Effect of GnRH agonist before IVF on outcomes in infertile endometriosis patients: a randomized controlled trial

Ana M Monzó is an obstetrics and gynaecology physician in infertility and assisted reproductive technology at La Fe University Hospital in Valencia, Spain (2000–2019), Director of the Preimplantation Genetic Testing Programme at the Department of Reproductive Medicine and leads La Fe Teaching Programe of Residency (2008–2019). She has a doctorate degree in medicine (1994) and a master's degree in reproductive medicine.

Elisabet Rodríguez-Tárrega1,*, Ana M. Monzo1,3, Ramiro Quiroga1, Patrocinio Polo-Sánchez1, Pedro Fernández-Colom1, Mercedes Monterde-Estrada1, Edurne Novella-Maestre2,3, Antonio Pellicer2,4

KEY MESSAGE
In this clinical trial, 3 months of treatment with gonadotrophin releasing hormone agonist before starting an IVF cycle does not increase clinical pregnancy rates in infertile endometriosis patients. These results contradict the findings of previous studies and, therefore, further high-quality trials are needed.

ABSTRACT
Research question: Does 3-months of gonadotrophin releasing hormone agonist (GnRHa) treatment before IVF improve clinical pregnancy rate in infertile patients with endometriosis?

Design: Single-blind, placebo-controlled clinical trial of 200 infertile women with endometriosis assigned to use GnRHa (study group) or placebo (control group) for 3 months before IVF. Clinical, embryological outcomes and stimulation parameters were analysed. Clinical pregnancy rate was the primary endpoint. In a subgroup of 40 patients, follicular fluid levels of oestradiol, testosterone and androstendione were measured. Gene expression profile of CYP19A1 was analysed in cumulus and mural granulosa cells.

Results: Implantation or clinical pregnancy rate were not significantly different between the two groups. Clinical pregnancy rates were 25.3% and 33.7% in the study and control groups, respectively (P = 0.212). Cumulative live birth rate was not significantly different: 22.0% (95% CI 13.0 to 31.0) in the study group and 33.7% (95% CI 24.0 to 44.0) in the control group (P = 0.077). Ovarian stimulation was significantly longer and total dose of gonadotrophins significantly higher in the study group (both P < 0.001). Serum oestradiol levels on the day of HCG were significantly lower in the study group (P = 0.001). Cancellation rate was significantly higher in the study group (P = 0.042), whereas cleavage embryos were significantly more numerous in the control group (P = 0.023). No significant differences in the expression of CYP19A1 gene in mural or cumulus granulosa cells or steroid levels in follicular fluid between the two groups were observed, but testosterone was significantly lower in the study group (P < 0.001).

Conclusion: Three-months of GnRHs treatment before IVF does not improve clinical pregnancy rate in women with endometriosis.

KEYWORDS
Aromatase
cYP19A1 gene
Endometriosis
GnRH agonist
IVF
INTRODUCTION

Substantial evidence exists for an association between endometriosis and infertility; however, a causal relationship has not yet been established. The mechanisms for endometriosis-related infertility are not fully understood and seem to vary in different endometriosis stages (Fadhlouï et al., 2014). Women with endometriosis often need assisted reproductive technology to improve the chance of pregnancy (Fadhlouï et al., 2014), and several studies have suggested lower pregnancy outcomes (Barnhart et al., 2002). Nevertheless, other investigators have reported comparable pregnancy rates (Surrey, 2013).

It has been reported that endometriotic implants secrete pro-inflammatory cytokines (interleukin-1b, 8, 6 and TNFα), which attract macrophages producing an inflammatory state that may compromise folliculogenesis, egg or embryo quality, embryonic development and implantation (Garrido et al., 2000).

Aromatase plays an important role on the oocyte competence, which may be impaired in women with endometriosis. Several researchers (Harlow et al., 1996; De Abreu et al., 2006) have indicated that defects in granulosa cell steroidogenesis may cause a toxic effect on gametes and embryos associated with endometriosis. This could affect oocyte function explaining its fertilizing capacity reduction and subsequent competence of the corpus luteum, leading to subfertility. CYP19A1 gene expression (which encodes aromatase) is down-regulated in patients with endometriosis (Barcelos et al., 2015). Differential expression in mural or cumulus granulosa cell is controversial (De Abreu et al., 2012; Lu et al., 2012), and only one study evaluates its expression in cumulus granulosa cells.

