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ARTICLE

A comparison of two months pretreatment with GnRH agonists with or without an aromatase inhibitor in women with ultrasound-diagnosed ovarian endometriomas undergoing IVF

BIOGRAPHY

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KEY MESSAGE

Among women with endometriomas, ovarian reserve tests and clinical pregnancy outcomes can be improved with several months of pretreatment with gonadotrophin-releasing hormone agonist combined with an aromatase inhibitor.

ABSTRACT

Research question: Does the addition of an aromatase inhibitor improve IVF outcomes in women with endometriomas when pretreating them with gonadotrophin-releasing hormone agonists?

Design: Retrospective two-centre cohort study involving 126 women aged 21–39 years who failed a previous IVF cycle and all subsequent embryo transfers and had sonographic evidence of endometriomas. Women were non-randomly assigned to either 3.75 mg intramuscular depo-leuprolide treatment alone or in combination with 5 mg of oral letrozole daily for 60 days prior to undergoing a fresh IVF cycle. Main outcome measures included clinical pregnancy rate and ongoing pregnancy rate after 24 weeks' gestation.

Results: Prior to treatment, antral follicle count (AFC), basal serum FSH and endometrioma diameter did not differ between groups. After treatment, AFC differed between letrozole and non-letrozole-treated groups (10.3 ± 2.0 versus 6.4 ± 2.5; $P = 0.0001$), as did mean endometrioma maximum diameter (1.8 ± 0.4 cm versus 3.2 ± 0.8 cm; $P = 0.0001$). At IVF, the gonadotrophin dose used was significantly lower in letrozole-treated subjects (2079 ± 1119 versus 3716 ± 1314; $P = 0.0001$), the number of mature oocytes collected was greater (9.1 ± 2.4 versus 4.0 ± 1.7; $P = 0.0001$), as were the number of two-pronuclear embryos and number of blastocysts. The clinical pregnancy rate was significantly higher in the letrozole-treated group (50% versus 22%, $P = 0.003$), as was the live birth rate (40% versus 17%, $P = 0.008$).

Conclusions: The combination of depo-leuprolide acetate monthly for 60 days combined with daily letrozole has better clinical outcomes at IVF in women with endometriomas than depo-leuprolide acetate treatment alone.

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KEYWORDS

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INTRODUCTION

Endometriosis is a common condition in women with infertility, affecting anywhere from 25% to 50% depending on the quoted study (Pfeifer *et al.*, 2012). It is a disease characterized by the presence of endometrial tissue outside the uterus and when severe, can extend to the ovaries, forming cysts known as endometriomas (*De Ziegler et al., 2010*). Endometriosis is known to cause chronic inflammation and significant adhesions (*Bulletti et al., 2010*), which may hinder the ability to conceive. In women with endometriomas, ovarian ageing with reduction in follicle counts occurs at an accelerated pace compared to women without these cysts (Pfeifer *et al.*, 2012). It would be expected that this decrease in follicle count would result in a diminished response to gonadotrophin stimulation. Mechanisms associated with this loss of true or perceived ovarian reserve include mechanical compression, destruction of viable ovarian tissue or damage during surgical resection of the cyst. IVF pregnancy rates are reduced in women with endometriosis-associated infertility (*Barnhart et al., 2002*). Surgical excision of ovarian endometriomas, however, has had no benefit on IVF pregnancy rates (*Tsoumpou et al., 2009*). It is possible that when performing surgery to resect endometriomas, damage to the surrounding healthy ovarian tissue may occur that can decrease the ovarian response to stimulation (*Bedaiwy et al., 2009*), particularly in the presence of excessive cauterization.

