The role of letrozole in in vitro fertilization treatment: new remedy or old mirage?

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Aromatase inhibitors, particularly letrozole (LZ), are now established as first-line ovulation induction agents, offering an effective ovarian stimulation strategy to enhance outcomes of intrauterine insemination. In recent years, they have also emerged as potentially valuable adjuvants to gonadotropin ovarian stimulation for in vitro fertilization, particularly in fertility preservation in women with estrogen-responsive cancers. Their primary mechanism of action is to reduce the circulating estrogen levels by inhibiting androgen aromatization. Recent studies have provided evidence that this property may confer therapeutic advantages in other patients undergoing in vitro fertilization. In this study, evidence supporting the role of adjuvant LZ in poor responders, as a moderator of ovarian hyperstimulation syndrome symptoms, and an agent for improving the luteal phase after ovarian stimulation is reviewed. The use of LZ for endometrial preparation in the frozen-thawed embryo transfer cycle is also considered. (Fertil Steril® 2025;123:41–9. ©2024 by American Society for Reproductive Medicine.)

Key Words: Letrozole, aromatase inhibitor, artificial reproductive technology, IVF, ovulatory disorders

Modern fertility treatments now offer real hope to millions of couples struggling with infertility. Although ovarian stimulation regimens are now mature, opportunities to further improve outcomes by ameliorating detrimental effects associated with supraphysiological endocrine responses require exploration. Cotreatment with aromatase inhibitors, such as letrozole (LZ), represents a promising strategy. This article reviews how LZ is developing a promising role in fertility treatments beyond ovulation induction (OI), considers the clinical potential of LZ as a "multipurpose" adjuvant agent in in vitro fertilization (IVF), and presents recent evidence suggesting it to, indeed, represent a "new remedy."

MECHANISM OF ACTION

Over the past 2 decades, LZ, a third-generation nonsteroidal aromatase inhibitor, has proven to be a drug of considerable utility for OI and other fertility treatments (1, 2). Its primary mechanism involves reducing circulating estrogens by reversibly binding to the heme group of the cytochrome p450 subunit of the CYP19 aromatase enzyme, thereby potently blocking the conversion of androstenedione to estrone and testosterone to estradiol (E2). The aromatase enzyme is highly expressed in the granulosa cells of the ovaries, especially the preovulatory follicles, but is also located in the bone, placenta, brain, and adipose tissue (3–5) (Fig. 1).

The decrease in circulating estrogens results in diminished negative feedback on the hypothalamic-pituitary axis. Consequently, there is an increase in the secretion of endogenous follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which stimulates follicular growth and enhances ovulation. Letrozole also increases intraovarian androgen levels by inhibiting the aromatization of testosterone and androstenedione, working synergistically with the elevated LH levels to stimulate androgen production from pregnenolone in the theca cells (6–9). This is evident from the midfollicular phase, whereas the dehydroepiandrosterone sulfate levels remained unaffected, indicating a less significant adrenal contribution (10). The overall increase in androgen levels, along with elevated gonadotropin level and enhanced FSH receptor (FSHR) expression, likely promotes earlier follicular recruitment (11, 12).

PHARMACOKINETICS OF LZ

Letrozole is generally available as tablets (2.5 mg) that are fully absorbed from the intestine regardless of food intake. Letrozole is primarily metabolized in the liver and has a half-life of approximately 2–4 days, which is significantly shorter than that of other antiestrogens, such as clomiphene citrate (CC) (13). Once steady state is achieved (after approximately 14

Received August 30, 2024; revised October 22, 2024; accepted October 24, 2024; published online October 31, 2024.

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Fertil Steril® Vol. 123, No. 1, January 2025 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2024.10.040

days), the effect of LZ is estimated to last 9–10 days after discontinuation. However, this duration is likely shorter in fertility treatments, where the treatment duration often does not reach a steady state. An effect from follicular phase treatment on serum E2 and progesterone (P4) levels has been observed midluteal phase. However, this effect does not extend into subsequent cycles (10, 14, 15).