Many investigators have attempted to describe and validate pre- and co-treatments that improve IVF outcomes. Published research in this area, however, is difficult to interpret, mainly owing to shortcomings in the study design or methodology. Few prospective studies, however, have compared gonadotrophin releasing hormone (GnRH) analogues before treatment with no treatment before IVF (Dicker et al., 1990; Nakamura et al., 1992; Marcus and Edwards, 1994; Ruiz-Velasco and Allende, 1998; Rickes et al., 2002; Surrey et al., 2002; Sallam et al., 2006; Declerq et al., 2016). On the basis of the results of the meta-analysis by Sallam et al. (2006), 3–6 months of pituitary downregulation with GnRH agonist (GnRHa) before IVF have been recommended for women with endometriosis-associated infertility (level B evidence) (ESHRE Endometriosis Guideline Development Group, 2013).

A Cochrane review of long-term GnRHa therapy before IVF has recently been updated. Again, this review raises important questions about the benefits of long-term GnRHa therapy compared with no pre-treatment before IVF and intracytoplasmic sperm injection (ICSII) in women with endometriosis.

Continuous pituitary suppression with GnRHa decreases ovarian steroidogenesis; therefore, existing endometriotic lesions lack their main growth stimulus. These drugs also have a direct effect on endometrial cells growth by other mechanisms (Hashin, 2012). Therefore, GnRHa pre-treatment could reverse hostile proinflammatory peritoneal environment and other deleterious effects associated with endometriosis, such as poor folliculogenesis resulting in reduced oocyte quality and impaired endometrial receptivity. These effects could improve clinical outcomes after IVF.

On the other hand, the relationship between aromatase gene expression and the benefits of GnRHa treatment in these patients has not yet been elucidated. The aim of the present randomized controlled trial was to evaluate the effect of GnRHa treatment over 3 months on IVF outcome in terms of clinical pregnancy rate, and to establish whether CYP19A1 gene expression and follicular steroidogenesis in granulosa cells are influenced by this treatment.

MATERIALS AND METHODS

Design

This prospective, randomized, single-blinded, placebo-controlled clinical trial was conducted at an Assisted Reproduction Unit in a public tertiary care University Hospital. The trial was approved by the hospital Research Ethics Board on 26 October 2010 (reference number: END01V-010 2010-022216-39) and Health Authority approval was also obtained on 8 November 2010 (Spanish Agency for Drugs and Medicinal Devices, reference: MUH/CLIN). All participants signed written informed consent. The trial was registered at ClinicalTrial.gov reference number: NCT01581359.

Participants

Between March 2012 and March 2015, a cohort of 200 consecutive patients with indication for IVF with or without ICSI treatment diagnosed with endometriosis stage I to IV, according to the American Fertility Society criteria (Così et al., 1997), were included in the study and randomized to two groups: a study group and a control group. All patients were white.

The included patients met the following criteria: patients under the age of 40 years with laparoscopic diagnosis and surgical resection of endometriosis carried out 1 year before inclusion in this study or ovarian endometriotic cysts observed by ultrasound at the beginning of the study, body mass index (BMI) lower than 28 kg/m², and infertility with indication for IVF or ICSI.

In 273% of patients in whom no surgical indication before IVF was considered, endometriosis was diagnosed on transvaginal ultrasound as described by Exacoustos et al. (2014). An endometrioma was defined by visualization of a unicocular or multilocular (less than five locules) ovarian cyst with regular margins and ground-glass echogenicity of the cyst fluid, usually poorly vascularized. The presence of the cyst was confirmed on at least two separate examinations carried out at least 1 month apart to rule out other haemorrhagic cysts that could be confused with an endometrioma. All these patients also presented with clinical symptoms of dysmenorrhoea and chronic pelvic pain.

Exclusion criteria were FSH measured in early follicular phase greater than 12 IU/I and patients with major disorders that would make carrying a pregnancy inadvisable.

Sample size

The sample size was calculated for a superiority trial of GnRHa over placebo for a twofold increase in the primary outcome measure (clinical pregnancy rate), from 20–40%. Calculation indicated that the minimal sample should
have been 180 patients (90 in each arm) to have an 80% chance of detecting differences, at a significance level of 0.05. Assuming a 10% rate of withdrawal, it was planned to enrol 200 patients.

**Randomization**
Randomization was carried out by a statistician with no clinical involvement in the trial, through a computer programme using a random block sequence. Patients were allocated in blocks of four and assigned to each group through opaque-sealed envelopes, which were opened only after the patient had given written consent. Two researchers were responsible for assignment, enrolment, administration of pre-IVF injections and follow-up. Treating physicians, embryologists and patients were blinded for the randomization results during the study until the end of the follow-up.