Gonadotrophin-releasing hormone (GnRH) agonists diminish the impact of endometriotic lesions by inhibiting secretion of FSH, preventing ovarian production of oestrogen and creating a hypoestrogenic state. Because endometriosis is maintained by oestrogen, these agents therefore block further development and maintenance of this disease (*Olive, 2008*). Several studies have evaluated medical pretreatment of women with endometriosis with 3–6 months of ovarian suppression using GnRH agonists prior to undergoing IVF. These studies have demonstrated higher pregnancy rates compared with no pretreatment (*Surrey et al., 2002*). This finding has been maintained at meta-analysis (*Sallam et al., 2006*). Because 3–6 months of pretreatment, followed by a period of menstrual cycle recovery, causes a long delay in care, many IVF centres use a GnRH agonist for only 2 months, although the efficacy when used for this duration is unknown. GnRH agonists have not been shown to alter endometrioma size. The mechanism of improvement of pregnancy rates at IVF with GnRH agonist pretreatment is unknown, but theories include normalization of the inflammatory milieu and increased expression of beta integrins (*Khine et al., 2016; Nirgianakis et al., 2013; Surrey et al., 2002*).

Aromatase inhibitors affect oestrogen production from androgenic precursors by interfering with aromatization, a mechanism different from that of GnRH agonists. Elevated levels of aromatase have been found in ovarian endometriomas when compared with other endometriotic lesions (*Maggiore et al., 2017; Pavone and Bulun, 2012*). Aromatase inhibitors have been successful in reducing endometriosis-related pelvic pain (*Attar and Bulun, 2006*). The combination of an aromatase inhibitor, specifically letrozole, with a GnRH agonist has been found to be more effective at treating endometriosis-associated pain than either alone (*Lossl et al., 2009*). One study using aromatase inhibitors in women not undergoing IVF demonstrated a reduction in size of the endometriomas (Agarwal and Foster, 2014). Reduction of endometrioma size is not usually seen with GnRH agonist therapy.

To date, no studies have compared the pretreatment of GnRH agonists with and without letrozole prior to IVF in women with endometriomas. This study was performed to compare the effect of these two treatments on IVF outcomes.

MATERIALS AND METHODS

Study design – participants

This retrospective two-centre cohort study was performed between June 2011 and January 2016. Patients meeting the following inclusion criteria were included in the study: female age 21 to 39 years; those having sonographic evidence of ground-glass appearance cysts consistent with endometriomas; and the persistence of these cysts for at least 3 months without decrease in size. All included subjects had failed a previous IVF cycle and all resultant frozen embryo transfers. None of the subjects had unremoved hydrosalpinges, intra-cavitary fibroids or polyps or severe male factor infertility. All subjects were pre-evaluated with a hysteroscopy, a normal serum thyroid-stimulating hormone and prolactin according to the assay used. None of the subjects had previous surgical treatment of the endometriosis.

Exclusion criteria included women aged 40 years or more, lack of an endometrioma, first IVF cycle or its resulting frozen embryo transfer cycles, severe male factor infertility requiring surgical testicular manipulation or less than 5 million total motile sperm count.

Sixty-two women with endometriomas were offered depo-leuprolide acetate and 54 of these women completed care. Sixty-four women with endometriomas were prescribed both depo-leuprolide acetate and letrozole and 50 of these women completed care. None of the women failed to complete the GnRH agonist or aromatase inhibitor treatment and IVF once started, however, women in each group chose not to continue IVF with us and did not undergo the pretreatment suppression therapy. Names were recorded prospectively pre-care. Subsequently, retrospective IRB approval was obtained to analyse the data.

Interventions

The women included in this study were treated at a university IVF centre and a private IVF centre, both located in Montreal, Canada, and were non-randomly assigned to one of two treatment groups before undergoing a fresh IVF cycle.

