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ORIGINAL ARTICLE

Does adjuvant letrozole reduce uterine peristalsis prior to fresh embryo transfer?

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STUDY QUESTION: Does adjuvant letrozole in ovarian stimulation for IVF decrease the uterine peristalsis frequency (UPF) prior to fresh embryo transfer (ET)?

SUMMARY ANSWER: Adjuvant letrozole in ovarian stimulation for IVF does not reduce the UPF significantly prior to fresh ET.

WHAT IS KNOWN ALREADY: Throughout the cycle, uterine peristalsis aids spermatozoa transport to the fallopian tube and may affect implantation. At fresh ET, UPF is negatively correlated with implantation and clinical pregnancy rates and is believed to be modulated by oestradiol and progesterone. High levels of oestradiol, from multiple follicular development, in ovarian stimulation have been reported to increase UPF, whereas progesterone is considered to be an utero-relaxant. The influence of androgens is unclear. Co-treatment with letrozole during gonadotropin ovarian stimulation limits the supra-physiological oestradiol rise and may therefore reduce UPF prior to fresh ET.

STUDY DESIGN, SIZE, DURATION: This study was carried out on subjects participating in a single-centre double-blinded randomized controlled trial of the impact of letrozole on follicle development and endocrine profiles, and investigated the impact of adjuvant letrozole in ovarian stimulation for IVF on UPF prior to fresh ET and the correlations of UPF with endocrine markers. Between 2016 and 2017, 39 women expected to be normal responders were randomized to co-treatment with letrozole or placebo. Of these, 33 women completed this element of the study. The study was carried out according to the Helsinki Declaration and the ICH-Good-Clinical-Practice.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Eligible women were randomized 1:1 to adjuvant treatment with letrozole 5 mg/day or placebo in an antagonist protocol using a fixed dose of recombinant (r) FSH 150 IU/day. Final maturation was triggered with hCG 6500 IU and luteal support with vaginal progesterone was administered from the day following oocyte aspiration. Less than 1 h prior to fresh ET, 6-min duration transvaginal ultrasound recordings of the uterus in sagittal section were performed and blood samples were drawn.

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 33 women completed the study (letrozole n = 17; placebo n = 16). Age, BMI and ovarian reserve markers were similar between the groups. On the day of ET, serum oestradiol levels were significantly suppressed in the letrozole group to a mean of 867 ± 827 pmol/l compared to 3110 ± 1528 pmol/l in the placebo group ($P < 0.001$). Mean UPF prior to fresh ET did not differ between the intervention and placebo group (3.3 ± 0.36 versus 3.5 ± 0.51 per minute respectively, $P = 0.108$). UPF was assessed and agreed by two observers who were blinded to adjuvant treatment. Two patients were excluded due to poor quality of the ultrasound recordings. Supra-physiological serum oestradiol in the placebo group were negatively correlated with UPF ($P = 0.014$; $R = –0.62$), but the more physiological serum oestradiol levels in the letrozole group showed no correlation with UPF ($P = 0.567$; $R = 0.15$). Serum progesterone levels were similar in both groups and did not show any significant correlation with UPF. Testosterone

[†]Deceased.

levels were significantly higher in the letrozole group ($P = 0.005$) and showed a non-significant trend that negatively correlated with UPF in the placebo group (P-value = 0.071, $R = -0.48$).

LIMITATIONS, REASONS FOR CAUTION: Limitations of the study included the limited sample size and the lack of a power calculation specifically determined for this endpoint.

WIDER IMPLICATIONS OF THE FINDINGS: The supra-physiological levels of oestradiol generated during ovarian stimulation were significantly suppressed in the intervention group. However, UPF prior to fresh ET was similar in both groups. Modulating the luteal phase sex steroids with adjuvant letrozole had little measured impact on UPF. Any beneficial effect of adjuvant letrozole during ovarian stimulation is unlikely to be due to significant modulation of UPF.

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TRIAL REGISTRATION NUMBER: Clinicaltrials.gov: NCT02939898, EudraCT no.: 2015-005683-41.

Key words: endometrium / ovarian stimulation / luteal phase / endocrinology / ultrasound / oestrogen / progesterone / assisted reproduction

WHAT DOES THIS MEAN FOR PATIENTS?

The uterus undergoes small contractions that change in frequency and character through the menstrual cycle. They may help the sperm to reach the fallopian tube around ovulation, but an increased uterine contraction frequency at the time of transferring the embryo after IVF has been observed and may decrease the chance of pregnancy. These uterine contractions are believed to be regulated by the female hormones oestradiol and progesterone, which respectively increase and decrease the contractions. Oestradiol can reach very high levels after ovarian stimulation for IVF due to the development of several follicles all producing this hormone. It is possible to reduce oestradiol levels during ovarian stimulation, with a medication that reduces its production. In this study, we investigated whether the suppression of oestradiol during ovarian stimulation could decrease the uterine contraction frequency at the time of transfer of the embryo in IVF treatment. Thirty-three women completed the study receiving either the treatment medication or placebo during ovarian stimulation. We found that oestradiol was normalized in the group that received the medication, but the uterine contraction frequencies were similar in the treatment and placebo group. Interestingly, high levels of oestradiol were actually found to decrease the uterine contraction frequency, whereas normal levels of oestradiol and all levels of progesterone showed no impact on the uterine contraction frequency. These findings are reassuring, but larger studies would help to confirm these findings, since the number of participants in this study was limited.

