchez et al., 2013).

The major and common role of ferritin is to bind Fe(II), oxidize it and sequester it in a safe form inside its large cavity. Since ferritin levels are modulated by the presence of iron, we hypothesized that the high levels of iron present in the endometrioma might in turn affect ferritin levels in neighboring follicles. We found that H ferritin levels detected in follicular fluids were higher than L ferritin levels. This is in contrast to what has been reported in serum samples from normal individuals in whom this ratio is inverted, but similar to what is detected in some malignancies (Torti and Torti, 2002). When individual follicles were analyzed, we found that H and L ferritin levels were higher in follicular fluids of follicles proximal to the endometrioma. In our analysis each follicle was considered as an independent variable since all the women studied were homogeneous in terms of age, ovarian reserve and etiology of infertility (endometriosis/endometrioma).

Iron metabolism is controlled by a series of transcriptionally regulated factors that orchestrate the cellular defense against iron, stress and inflammation by modulating the expression of H ferritin and TfR1 (Henzte 1996; Eisenstein, 2000). Iron overload in the endometrioma also perturbs iron metabolism in the luteinized granulosa cells in proximal follicles, as demonstrated by high levels of H ferritin and TfR1 mRNA expression in these cells. Ferritin has not only a central role in acting as an iron buffer that stores the excess to deliver it when needed, but also acts in the control of the pro-oxidant activity of the metal (Arosio and Levi, 2010). In addition to iron overload, ferritin expression is regulated by a variety of conditions associated with oxidative stress that act directly on gene expression. In particular, H ferritin is a component of the organismal acute phase response to stress, injury, and infection (Arosio and Levi, 2002; Torti and Torti, 2002). Since the endometrioma contains high levels of oxygen radicals and inflammatory cytokines, including interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)-α, up-regulation of H ferritin expression might help to contain cytokine-induced injury in the proximal follicles (Prefumo et al., 2002).

Iron can be delivered to granulosa cells by soluble transferrin, a transporter that captures iron released in the interstitial space. The binding of iron-laden transferrin to the cell-surface TfR1 results in endocytosis and...
IL-6 and TNF-
TfR1 has been reported under inflammatory conditions (presence of regulated (Wang and Pantapoulus, 2011). However, an up-regulation of uptake of the metal cargo. Upon iron overload, TfR1 is normally down-regulated (Wang and Pantapoulus, 2011). However, an up-regulation of TfR1 has been reported under inflammatory conditions (presence of IL-6 and TNF-α) (Wang et al., 2013). Therefore, the observed TfR1 up-regulation on luteinized granulosa cells of follicles adjacent to the endometrioma might be induced by the inflammatory microenvironment, masking the expected decrease due to iron overload. We cannot rule out that a minor inflammatory cell contamination might contribute to the high levels of H ferritin and TfR1 observed in the granulosa cell preparations (Piquette et al., 1994).

Soluble factors in the follicular fluid of patients with endometriosis can disturb folliculogenesis and oocyte growth affecting the outcome of IVF (Pellicer et al., 1987; Andersen 1993; Bahiyar et al., 1998; Garrido et al., 2000). We showed that the oocyte retrieval rate was significantly reduced in the affected ovary compared with the healthy contralateral ovary. In fact, not all of the follicles close to the endometrioma contained an oocyte, as shown by the analysis of oocyte numbers normalized to punctured follicles. This was an indication of the toxic influence of the endometrioma content. Studies on the effect of an endometrioma on the quality of oocytes have reported contradictory results. Some authors showed that endometriosis affected total oocyte number (Suzuki et al., 2005), while others have shown that the presence of an endometrioma did not reduce the total number of retrieved oocytes during IVF treatments (Almog et al., 2011; Kiran et al., 2012). These contradictory sets of results might be explained by the fact that the contralateral intact ovary might adequately compensate for the reduced function of the affected ovary. A more accurate comparison of ovarian response in the endometrioma-affected ovary compared with the contralateral healthy ovary in the same women with unilateral ovarian endometriomas, who did not undergo previous ovarian surgery, has shown a reduced response to ovarian hyperstimulation with significantly fewer oocytes retrieved from the affected ovary (Somigliana et al., 2006). In addition to iron-mediated damage, the ovarian response in the endometrioma-affected ovary might also be influenced by the ovarian interstitial microvascular injury, as supported by the finding of blood flow changes within ovarian interstitial arteries (Qiu et al., 2012).