Women who presented with cysts consistent with endometriomas were offered treatment with either intramuscular depo-leuprolide acetate 3.75 mg every 30 days for two injections starting cycle day 1 to 6, or a combination of intramuscular depo-leuprolide acetate 3.75 mg every 30 days for two injections, as well as oral letrozole 5 mg daily for 60 days to start with the first shot of depo-leuprolide acetate. Patients were allowed to spontaneously resume normal menses, at which point an oral contraceptive (35 μg ethanyl oestradiol, Marvelon, several makers, Canada) pretreated GnRH agonist long protocol was initiated. For a more in-depth description of the protocol see *Dahan et al. (2014)*. When at least two follicles

reached a mean diameter of 18 mm, 10,000 IU of urinary human chorionic gonadotrophin (HCG) was given. Oocyte retrieval was then completed 36 hours later. IVF was performed and embryos were transferred on day 3 or 5 based on embryology protocols. In order to have a day 5 transfer, two good-quality day 3 embryos were required (6 to 9 cells of grade 1 or 2 quality). Vaginal progesterone (endometrin 100 mg three times daily, Ferring Canada) was administered from the day of embryo transfer until a negative pregnancy test or 12 weeks of pregnancy.

Ultrasonography

Vaginal ultrasound was performed on cycle day 2 to 5 for baseline determination, on treatment day 3, and as indicated by protocol and stimulation levels. All subjects underwent two ultrasounds 3 months apart before their first IVF cycle and an ultrasound after completing 2 months of GnRH agonist down-regulation. A reproductive endocrinology physician or trained ultrasound technician determined the largest endometrioma diameter by measuring cyst length in three diameters using a transvaginal probe with a Voluson 8 ultrasound machine (General Electric, USA).

Objectives and outcomes

The primary outcome measure was clinical pregnancy rate and live birth rate after reaching 24 weeks' gestation. Secondary outcome measures included antral follicle count (AFC), largest mean endometrioma diameter, total gonadotrophin dose administered, number of mature oocytes collected, number of two-pronuclear (2PN) embryos and number of blastocysts between women treated with depo-leuprolide alone, or treated with both depo-leuprolide and letrozole. A clinical pregnancy was defined as the presence of one or more gestational sacs at 2 weeks after a positive HCG test with a fetal heartbeat seen on ultrasound. Pregnancy test was performed at 16 days embryo age.

Statistical methods

Statistical analysis was carried out using SPSS 23 (IBM Corp., USA) with the unpaired or paired t-test as indicated for continuous data and the chi-squared test for categorical data. Continuous data were verified for normalcy using the Kolmogorov–Smirnov test. None of these variables failed to have a normal distribution. A two-sided *P*-value of ≤0.05 was accepted as statistically significant. Data are presented by the mean with the corresponding standard deviation or percentage. A power analysis was performed for two independent groups with 1 to 1 enrolment with an alpha error of 5% and 80% power to detect a difference (beta 0.2). Clinical pregnancy rates were determined to be 60% and 30% based on standard rates in our clinic. It was determined that a minimum of 84 patients (42 in each group) should be enrolled.

Ethical approval

Patients were non-randomly offered one of the two treatment protocols as part of clinical practice. They were prospectively enrolled into the database at that point. Approval for this study was obtained through the McGill University Health Centre Institutional Review Board (#4145).

RESULTS

Sixty-two women with endometriomas were offered depo-leuprolide acetate and 54 of these women completed care. Sixty-four women with endometriomas were offered both depo-leuprolide acetate and letrozole and 50 of these women completed care.

Baseline clinical characteristics of both treatment groups are summarized in TABLE 1. Both groups were homogeneous in terms of age ($P = 0.54$), AFC prior to oestrogen suppression medical treatment ($P = 0.25$) and basal serum FSH concentration ($P = 0.15$). In addition, no significant differences were detected in mean largest endometrioma diameter before treatment ($P = 0.47$) or in number of failed embryo transfers ($P = 1.00$).

Results of the treatment comparisons are shown in TABLE 2. After depo-leuprolide acetate treatment, the AFC differed between the letrozole (10.3 ± 2.0) and non-letrozole (6.4 ± 2.5) treated groups ($P = 0.0001$). The mean endometrioma maximum diameter was also significantly different ($P = 0.0001$) between the two groups, with a greater decrease seen in the letrozole-treated women (1.8 ± 0.4 cm versus 3.2 ± 0.8 cm).