Introduction

There continues to be much interest in identifying interventions that may modulate uterine peristalsis frequency (UPF) at the time of embryo transfer (ET), as this has been implicated as a factor determining the likelihood of successful IVF treatment (Griesinger *et al.*, 2021). UPF changes throughout the cycle, increasing through the follicular phase and decreasing from the early to late luteal phase (Kunz *et al.*, 1996; Ijland *et al.*, 1997; Fanchin *et al.*, 2001a; Kuijsters *et al.*, 2017). Active uterine peristalsis is considered to assist the transport of spermatozoa to the fallopian tube at ovulation before decreasing to facilitate embryo implantation in the mid-luteal phase. A suboptimal UPF may impact fertility and treatment outcomes (Kuijsters *et al.*, 2017). Ovarian stimulation for both intrauterine insemination and IVF has been demonstrated to be associated with an increase in mid-luteal phase UPF compared to natural cycles (Fanchin *et al.*, 1998; Ijland *et al.*, 1998; Eytan *et al.*, 2001; Zhu *et al.*, 2012). A higher UPF at the time of fresh ET is reported to be negatively correlated with implantation- and clinical pregnancy rates (Fanchin *et al.*, 1998; Zhu *et al.*, 2014; Chung *et al.*, 2017). The same applies in frozen–thaw ET cycles (Zhu *et al.*, 2014).

UPF is believed to be primarily modulated by oestradiol (Oike *et al.*, 1990; Ijland *et al.*, 1998; Kunz *et al.*, 1998; Bulletti and de Ziegler, 2005) and progesterone (Oike *et al.*, 1990; Fanchin *et al.*, 2001a; van Gestel *et al.*, 2003; Bulletti and de Ziegler, 2005). Ovarian stimulation for IVF usually results in supraphysiological levels of oestradiol, and this is thought to underlie the observed increased uterine peristalsis (Ijland *et al.*, 1998; Casper *et al.*, 2017). Progesterone, however, is thought to have utero-relaxing properties (Fanchin *et al.*, 1998, 2001b; Bulletti and de Ziegler, 2005; Kuijsters *et al.*, 2017).

The high serum oestradiol levels that arise at the folliculo-luteal phase transition as a consequence of ovarian stimulation act to suppress pituitary gonadotropin secretion, necessitating luteal phase support. The use of adjuvant letrozole, an aromatase inhibitor, during ovarian stimulation, limits the supraphysiological rise in oestradiol normally observed, leading to a higher endogenous gonadotropin secretion in the luteal phase (Bülow *et al.*, 2022b). This may increase serum progesterone levels in the luteal phase. Interest in the possible role of

letrozole as an ovulation agent and adjuvant to gonadotropin stimulation in IVF has grown in recent years. Indeed, it is now recommended as a first-line treatment in ovulation induction (Franik *et al.*, 2018). However, the role of adjuvant letrozole in IVF is less established. A Cochrane review from 2017 found no evidence of increased live birth rates when using adjuvant letrozole compared to gonadotropins, in mostly poor responders. However, very few studies were included (Kamath *et al.*, 2017). More recently, a systematic review of the evidence for an adjuvant role of letrozole in IVF concluded that while anticipated poor responders may benefit from letrozole co-treatment, there remains insufficient evidence to support its use in normal and high responders (Bülow *et al.*, 2022a).

Given the established suppressive effect of letrozole on oestradiol levels during ovarian stimulation with gonadotropins, it can be hypothesized that, when used in this context, it may decrease UPF prior to fresh ET. The primary aim of this study was therefore to measure UPF prior to fresh ET after administration of adjuvant letrozole versus placebo during ovarian stimulation for IVF. The secondary aim was to investigate correlations between serum sex steroid levels and UPF prior to fresh ET.

Materials and methods

Study design

This study was carried out on subjects participating in a single-centre double-blinded randomized controlled trial (RCT) of the impact of letrozole on follicle development and endocrine profiles (EudraCT No.: 2015-005683-41 and clinicaltrials.gov No: NCT02939898). The trial was conducted according to ICH-Good Clinical Practice and the Declaration of Helsinki. The study was approved by the 'Danish Health Authority and Medicines Agency', approval no.: 2015-005683-41 and 'The Danish Data Protection Agency', approval no.: HGH-2016-033, I-Suite: 04482.

Ethical approval

This study was approved by 'The Regional Scientific Ethics Committee', approval no.: VEK NR: H-15021852 before starting inclusion. All patients gave their written consent before their inclusion in this study after receiving written and verbal information.