**Procedures**
Patients in the study group received three subcutaneous injections of a long-acting GnRHa (3.75 mg of triptorelin acetate [Gonapeptyl Depot®]) (Ferring Pharmaceuticals, Madrid, Spain) on a monthly basis. Control group received three injections of saline with the same monthly basis. Injections were administered on the first or second cycle day, and then 28 and 56 days after the first injection. Ovarian stimulation was started 80+ or 28 and 56 days after the first injection.

Follicular growth monitoring was carried out three times a day, at 8 a.m., 12 noon and 4 p.m. until HCG administration. Follicular growth monitoring was carried out by vaginal ultrasound (mean size and endometrial thickness). Transvaginal ultrasound-guided oocyte retrieval, under venous sedation, was carried out 36 h after the administration of 250 µg of HCG (Ovitrelle 250 µg) (Merck, Madrid, Spain) when at least one follicle measured over 7 mm and three or more follicles reached a mean diameter of 16 mm.

No more than two embryos per patient were transferred on day 2 or 3 of culture. The morphologic embryo quality was assessed in four categories (A, B, C or D), according to ASEPBR (Asociación para el Estudio de la Biología de la Reproducción) criteria, A being the best embryo quality score, and A and B regarded as good quality (Hurtado de Mendoza and Ten, 2015).

Fresh embryo transfers were carried out only if the progesterone level was less than 1.5 ng/ml on the trigger day. Embryo transfers were carried out under pelvic ultrasound. In cases in which the entire cohort of embryos was cryopreserved, or if the remaining embryos were viable, these embryos were vitrified using a lock system disposal (Cryotip®) (Irvine Scientific, Santa Ana, USA) and the medium VitrificationFreeze Kit® (Irvine Scientific, Santa Ana, USA). Good-quality embryos were vitrified at the cleavage or blastocyst stage. Vitrified embryos were transferred after warming in later cycles using endometrial priming with oral oestradiol valerate (Meriestra 1 mg tablets) (Novartis, Barcelona, Spain). Starting dose of 2 mg on day 2 of a menstrual cycle was increased by 2 mg/day every 4 days until 6 mg/day. Ultrasound scan and hormone level measurement (oestradiol and progesterone) were carried out 12 days after the start of oestradiol administration. Embryo warming and transfer were scheduled when endometrial thickness reached at least 7 mm and progesterone level remain less than 1.5 ng/ml.

For fresh transfers, the luteal phase was supported by twice-daily vaginal application of 200 mg of micronized progesterone (Progeffik tablets 200 mg) (Effik Laboratories, Madrid, Spain) or Utrogestan tablets 200 mg (SEID, Barcelona, Spain) starting the day after egg retrieval. For cryopreserved embryo transfer, progesterone supplementation (200 mg three times a day) started when monitoring was adequate and one or two embryos were warmed and transferred 2, 3 or 5 days after the start of progesterone according to the day in which the embryos were vitrified.

All fresh and 34 subsequent cryopreserved embryo transfers were

**FIGURE 1** Study protocol. GnRHa, gonadotrophin-releasing hormone agonists.
Granulosa cells were isolated and CGC of a sub-group of 40 patients was studied in the human MGC and mural granulosa cells and cumulus CYP19A1 gene expression in human and MGC were also stored at –80°C in nitrogen until RNA extraction. Follicular fluid of the leading follicle from the oocyte by microdissection granulosa cells (CGC) were separated immediately identified and separated to the manufacturer’s protocol. Complementary DNAs were prepared using 500 ng of RNA and reverse transcription polymerase chain reaction (PCR) High Capacity cDNA Reverse Transcription (Applied Biosystem, Warrington, UK).

The follow-up period ceased 1 year after the last patient was randomized (March 2016).

Information on the outcome of pregnancy, obstetrical and perinatal complications was obtained by means of reviewing medical records.

Follicular fluid of the leading follicle from each ovary of the last 40 consecutive patients included in the study was first and individually aspirated for CYP19A1 gene expression in granulosa cells and hormone concentrations.

**Follicular fluid and granulosa cell samples collection**

Cumulus-oocyte complex (COC) was immediately identified and separated from the follicular fluid. Cumulus granulosa cells (CGC) were separated from the oocyte by microdissection using two insulin needles placed within a cryotube and immediately frozen in liquid nitrogen until RNA extraction.

Only clear follicular fluid free of blood and presenting a metaphase II (MII) oocyte was considered adequate for analysis. Follicular fluid sample was centrifuged at 300 g for 7 min, cell components were identified as mural granulosa cells (MGC) and were removed from the follicular fluid. Follicular fluid and MGC were also stored at –80°C in individual tubes for further analysis.