At IVF, the gonadotrophin dose administered was significantly less in the letrozole-treated subjects (2079 ± 1119 versus 3716 ± 1314), while the number of mature oocytes collected was significantly greater (9.1 ± 2.4 versus 4.0 ± 1.7). In terms of embryological outcomes, the number of 2PN embryos was significantly greater in the women who received

TABLE 1 DEMOGRAPHICS OF WOMEN WITH ENDOMETRIOMAS TREATED WITH A GONADOTROPIN-RELEASING HORMONE AGONIST ± LETROZOLE INHIBITION

	Depo-leuprolide + letrozole (*n* = 50)	Depo-leuprolide alone (*n* = 54)	*P*-value
Female age (years)	34.6 ± 3.4	34.2 ± 3.2	0.54
AFC pretreatment	6.2 ± 1.9	6.7 ± 2.4	0.25
Basal serum FSH (IU/l)	11.3 ± 2.3	10.6 ± 2.6	0.15
Mean largest endometrioma diameter (cm)	3.5 ± 0.7	3.4 ± 0.7	0.47
Failed previous embryo transfers	1.4 ± 0.2	1.4 ± 0.2	1.00
Previous pregnancies	0.6 ± 0.7	0.7 ± 0.5	0.41
Previous deliveries	0.4 ± 0.7	0.6 ± 0.6	0.12
Male age (years)	38.2 ± 4.3	39.3 ± 5.2	0.25

AFC = antral follicle count.

TABLE 2 POST-TREATMENT RESULTS AND PREGNANCY OUTCOMES IN WOMEN WITH ENDOMETRIOMAS TREATED WITH A GONADOTROPIN-RELEASING HORMONE AGONIST ± LETROZOLE INHIBITION

	Depo-leuprolide + letrozole (*n* = 50)	Depo-leuprolide alone (*n* = 54)	*P*-value
AFC	10.3 ± 2.0	6.4 ± 2.5	0.0001
Mean maximum endometrioma diameter (cm)	1.8 ± 0.4	3.2 ± 0.8	0.0001
Total gonadotrophin dose (IU)	2079 ± 1119	3716 ± 1314	0.0001
Number of MII oocytes collected	9.1 ± 2.4	4.0 ± 1.7	0.0001
Number of 2PN embryos	7.3 ± 2.9	1.3 ± 1.0	0.0001
Number of blastocysts	3.1 ± 1.1	0.6 ± 0.2	0.0001
Number of transferred embryos	1.3 ± 0.5	1.4 ± 0.6	0.36
Number of frozen blastocysts	1.3 ± 1.0	0.2 ± 0.4	0.0001
Implantation rate (%)	46	29	0.03
Clinical pregnancy	25/50	12/54	0.003
Live birth rate past 24 weeks' gestation	20/50	9/54	0.008
Miscarriage rate per patient	10/50	11/54	0.96
Miscarriage rate per pregnancy	10/35	11/23	0.67

2PN = two-pronuclear; AFC = antral follicle count; MII = metaphase II.

the addition of letrozole (7.3 ± 2.9 versus 1.3 ± 1.0), as was the number of blastocysts and frozen blastocysts. The number of transferred embryos in the two groups were similar (1.3 ± 0.5 versus 1.4 ± 0.6).

The clinical pregnancy rate was significantly higher ($P = 0.003$) in the letrozole-treated group (50%) as compared with the non-letrozole-treated group (22%). The live birth rate past 24 weeks' gestation was also significantly higher ($P = 0.008$) in women who were pretreated with the addition of letrozole (40% versus 17%). Of note, none of the subjects had complete resolution of their endometriomas with letrozole treatment.