Study population

From August 2016 to November 2017, women visiting the fertility clinic for IVF or ICSI were invited to participate by a clinician or a research nurse and were included in the study by a clinician. After receiving written informed consent, a total of 39 women were randomized to adjuvant letrozole or placebo and 33 completed the study (letrozole n = 17; placebo n = 16). Inclusion criteria included eligibility for IVF or ICSI according to local criteria, regular cycle of 21–35 days, age <41 years, and a level of anti-Müllerian hormone (AMH) of 8–32 pmol/l. Patients with polycystic ovarian syndrome, fertility preservation, previous ovarian stimulation with <4 oocytes obtained or allergy towards letrozole were excluded from the study. Subjects were excluded from the study after randomization in case of withdrawal of consent. The cycle was terminated in case of lack of compliance with medication or on medical instruction for clinical reasons including severe adverse events or adverse reactions with letrozole/placebo.

Randomization and blinding

Study participants were randomized by a clinician or a research nurse into blocks of six in a 1:1 ratio on the day of commencing gonadotrophin ovarian stimulation on cycle Day 2 or 3 to receive either intervention with letrozole or placebo. A certified pharmacy had prepared identical capsules of letrozole and placebo, packed and labelled the containers with serial numbers and also provided the sealed envelopes with allocation information. The assignment of the containers started with the lowest numbers. The allocation sequence was computer generated by the pharmacy and remained unknown until the end of the study and until all outcomes had been measured, as there were no events that required unblinding. The study was double-blinded for study participants, clinicians and other staff for the duration of the trial.

Study protocol

Ovarian stimulation was carried out using adjuvant letrozole 5 mg/day or placebo, then a fixed daily dose of rFSH 150 IU/day (Gonal-F®) starting on cycle Day 2 or 3 and continued until the day before hCG triggering of final oocyte maturation. GnRH antagonist (Orgalutran®) 0.25 mg/day was commenced from stimulation Day 5 to prevent premature luteinization. Final maturation was triggered with hCG 6500 IU (Ovitrelle®) when there were ≥ two follicles ≥17 mm. Luteal support with either daily vaginal Crinone® 90 mg or Lutinus® 100 mg three times daily was initiated the day after oocyte aspiration. ET was performed on Day 2, 3 or 5 after oocyte aspiration. The decision about which day to transfer was decided in agreement by the treating clinician and embryologist.

The primary outcome was the UPF prior to fresh ET. Secondary outcomes were oestradiol, progesterone, testosterone and androstenedione levels prior to fresh ET and their correlations with UPF.

Measurements of uterine peristalsis

All transvaginal ultrasound scans (TVUS) to measure UPF were performed by the same clinician <1 h prior to ET. The women rested for at least 5 min before the probe was introduced gently with the patient lying in the lithotomy position. The uterus was imaged in the mid-sagittal plane with a 7.5 MHz transvaginal transducer (Voluson™E6, GE Healthcare, Chicago, IL, USA) and images were recorded in real-time for 6 min. During the recording, women were requested not to move, speak or take any deep breaths.

After completion of inclusion to the study, the recordings underwent manual quantitative assessment (Zhu *et al.*, 2014) for UPF by two independent observers who were blinded to adjuvant treatment. The UPF was assessed by viewing the 6-min recordings at 20× natural playback speed and was calculated as the mean number contractions per minute after counting the total number and dividing by the duration of the recording. A contraction was defined as a clear wavelike movement of the endometrium. To assist with standardization and uniformity, assessments of UPF were carried out by the two observers the same way, with both viewing the recording playbacks simultaneously. The videos could be played back repeatedly until each

observer had clarified their assessment. In case of discrepancies in the UPF measurement, the recording was replayed and UPF was recounted until agreement was reached between the two observers. Two of the recordings were of insufficient quality for analysis and were excluded.

Hormonal analysis

Venous blood samples were drawn <1 h prior to fresh ET in the same visit as the TVUS examination. Serum endocrinology were measured using immunoassay analyser Elecsys/Cobas® (Roche Diagnostics, Mannheim, Germany). Progesterone was diluted 1:10 before analysis.

Statistical analyses

A sample size calculation was not performed in the main RCT, as it was an exploratory investigation of the endocrine and paracrine impact of adjuvant letrozole without one fixed endpoint (EudraCT No.: 2015-005683-41 and clinicaltrials.gov No: NCT02939898). Categorical data are presented as numbers and percentages and compared by the Chi-square test. Continuous data are presented as mean (±SD) and differences between the two groups were assessed using Student's paired *t*-test. Continuous data are also presented as median (interquartile range) in the tables. A sensitivity analysis of UPF on only Day 3 transfers was also performed. Normal distribution testing was conducted using histograms and QQ plots. Correlation of hormonal levels with UPF were assessed using a linear model and visualized in scatter plots. *P*-values <0.05 were considered statistically significant. IBM SPSS version 27 was used for statistical analyses.