**CYP19A1 gene expression in human mural granulosa cells and cumulus granulosa cells**

Gene expression profile of CYP19A1 was studied in the human MGC and CGC of a sub-group of 40 patients. Granulosa cells were isolated and processed according to the method described by Toyta et al. (2000). Total RNA was extracted by using Quick-RNA™ MicroPrep kit (ZyBio Research Corp., Irvine, CA, USA). Total RNA was used to normalize the target gene cycle threshold values. The PCR conditions and expression of final results were carried out as previously described by Novella-Maestre et al. (2010, 2015).

**Testosterone, androstenedione and oestradiol concentrations in follicular fluid**

Total testosterone, oestradiol and androstenedione concentrations were determined based on an electrochemiluminescence immunoassay using Architect Estradiol, Architect 2nd Generation Testosterone System (Abbott Diagnostics, Chicago IL, USA) and IMMULITE 2000 Androstenedione (Siemans Healthineers, Erlangen, Germany) kits, respectively, according to the manufacturer’s instructions. The intra- and inter-assay coefficients of variation of these assays were less than 10%.

**Outcome measurements**

The primary endpoint of the study was clinical pregnancy rate (CPR) per started cycle. Clinical pregnancy was defined as a pregnancy with a positive fetal heartbeat at 6–7 gestational weeks.

Secondary outcomes were as follows: cumulative CPR per patient; implantation rate; miscarriage rate; cumulative live birth rate; and multiple pregnancy rate. Variables related to ovarian stimulation include oestradiol levels; number of MII oocytes; number of embryos; and embryo quality. Fertilization rate was defined as number of fertilized oocytes per number of oocytes that underwent IVF/ICSI. Implantation rate was defined as number of gestational sacs per number of transferred embryos per patient. Miscarriage rate was defined as the number of spontaneous clinical pregnancy losses up to 12 gestational weeks per number of clinical pregnancies. Multiple live births have been counted as one live birth event in cumulative live birth rate. Moreover, the CYP19A1 gene expression in granulosa cells and the intrafollicular levels of oestradiol, testosterone and androstenedione were evaluated.

**Statistical analysis**

Data were analysed per protocol, including all patients who received the allocated treatment during 3 subsequent months and started ovarian stimulation. Normal distribution of data was verified. Mann–Whitney U test, t-test or chi-squared test were used to compare treatment groups. 95% confidence intervals for the difference in proportions were estimated using exact binomial confidence limits.

For the primary endpoint (clinical pregnancy rate), a multivariable regression model was also applied adjusting for the covariates age, duration of infertility, BMI, classification of endometriosis and endometriosis surgery. IBM SPSS Statics (version 17) software was used to analyse the data. Significance level was set to 0.05.

**RESULTS**

A total of 239 patients were screened for the study and 200 were successfully enrolled and randomized according to the study protocol (figure 2). Among the 200 randomized patients, 183 completed the study and were included for statistical analysis, 91 in the study group and 92 in the control group. Seventeen patients were excluded owing to selection failure (n = 2), voluntary drop out (n = 6), protocol deviation (n = 7) or mild adverse events (n = 2). Adverse events were rash, swelling and pain in the skin after the GnRHa injection in two patients in the study group. Twenty-three patients in this group had mild symptoms of hypoestrogenism such as hot flashes, but these side-effects were well tolerated, as reported during the clinical check-up. All included patients completed the follow-up.

No statistical differences were observed in baseline clinical characteristics between the two groups (table 1). No statistical differences were found in the percentage of patients who underwent laparoscopic surgery before the study...
between the two groups (71.4% [65/91] in the study group and 73.9% [68/92] in the control group; \(P = 0.706\) (TABLE 1).

As described previously, patients with no surgical diagnosis of endometriosis (27.3%) were assessed by ultrasound and clinical criteria. At the enrolment, 84.6% (77/91) of patients in the study group and 80.4% (74/92) of patients in the control group had at least one ovarian endometrioma (\(P = 0.457\)). Of these, 33.8% (26/77) in the study group and 36.5% (27/74) in the control group presented with more than one endometrioma. All patients who had not undergone previous surgery (26 in study group and 24 in control group) had at least one endometrioma at the time of IVF treatment.

Patients who had undergone previous surgical treatment (\(n = 133\)) were classified according to American Fertility Society/American Society for Reproductive Medicine classification system in four stages of endometriosis (Conis et al., 1997). Distribution of all patients was similar between the two groups (\(P = 0.436\) (TABLE 1).

The number of stimulation days was significantly higher in the study group compared with the control group (\(P < 0.001\)). The total dose of gonadotrophins administered was also significantly higher in the study group (\(P < 0.001\)). Oestradiol levels on the day of HCG administration were significantly lower in the study group, compared with the control group (\(P = 0.001\) (TABLE 2).