A comparison of IVF results in the same population with no pretreatment and those treated with letrozole and depo-leuprolide is presented in TABLE 3. AFC improved (6.2 ± 1.9 versus 10.3 ± 2.0) and maximum endometrioma diameter decreased (3.5 ± 0.7 versus 1.8 ± 0.4). In addition, the dose of gonadotrophins decreased significantly (3584 ± 1289 versus 2070 ± 1119) and stimulation parameters such as number of mature oocytes (3.2 ± 1.9 versus 9.1 ± 2.4), number of 2PN embryos (1.4 ± 1.2 versus 7.3 ± 2.9) and number of blastocysts (0.7 ± 0.5 versus 3.1 ± 1.1) improved significantly. TABLE 4 presents the same comparison in the group treated with depo-leuprolide alone. It can be seen that none of the parameters improved after suppression with depo-leuprolide. As expected, the dose of gonadotrophins was increased to try to obtain a greater stimulation. However, the number of blastocysts obtained coincidentally decreased. TABLE 4 also shows the *P*-values of the comparisons of the pre-suppression IVF stimulation parameters in the group that went on to be treated with GnRH agonist with (means listed in TABLE 3) or without letrozole (means listed in TABLE 4). As can be seen, their first IVF stimulations resulted in very similar outcomes, with no statistical differences noted.

DISCUSSION

The optimal treatment of endometriosis prior to undergoing IVF remains a challenge for healthcare practitioners. In particular, women with endometriomas represent a unique group of patients that are difficult to treat. Endometriomas cause ovarian damage through multiple

TABLE 3 COMPARISON OF IVF RESULTS AND OVARIAN RESERVE PARAMETERS PRE (FIRST IVF) AND POST (SECOND IVF) TREATMENT WITH LETROZOLE AND GONADOTROPHIN-RELEASING HORMONE AGONIST IN THE SAME SUBJECTS

	First IVF with no pretreatment (*n* = 50)	Second IVF after letrozole and GnRH agonist pretreatment (n = 50)	*P*-value
AFC	6.2 ± 1.9	10.3 ± 2.0	0.0001
Maximum endometrioma diameter (cm)	3.5 ± 0.7	1.8 ± 0.4	0.0001
Total gonadotrophin dose (IU)	3584 ± 1289	2079 ± 1119	0.0001
Number of MII oocytes collected	3.2 ± 1.9	9.1 ± 2.4	0.0001
Number of 2PN embryos	1.4 ± 1.2	7.3 ± 2.9	0.0001
Number of blastocysts	0.7 ± 0.5	3.1 ± 1.1	0.0001

2PN = two-pronuclear; AFC = antral follicle count; MII = metaphase II.

TABLE 4 COMPARISON OF IVF RESULTS AND OVARIAN RESERVE PARAMETERS PRE (FIRST IVF) AND POST (SECOND IVF) TREATMENT WITH GONADOTROPHIN-RELEASING HORMONE AGONIST ALONE IN THE SAME SUBJECTS

	First IVF with no pretreatment (*n* = 54)	Second IVF after GnRH agonist pretreatment (*n* = 54)	*P*-value	*P*-value comparing first IVF with no pretreatment in subjects with (*n* = 50) and without (*n* = 54) letrozole
AFC	6.7 ± 2.4	6.4 ± 2.5	0.25	0.25
Maximum endometrioma diameter (cm)	3.4 ± 0.7	3.2 ± 0.8	0.09	0.47
Total gonadotrophin dose (IU)	3274 ± 1556	3716 ± 1314	0.04	0.27
Number of MII oocytes collected	3.6 ± 1.6	4.0 ± 1.7	0.13	0.25
Number of 2PN embryos	1.3 ± 0.9	1.3 ± 1.0	0.96	0.63
Number of blastocysts	0.7 ± 0.3	0.6 ± 0.2	0.01	1.0

2PN = two-pronuclear; AFC = antral follicle count; MII = metaphase II.

mechanisms. These cysts contain proteolytic enzymes, reactive oxygen species and inflammatory molecules that can impair ovarian function (*Hamdan et al., 2015*). Follicular density also appears to be lower in ovarian tissue surrounding endometriomas (Garcia-Velasco and Somigliana, 2008). Recent studies have generated concern that surgical treatment of endometriomas could be harmful to ovarian reserve (*Bedaiwy et al., 2017; Esinler et al., 2006; Hamdan et al., 2015*; Somigliana *et al.*, 2005). The presence of ovarian endometriomas at the time of oocyte retrieval, however, may pose a risk of pelvic infection (*Maggiore et al., 2017*), particularly if the needle must be passed through the cyst.