Results

Forty-five women were assessed for eligibility and included in the study (Fig. 1). Six women were excluded before randomization because of withdrawal of consent (n = 1), spontaneous pregnancy (n = 2), hypertension requiring treatment before IVF (n = 1), hydrosalpinx (n = 1) or partner azoospermia requiring assessment prior to IVF (n = 1). A total of 39 women were randomized to receive adjuvant letrozole (n = 19) or placebo (n = 20). Six women discontinued the study protocol due

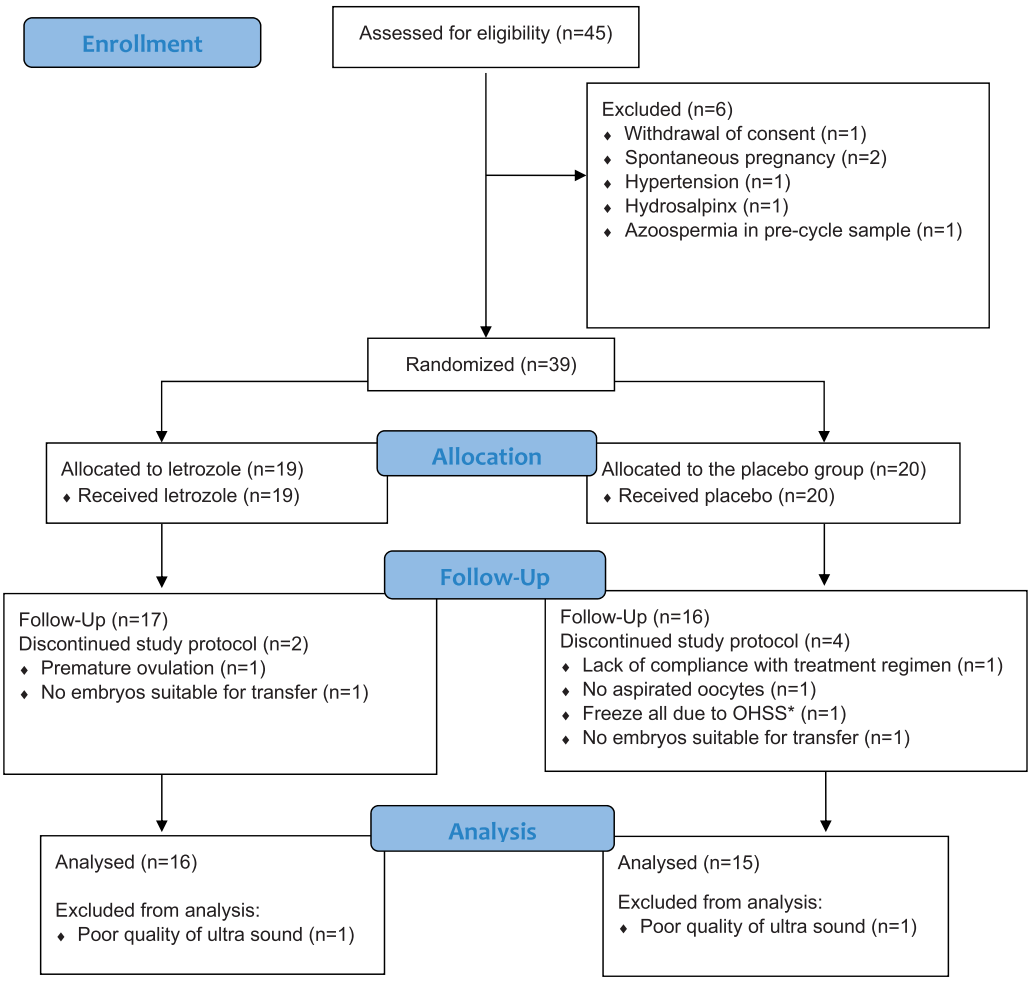

Figure 1. CONSORT flow diagram.

to premature ovulation (n = 1), no embryos suitable for transfer (n = 2), non-compliance with treatment regimen (n = 1), no oocytes aspirated (n = 1) or because all embryos were frozen due to their being at high risk of developing ovarian hyperstimulation syndrome (n = 1). A total of 33 women completed the study, but data from one woman in each group were excluded due to uncertainty of the UPF as a result of insufficient quality of the ultrasound recording.

Baseline and demographic characteristics

No differences were noted in baseline and demographic characteristics between subjects in the letrozole and the placebo group (Table I). The mean age (±SD) in each group was 31.6 (±4.2) years and 31.5 (±3.2) years, respectively. Mean BMI (±SD) was 22.7 (±3.3) kg/m^2 and 23.2 (±4.0) kg/m^2, respectively. Mean AMH levels were 19.5 (±8.2) pmol/l and 17.1 (±6.0) pmol/l, respectively. Antral follicle count, cycle length, fertility duration, causes of infertility and number of previous IVF/ICSI revealed no significant difference between the groups. The distribution for ET dates were the same between the two groups, with 74% being carried out 3 days after oocyte aspiration.