Cancellation rate was significantly higher in the study group (\(n = 10\) [11%]) than in the control group (\(n = 3\) [3.3%], \(P = 0.042\)). The reasons for cancellation were poor ovarian response (\(n = 11\)), drug administration error during ovarian stimulation (\(n = 1\)) and risk of OHSS (\(n = 1\)).

Only one pregnant patient in the control group was diagnosed with late OHSS and required hospital admission and treatment with support measures until resolution. After that, she had an ongoing pregnancy and delivered at term a healthy newborn. No patient in the study group suffered from OHSS.

The number of total oocytes and MII oocytes did not differ between the two groups (MII oocytes: 4.47 ± 3.03 in the study group versus 5.35 ± 3.08 in the control group, \(P = 0.064\)). In contrast, immature oocytes (metaphase I and prophase I oocytes) were significantly more numerous in the study group compared with the control group (\(P = 0.003\) (TABLE 2).

The number of embryos transferred and vitrified did not show any statistical differences between the two groups. The number of cleavage embryos was significantly higher in the control group (\(P = 0.023\) (TABLE 2). No significant differences were found between the two groups regarding embryo quality (data not shown).

A total of 142 fresh embryo transfers were carried out, 67 (73.6%) in the study group and 75 (81.5%) in the control.
TABLE 1 DEMOGRAPHIC CHARACTERISTICS AND ENDOMETRIOSIS VARIABLES IN THE STUDY (GNRHA) AND CONTROL (PLACEBO) GROUPS

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Study group (n = 91)</th>
<th>Control group (n = 92)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age, years</td>
<td>33.86 ± 3.08</td>
<td>33.72 ± 3.25</td>
<td>0.765</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.85 ± 2.86</td>
<td>22.42 ± 2.78</td>
<td>0.167</td>
</tr>
<tr>
<td>Basal FSH, IU/l</td>
<td>7.01 ± 1.91</td>
<td>7.17 ± 1.81</td>
<td>0.580</td>
</tr>
<tr>
<td>Duration infertility, years</td>
<td>2.91 ± 1.40</td>
<td>2.93 ± 1.55</td>
<td>0.914</td>
</tr>
<tr>
<td>Endometriosis variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients who have undergone previous surgery, n (%)</td>
<td>65 (71.4)</td>
<td>68 (73.9)</td>
<td>0.706</td>
</tr>
<tr>
<td>ASRM stage I-II, n (%)</td>
<td>15 (23.1)</td>
<td>12 (17.6)</td>
<td>0.436</td>
</tr>
<tr>
<td>ASRM stage III-IV, n (%)</td>
<td>50 (76.9)</td>
<td>56 (82.4)</td>
<td></td>
</tr>
<tr>
<td>Patients who have not undergone previous surgery, n (%)</td>
<td>26 (28.6)</td>
<td>24 (26.1)</td>
<td>0.726</td>
</tr>
</tbody>
</table>

a Values of clinical characteristics are mean ± standard deviation, t-test.
b Values of endometriosis variables are n (%), chi-squared test.

ASRM, American Society for Reproductive Medicine; BMI, body mass index; GnrHa, gonadotrophin-releasing hormone agonist.

group (P = 0.135). Additionally, 25 patients in the control group yield 62 embryos that were cryopreserved and 20 patients in the study group obtained 57 embryos that were cryopreserved. Thirty-four vitrified–warmed embryo transfers were also carried out, 14 in 11 study group patients (22 embryos), and 20 in 15 control group patients (32 embryos) (P = 0.234). The remaining embryos (35 embryos in study group and 30 in control group) were still cryopreserved at the end of the follow-up period.

Cycle outcomes are presented in TABLE 3. Implantation rate did not show statistical differences between the two groups. In the study group, CPR per started cycle was 25.3% (23/91) in the and in the control group it was 33.7% (31/92) (P = 0.212). No statistical differences were found in cumulative CPR per patient between both groups (27.5% in the study group and 40.2% in the control group, P = 0.144). No significant differences were found in the miscarriage rate (TABLE 3).

Twin pregnancies at the first ultrasound were significantly higher in the control group than in the study group, when only fresh transfers were considered: 32.3% (10/31) in the control group versus 8.7% (2/23) in the study group; P = 0.039, OR 3.71 (95% CI 1.16 to 10.05). No differences were demonstrated when fresh plus cryopreserved embryo transfers were considered (data not shown).