We found that Day 3 embryo quality was not affected by the presence of an endometrioma. Iron intoxication did not seem to affect embryo quality since no correlation was observed between the levels of ferritin or TfR1 expression in individual follicles and embryo quality. This might reflect the fact that even though iron overload affected oocyte development, those mature oocytes retrieved were capable of generating good quality embryos. Our results are similar to those reported by Suzuki et al. (2005), who found that women affected with endometriosis had a reduced total oocyte number compared with non-endometriotic control women but embryo quality and pregnancy outcome were not affected. More recently, it has been demonstrated that the presence of bilateral endometriomas during IVF treatment was not associated with reduced embryo quality (Reinblatt et al., 2011).

Our findings show that iron and its major storage proteins represent molecular signs of the gonadotoxic insult to individual follicles developing adjacent to the cyst during IVF cycles. This is reflected by the low rate of oocyte retrieval from endometrioma-proximal follicles. These results provide novel information suggesting that when surgical removal of the endometrioma is not an option, follicle aspiration at sites distant from the endometrioma might increase the probability to retrieve competent oocytes. Even if during oocyte retrieval the access to the endometrioma-affected ovary is technically difficult in some patients, especially in women with previous surgery, assisted reproduction technology (ART) is to be preferred over a second surgical intervention. In particular, pre-ART treatment with oral contraceptives has been shown to improve ART outcome in case of ovarian endometriosis, mainly if endometriomas are present at the time of oocyte retrieval (de Ziegler et al., 2010). Further investigation is needed to clarify whether and how the medical therapy, or specific strategies of the surgical therapy, may limit or slow the damage caused by the cyst.

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**Authors’ roles**

A.M.S. performed all the experimental work, performed the statistical analysis and contributed to the preparation of the manuscript. E.P. conceived the scientific idea, performed all clinical procedures and contributed to the preparation of the manuscript. L.C. managed the clinical and laboratory data of the IVF patients. P.S. and S.L. contributed to data analysis and interpretation. P.V. conceived the scientific idea and contributed to the preparation of the manuscript. M.C. contributed to the preparation of the manuscript. P.P.-B. conceived the scientific idea, supervised the project and prepared the manuscript.

### Table I

**H and L ferritin protein levels in human follicular fluid (FF), and transferrin receptor 1 (TfR1) and H ferritin transcript levels in luteinized granulosa cells (LGC) of follicles whose oocytes resulted in top, good or poor quality Day 3 embryos upon IVF.**

<table>
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<tr>
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<th>Day 3 embryo quality</th>
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<tr>
<td></td>
<td>Top (n = 15)</td>
<td>Good (n = 8)</td>
<td>Poor (n = 16)</td>
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<tr>
<td>H ferritin protein levels in FF (ng/mg of total protein)</td>
<td>1.11 ± 0.15</td>
<td>1.48 ± 0.26</td>
<td>0.89 ± 0.17</td>
<td>0.13</td>
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<tr>
<td>L ferritin protein levels in FF (ng/mg of total protein)</td>
<td>0.16 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>0.14</td>
<td></td>
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<tr>
<td>TfR1 mRNA levels in LGCs (relative expression x 10^{-2})</td>
<td>6.05 ± 2.64</td>
<td>9.80 ± 1.69</td>
<td>6.94 ± 2.81</td>
<td>0.5</td>
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<tr>
<td>H ferritin mRNA levels in LGCs (relative expression x 10^{-2})</td>
<td>2.66 ± 1.01</td>
<td>2.09 ± 0.26</td>
<td>3.40 ± 0.94</td>
<td>0.53</td>
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Data are presented as mean ± SEM. Statistical analysis was performed using Mann–Whitney test.
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Conflict of interest

The authors have no competing financial interests in relation to the content of this research paper.

References