Uterine peristaltic frequency

The mean UPF (±SD) measured 1 h prior to ET is shown in Table II and visualized in a boxplot in Fig. 2. In the letrozole group, mean UPF was 3.3 (±0.4) per minute (range 2.7–4.2 per min), and in the placebo group, mean UPF was 3.5 (±0.5) per minute (range 2.8–4.5 per min) ($P = 0.108$).

Table I Demographic data and baseline characteristics.

	Letrozole (n = 16)		Placebo (n = 15)	
	Mean (±SD)	Median (IQR)	Mean (±SD)	Median (IQR)
Age (years)	31.6 ± 4.2	31.2 (29.2–35.1)	31.5 ± 3.2	30.1 (29.0–33.0)
BMI (kg/m^2)	22.7 ± 3.3	22.5 (19.8–24.8)	23.2 ± 4.0	21.3 (20.1–26.0)
Anti-Müllerian hormone (pmol/l)	19.5 ± 8.2	19.0 (11.8–25.0)	17.1 ± 6.0	14.0 (13.0–22.0)
Antral follicle count (n)	21.1 ± 7.5	20.5 (15.3–25.8)	20.6 ± 6.1	22.0 (16.0–26.0)
Cycle length (days)	29.0 ± 2.2	28.3 (28.0–29.4)	28.6 ± 2.0	28.5 (28.0–30.0)
Duration of infertility (months)	29.3 ± 16.8	24.0 (20.0–36.0)	26.4 ± 12.1	24.0 (18.0–36.0)
Number of previous IVF/ICSI treatments	0.1 ± 0.3	0.0 (0.0–0.0)	0.0 ± 0.0	0.0 (0.0–0.0)
Smokers (n/%)	2 (13)		0 (0)	
Causes of infertility				
Male factor				
Yes (n/%)	9 (56)		11 (73)	
No (n/%)	7 (44)		4 (27)	
Tubal factor				
Yes (n/%)	2 (13)		0 (0)	
No (n/%)	14 (88)		15 (100)	
Endometriosis				
Yes (n/%)	1 (6)		1 (7)	
No (n/%)	15 (94)		14 (93)	
Single or female partner				
Yes (n/%)	0 (0)		1 (7)	
No (n/%)	16 (100)		14 (93)	
Unexplained				
Yes (n/%)	5 (31)		3 (20)	
No (n/%)	11 (69)		12 (80)	
Other causes				
Yes (n/%)	3 (19)		6 (40)	
No (n/%)	13 (81)		9 (60)	
Transfer dates				
Two days after aspiration (n/%)	2 (13)		1 (7)	
Three days after aspiration (n/%)	12 (75)		11 (73)	
Five days after aspiration (n/%)	2 (13)		3 (20)	

Values are expressed as mean (±SD), median (interquartile range) or numbers (%).
The values were all non-significant.

Table II Outcomes.

	Letrozole (n = 16)		Placebo (n = 15)		P-value
	Mean (±SD)	Median (IQR)	Mean (±SD)	Median (IQR)	
Uterine peristaltic frequency (frequency/min)	3.3 ± 0.4	3.3 (3.0–3.5)	3.5 ± 0.5	3.5 (3.2–4.0)	0.108
Serum oestradiol (pmol/l)	867 ± 827	690 (293–1134)	3110 ± 1528	3030 (1410–4770)	<0.001
Serum progesterone (nmol/l)	362 ± 170	354 (235–411)	292 ± 125	303 (210–355)	0.207
Serum testosterone (nmol/l)	2.4 ± 1.1	2.3 (1.5–3.2)	1.4 ± 0.5	1.4 (1.0–2.0)	0.005
Serum androstenedione (nmol/l)	11.3 ± 5.3	10.0 (6.6–16.0)	5.9 ± 2.2	5.2 (4.3–7.2)	0.001
Oestradiol (pmol/l)/progesterone (nmol/l) ratio	2.5 ± 1.9	2.3 (0.9–3.4)	11.0 ± 3.7	10.3 (8.7–14.5)	<0.001
Clinical pregnancy rate (n/%)	5/16 (31.25)		5/15 (33.33)		0.602
Live birth rate (n/%)	5/16 (31.25)		5/15 (33.33)		0.602

Values are expressed as mean (±SD), median (interquartile range) or numbers (%).

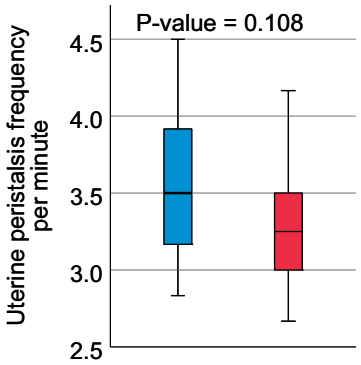

Figure 2. Uterine peristalsis frequency. The boxplot shows the uterine peristalsis frequency per minute in the placebo and letrozole group <1 h to fresh embryo transfer on Days 2, 3 or 5 after oocyte retrieval.