Cumulative live birth rate did not show a significant difference between the two groups: 22.0% (20/91, 95% CI 0.13 to 0.31) in the study group and 33.7% (31/92, 95% CI 0.24 to 0.44) in the control group (P = 0.077). Of 13 twin clinical pregnancies, two in the study group and 11 in the control group, 16 newborns were delivered (four in the study group and 14 in the control group).

A multivariable logistic regression analysis of selected covariates was carried out. Age, duration of infertility, BMI, classification of endometriosis and endometriosis surgery were not significant predictive factors associated with an increased probability of pregnancy.

No difference was found between the two groups in CYP19A1 gene expression in granulosa cells. Testosterone levels in follicular fluid was significantly lower in the control group compared with the control group (P < 0.001). Oestradiol level and androstenedione level in follicular fluid were not significantly different between study group and control group (Supplementary Table 1).

TABLE 2 DATA FROM OVARIAN STIMULATION AND IVF CYCLES IN THE STUDY (GNRHA) AND CONTROL (PLACEBO) GROUPS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group (n = 91)</th>
<th>Control group (n = 92)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dose of gonadotrophins, IU</td>
<td>30279 ± 974 (29522 to 3364.8)</td>
<td>23390 ± 673 (2707.5 to 2476.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stimulation, days</td>
<td>10.4 ± 2.6 (10.3 to 11.4)</td>
<td>90 ± 1.7 (86.6 to 94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oestradiol HCG day, pg/ml</td>
<td>1732.2 ± 889.6 (1726.6 to 2067.7)</td>
<td>2200.9 ± 806.9 (2072.5 to 2430.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Oocytes, total n</td>
<td>7.30 ± 4.45 (6.2 to 8.1)</td>
<td>7.66 ± 4.26 (6.8 to 6.6)</td>
<td>0.594</td>
</tr>
<tr>
<td>MII oocytes</td>
<td>4.47 ± 3.03 (3.8 to 5.1)</td>
<td>5.35 ± 3.08 (4.7 to 6.0)</td>
<td>0.064</td>
</tr>
<tr>
<td>Immature oocytes, MII + PI</td>
<td>1.63 ± 0.25 (1.22 to 2.19)</td>
<td>0.76 ± 0.14 (0.50 to 1.07)</td>
<td>0.003</td>
</tr>
<tr>
<td>Cleavage stage embryos, n</td>
<td>3.21 ± 2.55 (2.7 to 3.8)</td>
<td>4.11 ± 2.51 (3.8 to 4.9)</td>
<td>0.023</td>
</tr>
<tr>
<td>Transferred embryos</td>
<td>1.81 ± 0.43 (1.69 to 1.90)</td>
<td>1.82 ± 0.52 (1.70 to 1.93)</td>
<td>0.835</td>
</tr>
<tr>
<td>Vitrified embryos</td>
<td>0.63 ± 1.46 (0.39 to 0.87)</td>
<td>0.67 ± 1.24 (0.40 to 0.91)</td>
<td>0.833</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (95% confidence interval).

GnrHa, gonadotrophin-releasing hormone agonist; MII, Metaphase II; MII, metaphase II; PI, prophase I.
In this study, 3 months of pre-treatment with GnRHa before IVF did not improve clinical pregnancy rate in women with endometriosis, as previously reported by Fàbregues et al. (1998) and Sõritsa et al. (2015). Nevertheless, cumulative live birth rate showed a difference of 11.7% in favour of no pre-treatment. This finding was considered clinically relevant, although the difference did not reach statistical significance (P = 0.077).

Moreover, cancellation rate was higher if GnRHa was administered, and more days and total dose of gonadotrophin were required. We detected lower oestradiol levels and higher immature oocytes number, as well as fewer number of embryos when patients were pre-treated with GnRHa.

Published research on the effects of GnRHa pre-treatment before IVF in endometriosis patients is widely heterogeneous, and recommendations must be carefully taken into account. The best evidence on this topic is provided by the last Cochrane systematic review (Georgiou et al., 2019), involving a total of 640 participants. Our clinical trial is one of the eight parallel-design, randomized controlled trials included in this review. In all these studies, GnRHa treatment for a period of 3–6 months was compared with no treatment before IVF in women with endometriosis. Contrary to previous findings (Sallam et al., 2006), the authors are uncertain whether long-term GnRHa therapy affects live birth (RR 0.48, 95% CI 0.26 to 0.87) or clinical pregnancy rates (RR 1.13, 95% CI 0.91 to 1.41) because the quality of the evidence ranged from very low to low quality. The main limitation was lack of blinding of seven out of eight studies (Dicker et al., 1992; Rickes et al., 2002; Tataro et al., 2009; Surrey et al., 2010; Decler et al., 2016; Kaponis et al., 2020; 2002), and data of two of them were not included in the meta-analysis owing to risk of bias or data not available (Tataro et al., 2009; Surrey et al., 2010).