Alternative therapeutic options for these patients include prolonged medical pretreatment with GnRH agonists (*Hashim, 2016; Surrey et al., 2002*). While GnRH agonists have proved valuable in endometriosis-related pain (*Surrey, 2013*), in general they do not result in endometrioma shrinkage (Takeuchi *et al.*, 2010).

One obstacle to GnRH agonist therapy is the initial flare phenomenon, characterized by a dramatic increase in LH, FSH and oestradiol that occurs before pituitary down-regulation, lasting for 10–14 days. This may be associated with worsening of endometriosis-related symptoms and hypothetical cyst growth. Importantly, the addition of an aromatase inhibitor to a GnRH agonist has been shown to prevent this flair effect (*Bedaiwy et al., 2009*).

Besides lowering oestradiol levels, GnRH agonists also act to decrease peritoneal fluid inflammatory proteins, which have been found in women with endometriosis and increase expression of pro-apoptotic proteins (*Nirgianakis et al., 2013*). This inflammatory state may impair fertility by having a toxic effect on embryos and impairing tubal motility (*Khine et al., 2016*). By reducing this hostile peritoneal fluid microenvironment, GnRH agonists may inhibit proliferation of ectopic endometrial cells, neutralize endometriotic activity and reduce the size of endometriotic lesions (*Nirgianakis et al., 2013*; Zikopoulos *et al.*, 2010). Their suppression of cytokine levels may also enhance the return of endometrial markers of implantation (*Surrey et al., 2002*). In theory, following prolonged down-regulation with a GnRH agonist, women with endometriosis undergoing fertility treatment are starting ovarian stimulation with a more 'controlled' disease state and therefore an increased chance of pregnancy (*Huhtinen et al., 2012*).

Aromatase inhibitors have also gained attention for the management of infertility in women with endometriosis. Aromatase enzyme expression and oestrogen concentrations are significantly higher in endometriomas as compared with peritoneal and deep infiltrating endometriotic lesions (*Bedaiwy et al., 2009; Hashim, 2012*). Aromatase inhibitors provide additional blockade of the extra-ovarian aromatase enzyme expressed in endometriotic implants, the endometrium of women with endometriosis, and importantly in endometriomas (*Bedaiwy et al., 2009*; Khine *et al.*, 2013; *Seal et al., 2011*).

While a positive effect of aromatase inhibitors on pelvic pain has been reported, their effect on endometrioma size has been less studied. One study of five women showed complete regression of recurrent endometriomas after a 6-month course of letrozole (2.5 mg) given in combination with oral contraceptive add-back (0.15 mg desogestrel and 0.03 mg ethinyl oestradiol) to prevent menopausal side effects and ovarian stimulation, which would be expected with unopposed letrozole (*Seal et al., 2011*). Another non-fertility study demonstrated that a 3-month treatment course of daily letrozole (5 mg) together with the progestin norethindrone acetate (5 mg) as add-back therapy resulted in a mean endometrioma volume decrease by 75% (*Agarwal and Foster, 2015*). Aromatase leads to oestrogen production in endometriotic lesions, which stimulates cyclooxygenase-2 and increases prostaglandin E_2 (PGE_2) formation. PGE_2 creates a positive feedback loop by stimulating further aromatase activity and expression in endometriomas (*Nothnick, 2011; Panay, 2008*). Aromatase dysregulation is an important mechanism in endometrioma formation and therefore it has been postulated that aromatase inhibition may reduce endometrioma size (*Maggiore et al., 2017*). Aromatase inhibitors have also been shown to attenuate the initial flare effects of GnRH agonist treatment (*Bedaiwy et al., 2009; Panay, 2008*), as previously mentioned.