In order to account for the different transfer days employed, a sensitivity analysis of UPF restricted to Day 3 transfers was performed (letrozole n = 12; placebo n = 11). It showed a mean UPF (±SD) of 3.3 (±0.4) per minute in the letrozole group and 3.6 (±0.5) per minute in the placebo group (P = 0.150).

Correlation of oestradiol and uterine peristaltic frequency

Mean serum oestradiol levels on the day of ET were significantly lower in the letrozole group at 867 (±827) pmol/l compared to 3110 (±1528) pmol/l in the placebo group (P < 0.001). The hormonal values measured on ET day in each group are presented in Table II and visualized in Fig. 3. The higher serum oestradiol levels observed in the placebo group were negatively correlated with UPF ($P = 0.014$; $R = -0.62$), but the more physiological serum oestradiol levels measured in the letrozole group showed no correlation with UPF ($P = 0.567$; $R = 0.15$). The hormonal correlations with UPF are visualized in the scatter plots in Fig. 4.

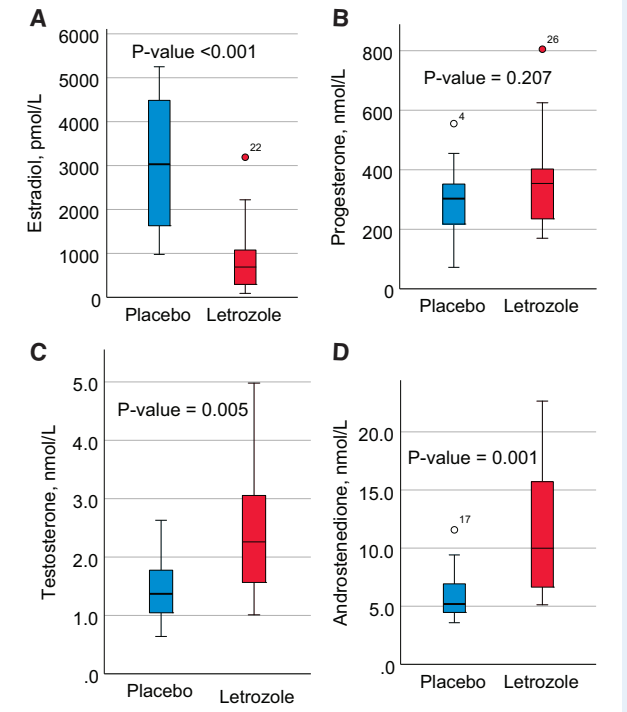

Figure 3. Hormonal levels. The boxplots show the serum hormonal levels in the placebo and letrozole group at the day of fresh embryo transfer on Days 2, 3 or 5 after oocyte retrieval. (**A**) Oestradiol. (**B**) Progesterone. (**C**) Testosterone. (**D**) Androstenedione.

Correlation of progesterone and uterine peristaltic frequency

Mean serum progesterone levels were moderately higher in the letrozole group, with a mean value of 362 (±170) nmol/l compared to