The most recent randomized controlled trial (Decler et al., 2016) did not find any benefit in surgically treated patients for peritoneal endometriosis (I–II ASRM stage), who were either assigned to 3 months of GnRHa treatment after surgery and subsequent IVF instead of immediate IVF after surgery. As observed in our study, and also by other groups (Nakamura et al., 1992; Mo et al., 2008), Decler et al. (2016) reported a longer duration of stimulation and higher amount of gonadotrophins required, possibly owing to the long hormonal suppression of the GnRHa administration.

As oocyte competence is well defined as the ability of the oocyte to complete maturation and undergo successful fertilization (Assali et al., 2008), the condition of poor oocyte quality might be clinically represented by a higher number of immature oocytes retrieved and a lower fertilization rate. In this sense, according to our results, GnRHa pre-treatment would lead to poorer oocyte quality in patients with endometriosis, although we found no differences in embryo quality between both groups. A recent study shows lower rates of mature oocyte and higher incidence of oocyte dysmorphisms in stimulated cycles with GnRHa regimen instead of antagonist, but similar clinical outcomes, so the effect of GnRHa on oocyte and embryo quality remains uncertain (Zanetti et al., 2019).

In the present randomized controlled trial, higher implantation and clinical pregnancy rates were not obtained in the study group. Moreover, multiple pregnancy rate was significantly lower in this group when only fresh transfers were considered. We suggest that long-acting GnRH could impair endometrial receptivity. These findings could be due to a change in the chemokines and growth factors expression patterns in endometrium induced by GnRHa pre-treatment, as previously described (Haouzi et al., 2010). Nevertheless, when cryotransfers were also considered, and therefore GnRHa had no effect on the endometrium, implantation rate was similar. These outcomes support the results of Van Der Houwen et al. (2014) who observed a positive effect on the ongoing pregnancy rate only when fresh plus cryopreserved embryos were considered, but no statistically significant differences were
observed. We are uncertain, however, of the effect of long-term GnRHa therapy on endometrial receptivity, as it is necessary to take into account the limitation of our reduced sample size, especially in cryopreserved embryo transfers.

One of the secondary outcomes of our study was to analyse the occurrence of differential CYP19A1 gene expression in granulosa cells in women who have or have not undergone treatment with GnRHa. It is known that endometriosis is an oestrogen-dependent inflammatory disease and aromatase (the key enzyme of oestrogen production) is expressed aberrantly in endometriotic lesions and in granulosa cells (Sanchez et al., 2016). Lower follicular fluid oestradiol and higher follicular fluid progesterone levels in patients with endometriosis compared with controls have been previously demonstrated, indicating that an impairment in steroidogenesis may directly affect the local oocyte environment and it has been proposed as a cause of diminished oocyte quality (Pellicer et al., 1998; Wunder et al., 2005). Barcelos et al. (2015) compared the expression of the CYP19A1 gene in CGC and oestradiol concentration in the follicular fluid of infertile women with and without endometriosis. Lower expression of this gene in the CGC of endometriosis group was observed, but no significant difference in the follicular fluid oestradiol concentration was demonstrated. This study suggested that reduced expression of CYP19A1 in CGC of women with endometriosis might play a role in the pathogenesis of endometriosis-related infertility. Authors hypothesized that the lower expression of CYP19A1 gene could reflect MGC activity that leads to oestradiol synthesis, whereas CGC could have less relevant role in steroidogenesis. Nevertheless, the results are still controversial.

De Abreu et al. (2012) studied the CYP19A1 gene expression in pooled MGC obtained after oocyte retrieval in 11 patients with endometriosis and 11 patients with tubal or male infertility without GnRH suppression, and no differences were observed. In contrast, Lu et al. (2012) found lower oestradiol concentration and less aromatase expression in granulosa cells of endometriosis patients compared with no-endometriosis patients.

Prolonged treatment with GnRHa suppresses pituitary gonadotrophin release and, consequently, FSH effect on aromatase synthesis. To the best of our knowledge, this is the first and only study that has evaluated the CYP19A1 gene expression in mural and cumulus granulosa cells obtained from single follicles in women with patients with 3 months of GnRHa pre-treatment compared with placebo. We found no differences in the levels of CYP19A1 gene expression between patients treated with GnRH or placebo in any granulosa cells samples.