One prospective randomized trial published on this dual therapy studied the combination of anastrozole and goserelin as compared with goserelin alone for 6 months following conservative surgery for endometriosis (*Soysal et al., 2004*). They demonstrated a

significantly increased pain-free interval and a reduction in symptom recurrence rates in patients after their surgery with this treatment. GnRH agonists used in isolation do not prevent oestrogen production in adipose tissue, skin and endometriotic foci because they do not block peripheral aromatization of androstenedione in adipose tissue and skin fibroblasts. Inflammation also induces aromatization in endometriotic foci. As a result, there is an ongoing peripheral supply of oestrogen to endometriotic implants and this is probably an important reason for the high failure rate among patients using GnRH agonists alone (*Soysal et al., 2004*).

While aromatase inhibitors given alone do not completely block ovarian steroidogenesis and may lead to ovarian stimulation and cyst formation, they are able to block extra-ovarian oestrogen production by inhibiting peripheral aromatization of androgens and suppressing the aberrant inflammation-induced aromatization in endometriotic foci. These agents therefore provide maximal oestrogen blockade when given in conjunction with a GnRH agonist (*Soysal et al., 2004*).

There is one uncontrolled pilot study investigating combined down-regulation by these two agents in women with endometriosis-related infertility undergoing IVF (*Lossl et al., 2009*). This study showed that the combination of anastrozole and goserelin significantly reduced endometrioma volume and serum Ca-125 levels (a marker of endometrioma activity) in a group of infertile patients with endometriomas undergoing IVF. Anastrozole is instrumental in selectively inhibiting aromatase, which is present in ovaries, adipose and endometriotic tissue. By suppressing oestrogen secretion, pituitary feedback is reduced and gonadotrophin secretion is stimulated. This can, however, be blocked by co-administering goserelin. Oestrogen biosynthesis is then maximally inhibited, which would theoretically contribute to endometrioma volume reduction, as these lesions are oestrogen dependent. While the authors also demonstrated that this treatment was compatible with pregnancy, they observed a high pregnancy loss rate. Importantly in this large study, pregnancy loss rate was not affected by the addition of the aromatase inhibitor. Comparisons with traditional treatments could not be done because the previously cited study did not have a control group.

To date, as far as can be determined, there are no studies comparing the use of a GnRH agonist and an aromatase inhibitor with suppression by a GnRH agonist alone before an IVF cycle in women with endometriomas.

The combination of depo-leuprolide acetate monthly for 60 days with daily letrozole was shown to have better clinical outcomes at IVF than depo-leuprolide acetate alone (the traditional treatment). Women who received the addition of letrozole demonstrated a higher AFC compared with those who were treated with only a GnRH agonist. Their mean endometrioma diameter was also significantly decreased. Regarding IVF cycle outcome, these women had more mature oocytes collected and a larger number of blastocysts created. A clinical pregnancy rate of 50% and an ongoing pregnancy rate of 40% were documented in this group, which was significantly greater than the 22% clinical pregnancy rate and 17% ongoing pregnancy rate in the non-letrozole-treated group. It is possible that the AFC and the stimulation parameters increased, because the presence of a large mass (the endometrioma) compresses the ovarian tissue, inactivating follicular recruitment. It is postulated that the decreased endometrioma volume seen with co-treatment of GnRH agonist and letrozole removed some of this inhibition.

Although both the ovarian reserve and ovarian stimulation parameters increased in the letrozole-treated group, part of the explanation for this may lie elsewhere. In a retrospective study of good responders treated with or without letrozole from the first day of stimulation of the IVF cycle, it was found that the group that received the aromatase inhibitor had more follicles, oocytes collected, metaphase II (MII) oocytes and blastocysts (*Haas et al., 2017*). This may be due to the increased androgen levels that would be expected with aromatase inhibition, causing increased expression of FSH receptors and the resultant increased stimulation. It is possible such a mechanism also contributed to the increased stimulation seen in our population co-treated with letrozole.