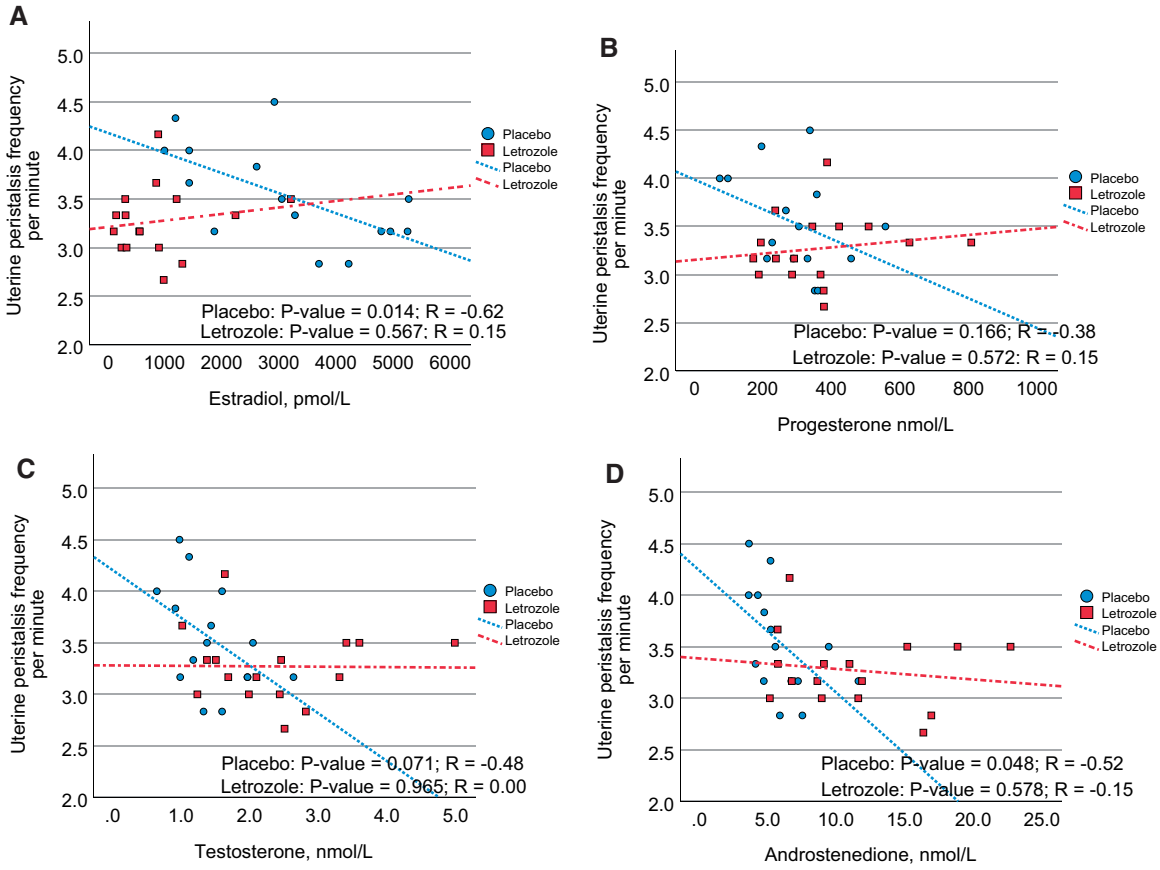

Figure 4. Hormonal correlation with uterine peristalsis frequency. The scatter plots show the hormonal correlations with the uterine peristalsis frequency <1 h to fresh embryo transfer in the placebo and letrozole group on Days 2, 3 or 5 after oocyte retrieval. (**A**) Oestradiol. (**B**) Progesterone. (**C**) Testosterone. (**D**) Androstenedione.

292 (±125) nmol/l in the placebo group ($P = 0.207$). Serum progesterone did not show any significant effect on the level of the UPF in the letrozole group ($P = 0.572$; $R = 0.15$) nor in the placebo group ($P = 0.166$; $R = –0.38$).

Correlation of oestradiol/progesterone ratio and UPF

The oestradiol (pmol/l)/progesterone (nmol/l) ratio (±SD) was significantly lower in the letrozole group measuring 2.5 (±1.9) compared to the placebo group with 11.0 (±3.7) ($P < 0.001$). However, the oestradiol/progesterone ratio showed no significant correlation with UPF ($P = 0.224$; $R = 0.37$).

Correlation of androgens and uterine peristaltic frequency

Mean testosterone and androstenedione levels were both significantly higher in the letrozole group compared to the placebo group ($P = 0.005$ and $P = 0.001$, respectively). A non-significant trend towards a negative correlation between testosterone and androstenedione and UPF was evident in the placebo group ($P = 0.07$; $R = –0.48$ and $P = 0.05$; $R = –0.52$, respectively). However, neither testosterone nor androstenedione levels showed any correlation with UPF in the letrozole group ($P = 0.965$; $R = –0.01$ and $P = 0.578$; $R = –0.15$, respectively).

Clinical pregnancy rate and live birth rate

Clinical pregnancy and live birth rates were 5/16 (31.25%) in the letrozole group for both outcomes and 5/15 (33.33%) in the placebo group for both outcomes ($P = 0.602$) (Table II).

Discussion

This study showed that even though adjuvant letrozole during ovarian stimulation with gonadotropins significantly decreased oestradiol levels and increased androgen levels measured on the day of ET, no significant impact on UPF was evident. To our knowledge, this is the first published study to assess the impact on mid-luteal UPF of adjuvant letrozole treatment given to reduce the endocrine disruption that characterizes gonadotrophin ovarian stimulation of multiple follicle development.

The strengths of this study include the interventional approach, randomized allocation and the involvement of two observers each blinded to allocation.

The main limitation in this study was the restricted sample size, which risks masking minor effects, and the lack of a power calculation specifically determined for this endpoint, which can lead to the possibility of a Type II error. The direction of uterine peristalsis was not assessed in this study.

Kunz et al. (1998) assessed the effect of blocking oestrogen receptors with clomiphene citrate, which was administered to prepare the endometrium for frozen–thawed ET and led to a supraphysiological level of oestradiol, but showed no significant difference in UPF compared with the natural cycle in the early, mid- and late follicular phase.

The reliability of visual assessment of UPF by TVUS has been questioned as being highly subjective and dependent of the skills of the observers compared to MRI or invasive intrauterine pressure measurements. However, in a recent study, six observers, with no experience of visual assessment of UPF, assessed 80 TVUS recordings and substantial agreement in the measurement of UPF was reported with an inter-observer agreement of 0.68 (Kuijsters et al., 2020).

The levels of serum oestradiol, which are usually increased to supraphysiological levels during ovarian stimulation for IVF were effectively suppressed by adjuvant letrozole. While no correlation between UPF and oestradiol levels was observed in this group, the supraphysiological levels of oestradiol in the placebo group were negatively correlated with UPF.