Oestradiol and androstenedione levels in follicular fluid did not show any differences in pre-treated versus placebo groups, but testosterone level was significantly lower in patients who had undergone GnRHa treatment. This decreased testosterone level could be explained by the suppressive GnRHa effect on LH; similarly, a decreased CYP19A1 gene expression would have been expected, as well as lower levels of oestradiol owing to FSH suppression; however, this hypothesis has not been demonstrated. Whether these observations truly reflect the quality and the competence of the inherent oocyte is yet to be elucidated.

The present study has several strengths. It is the first time a prospective, blinded, controlled with placebo and randomized design has been used in the subject area. Second, the study has been carried out in a single centre avoiding proceeding-related bias. Third, all included patients were similar in demographic characteristics. Finally, all the 183 participants included for per protocol analysis completed the follow-up.

The lack of a histologic diagnosis of endometriosis in 27.3% of patients can be considered as a weakness of the present study. In these cases, diagnosis was established based on ultrasound and clinical criteria. It could be a selection bias because of the potential risk of misclassification. The statistical analysis, however, controlled for surgically confirmed endometriosis offset this potential source of bias, because they showed similar results. In fact, only these patients were included in the updated Cochrane Review (Georgiou et al., 2019).

Surgical management of endometriosis before IVF is rather limited and debatable, as surgical excision is associated with a further decrease in ovarian reserve and does not seem to improve fertility outcomes (Somigliana et al., 2012; Ato et al., 2017). Nowadays, transvaginal ultrasound is the mainstay for the diagnosis of endometriomas. In a recent Cochrane review to compare surgical and ultrasound diagnosis of endometriosis, transvaginal ultrasound was found to have a sensitivity of 0.93 and a specificity of 0.96 for diagnosing endometriomas, corroborating our selection criteria (Nisenblat et al., 2016).

Another potential limitation of our study is the inclusion of women at any stage of endometriosis. We conducted the analysis by separate stages, and also by other secondary outcomes (data not shown), but the sample size of subgroups may be too small to find significant differences.

The lack of information about the presence of adenomyosis in our patients is another limitation. Adenomyosis has a detrimental effect on IFV clinical outcomes but a pre-treatment or a long protocol with GnRHa could be beneficial in these patients, as has been observed in some studies (Mijatovic et al., 2010; Younes and Tulandi, 2017). This issue has not been assessed in our clinical trial.

In conclusion, this clinical trial suggests that 3-months of pre-treatment with GnRHa before IFV cycle does not improve the clinical pregnancy rate in women with endometriosis. Also, the cancellation rate is higher compared with placebo, and more stimulation days and a higher dose of gonadotrophins are needed. After this treatment, oestradiol levels on the HCG day are lower, and more immature oocytes as well as less embryos are obtained. Aromatase gene expression in mural and cumulus granulosa cells is not affected by the GnRHa. For these reasons, in the absence of newer evidence, we do not recommend the pre-treatment with GnRH analogues before an IVF cycle in endometriosis patients to improve the clinical pregnancy rate. Further high-quality trials are necessary to determine the true benefit of long-term GnRHa therapy or other alternatives on infertility management of endometriosis patients.

ACKNOWLEDGEMENTS

The authors would like to thank the patients who participated in this study and the multidisciplinary team
REFERENCES


Hurtado de Mendoza, M.V., Ten, J. Criterios ASEEIR de valoración morfológica de oocitos, embriones tempranos y blastocistos humanos. Cuad. Embriol. 2015


Marcus, S.F., Edwards, R.G. High rates of pregnancy after long-term down-regulation at the Human Reproduction Unit, particularly Ms Pilar Palacios, the nurse who administered the injections of GnRHα or placebo, and Dr Maria Luisa Martinez-Triguero for hormone assays in follicular fluid. We also thank the Ferring Pharmaceuticals for granting the insurance to carry out this work. The authors are grateful for the professional English language editing to Mr Arash Javadinejad, English Instructor and Publication Editor at the Instituto de Investigación Sanitaria La Fe, Valencia, Spain. This study was supported by Ferring Pharmaceuticals (Madrid) which provided the insurance required for the patients included in this clinical trial. Ferring Pharmaceuticals was not involved in the study design, data analysis, writing or submission of the paper.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2020.06.020.


levels and embryo quality.

cycles of women with endometriosis: steroid follicular endocrine environment in stimulated

Bonilla-Musoles, F., Remohí, J., Simón, C. Cryopreserved Ovarian Tissue From Cancer Viability, and Restores AMH Levels in of Primordial Follicles, Preserves Follicular PTEN Inhibition Improves In Vitro Activation B., Díaz-García, C., Pellicer, A.

83: 866–873. doi:

Endometriosis Therapy1.


Received 2 August 2019; received in revised form 3 June 2020; accepted 30 June 2020.