It should be noted that this group had decreased ovarian reserve at baseline, as determined both by basal AFC, which was low, and serum basal FSH levels, which were high. All participants had failed one previous IVF cycle and all resultant frozen embryo transfers. As such, they were poor prognosis patients, which helps explain the relatively low pregnancy and ongoing pregnancy rates seen in these groups. It is notable that the AFC normalized after 2 months of treatment with GnRH agonist combined with the aromatase inhibitor. Being able to manage the cysts without the ovarian damage induced by surgery may represent a treatment advance for patients with decreased ovarian reserve and endometriomas. It has been noted that, as more patients have been treated with GnRH agonist combined with letrozole, some fail to shrink the endometriomas, and in one patient the endometriomas grew while on suppressive medications. Studies will be required to determine factors that predict failure to respond to this protocol. It has also been observed that one patient completely resolved a 4 cm and a second 6 cm endometrioma after treatment with 3 months of GnRH agonist and letrozole.

Adding an aromatase inhibitor to the GnRH agonist may result in further suppression of serum oestradiol levels, possibly increasing the symptoms associated with the hypoestrogenic state. However, none of the patients stopped treatment due to these symptoms. From experience, a slightly higher number of women co-treated with letrozole complain of bone pain. If this treatment becomes standard of care, a future study would be to investigate the relationship between symptomatology and treatment.

While baseline demographics and first IVF cycle stimulation parameters for the studied patients were comparable, the non-randomized allocation of subjects may mask a hidden bias in the present study. Patients were pretreated with only 2 months of medical therapy in order to not significantly delay their IVF therapy, and to make the delay more palatable to those involved. It should be noted that pregnancy rates were relatively low in the group treated with GnRH agonist alone. This is probably due to the low ovarian reserve and previous failed embryo transfers in this population. Nonetheless, this preliminary study allowed exploration

of important clinical outcomes in women undergoing medical management for endometriosis-related infertility. All patients underwent a long GnRH agonist protocol. This IVF protocol was chosen because it may provide higher live birth rates in women with endometriosis than the antagonist protocol (*Drakopoulos, 2018; Kolanska et al., 2017*). It should also be noted that in a randomized study, the long GnRH agonist protocol was found to result in more embryos than the antagonist protocol when used in poor responder patients (*Dakhly et al., 2016*).

Several other limitations exist with this study. The effect of transferring a euploid embryo on outcomes cannot be determined. Few patients undergo preimplantation genetic testing of their embryos and none of the patients in this study had preimplantation genetic testing. Nonetheless, it should be noted that given the results of the STAR Trial, the benefit of preimplantation genetic testing of embryos on ongoing pregnancy rates has come into question (*The STAR Trial, 2017*). Preimplantation genetic testing is cost prohibitive in our centre, costing almost 80% of an IVF cycle; this is another reason why it is rarely done.

It would also have been interesting to test the effect of a GnRH agonist and letrozole pretreatment on serum anti-Müllerian hormone levels. However, this test is expensive, costing approximately C$20, while most of our other assays cost 10 cents. In addition, by law, all costs must be paid by the centre and not the patient. As a result, AMH levels could not be tested. Finally, this was a cohort study and not a randomized controlled study and therefore undetected bias may exist, even though subjects were well matched.

It may be questioned why these agents were given prior to ovarian stimulation as opposed to after vitrification of embryos to minimize suppression of ovarian response, particularly in poor responders. However, initial experience with these agents suggested that ovarian reserve would improve and stimulation would possibly improve if these patients were treated pre-stimulation and given time to recover ovarian function. This improvement in ovarian reserve was also demonstrated in the previously published uncontrolled study (*Lossl et al., 2009*).

The findings from this study suggest that among women with endometriomas, ovarian reserve testing and clinical pregnancy outcomes can be improved with pretreatment of several months of a GnRH agonist combined with an aromatase inhibitor, as compared with a GnRH agonist alone. Data from a prospective randomized trial will help bolster these results.

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