The notion that oestradiol has a stimulatory effect on UPF derives from studies in the natural cycle during the follicular phase (Oike et al., 1990; Bulletti et al., 2000) or in the follicular phase in IVF. Kunz et al. (1998) reported supraphysiological levels of oestradiol and a higher UPF in the mid-follicular phase of IVF cycles compared to the natural cycle and a significant higher UPF in the early and mid-follicular IVF cycle. In that study, the authors also compared the natural cycle versus a hormone replacement therapy (HRT) cycle using a daily oral dose of oestradiol. While mid-follicular phase serum oestradiol levels were significant higher in the HRT group, no significant difference in late follicular phase uterine peristalsis was observed.

Previous studies have shown discrepant results regarding the impact of oestradiol on UPF in the luteal phase after ovarian stimulation. Over three studies, Fanchin et al. (1998, 2000, 2001a) observed no correlation between oestradiol levels and UPF on the day of ET in 220, 59, 43 IVF cycles, respectively. It was suggested that the uterus has a ceiling response to oestradiol in this regard, by which further increase in oestradiol will not change UPF. Zhu et al. (2012) compared UPF in natural cycles versus ovarian stimulation for IVF in a cohort study 2 days after oocyte aspiration plus 1 day after hCG trigger and found a positive correlation of oestradiol and UPF in the physiological level of oestradiol, but no correlation at the supraphysiological levels of oestradiol in contrast to this present study. However, it is not clear if the patients in this study were pooled across the natural and IVF cycle for analysis.

The mean serum progesterone level showed a trend towards a higher level in the letrozole group, which may reflect greater endogenous production secondary to reduced oestradiol suppression of LH (Bülow et al., 2022b). The majority of patients in both groups had the ET 3 days after oocyte aspiration, which meant that they had been exposed to vaginal progesterone for 2 days when blood were drawn. No significant correlation of serum progesterone and UPF was identified.

It has been previously reported that vaginal progesterone decreases the mid-luteal UPF from ~ 5 per min to under 2.5 per min after 2–4 days of vaginal progesterone in the natural cycle (Buleltti and de Ziegler, 2005). Previous studies have been inconsistent on the impact of progesterone on UPF on ET day in IVF in different research groups. Fanchin et al. (1998, 2000) observed a negative correlation of serum progesterone with UPF on ET day in 220 and 59 IVF cycles. However, the same group (Fanchin et al., 2001a) found no correlation of progesterone and uterine peristalsis in 43 IVF cycles on the day of hCG in the follicular phase, but on hCG day +4 and +7, serum progesterone correlated negatively with UPF. They also showed a significant negative correlation of progesterone with UPF on the day of ET (Day 2) in patients who started vaginal progesterone on the day of oocyte aspiration instead of on the day of ET (Fanchin et al., 2001b). Zhu et al. (2012) reported a significant negative correlation of progesterone and UPF at physiological levels of progesterone, but no correlation was shown with supraphysiological levels of progesterone 2 days after oocyte aspiration. However, what constituted a supraphysiological level of progesterone was not defined, although almost all patients had progesterone levels above the upper detection limit (>60 ng/ml) of the assay. Chung et al. (2017) showed significantly different levels of progesterone in pregnant versus non-pregnant women after fresh ET (Day 3) in a cohort study of 286 women, but there was no correlation of UPF and level of progesterone on the day of ET.

The oestradiol/progesterone ratio was significantly lower in the letrozole group but revealed no significant correlation with UPF.

Progesterone supplementation has an established role in IVF in supporting the luteal phase and implantation and is used as a standard in IVF. The findings of the present study present no reason to change recommendations regarding administration of vaginal progesterone for the purpose of decreasing UPF.

As would be expected due to the inhibition of aromatization to oestrogens by letrozole, testosterone and androstenedione levels were both significantly higher in the treatment group compared to the placebo group. Both showed a non-significant negative correlation with UPF at high levels in the placebo group only.

Adjuvant letrozole showed no significant impact on the clinical pregnancy or live birth rates. However, the study was not powered to look at these outcomes and larger studies would be required to assess this.

In conclusion, while adjuvant letrozole in gonadotropin ovarian stimulation for IVF prevents supraphysiological oestradiol levels but increases androgens, this study demonstrated no clear impact on UPF prior to fresh ET. However, given the limited study size, a minor effect of adjuvant letrozole on UPF cannot be ruled out. Large RCTs would be required to assess this and to determine any impact on clinical outcomes.

Data availability

The data are available to other researchers on reasonable request to the corresponding author.

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Authors' roles

A.K.W., S.O.S. and N.S.M. designed the study. Material preparation was performed by A.K.W. Data collection was performed by A.K.W. and M.D.H. Data analysis was performed by M.D.H., who also wrote the first draft of the manuscript. One of the authors, A.K.W. passed away before the data were analysed but had contributed substantially to this study. The rest of the authors participated in the interpretation of data, revisions and comments on the first draft of the manuscript and have read and approved the final manuscript for publication.

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Conflict of interest

The authors have no conflicts of interest to declare.

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