THERAPEUTIC INDICATIONS

Before LZ emerged as a fertility treatment option, it was primarily used in women with breast cancer as a chemotherapeutic agent because it was shown to slow or halt the growth of estrogen receptor–positive breast tumors because of its potent antiestrogenic effects (16, 17). This remains the primary context in which LZ is officially approved for use by major regulatory agencies such as the U.S. Food and Drug Administration or the European Medicines Agency. Although clinical applications in fertility have largely been adopted in an off-label setting, this has not prevented the recommendation of its use by national and international guidelines, and LZ is now an established agent across a range of fertility treatments (18, 19).

OI AND OVARIAN STIMULATION FOR INTRAUTERINE INSEMINATION

It is now almost 25 years since Mitwally and Casper (20) first proposed LZ as an OI agent acting through suppressing E2 negative feedback on the hypothalamic-pituitary axis and, thus, enhancing endogenous FSH release and stimulating follicular growth. Since these early studies, a large body of research has served to determine the place of LZ among the therapeutic repertoire for treating anovulatory or oligo-ovulatory infertility, and it is now considered by the European Society of Human Reproduction and Embryology and international guideline consensus groups to represent the first-line OI agent for anovulatory infertility (18, 21).

In couples with unexplained infertility and no clear ovulatory problem, outcomes after intrauterine insemination (IUI) or timed intercourse supported by LZ treatment and empirical LZ treatment have been studied in 14 randomized controlled trials (RCTs) and numerous observational studies. In a network meta-analysis, similar reproductive outcomes were shown when comparing LZ with CC and FSH/human menopausal gonadotropin (hMG) (22), and a similar conclusion was drawn by a Cochrane review of eight studies (23). However, mild stimulation with LZ tends to confer a lower risk of multiple pregnancies than FSH/hMG in couples with unexplained infertility (22). Although the use of LZ in these clinical contexts is not the focus of this study, its established role in OI and IUI has provided the confidence to explore the potential benefits that it may offer in IVF.

ADJUVANT LZ TO OVARIAN STIMULATION WITH GONADOTROPINS FOR IVF

In recent years, interest has grown in the role of LZ in IVF treatment. Adjuvant LZ has been proposed to mitigate the negative impact of the supraphysiological serum E2 levels with a possible positive impact on the oocyte, endometrium, and implantation (24). This property has led to its introduction as an adjuvant to FSH ovarian stimulation in women with estrogen-sensitive tumors, including breast cancer, who are undergoing fertility preservation treatment (25).

In contrast to tamoxifen or CC, which are also sometimes used as adjuvants in this context, the decreased E2 levels with LZ are obtained without affecting estrogen receptors in peripheral tissues, thus avoiding any detrimental effects on endometrium (13, 26). Letrozole has previously been shown in IVF to alter the sex steroid profile in both follicular fluid and serum, resulting in lower E2 and higher androgen levels in LZ-exposed women (10, 27). This shift in sex steroids toward higher androgens may result in increased FSHR expression (8). Furthermore, the suppressed E2 level reduces negative feedback on the hypothalamic-pituitary axis and increased the secretion of both FSH and LH (28). This may act in synergy to stimulate follicular growth and increase luteal phase endogenous LH, FSH-induced LH receptors, and P4 production (10, 14, 15). However, disrupting the endocrine milieu could have detrimental effects on reproductive functions and clinical outcomes, and most studies, thus far, have been underpowered to detect these effects.

A ROLE IN POOR RESPONDERS?

In the search for effective strategies to improve outcomes in low responders, adjuvant LZ has attracted attention. It has been proposed that by increasing precursor androgens and, hence, up-regulating follicular FSHR expression, LZ cotreatment may render recalcitrant follicles more sensitive to stimulation (8, 11, 28). Moreover, the higher endogenous FSH production generated by LZ from early in the follicular phase contributes to the total serum FSH levels and lower FSH consumption, as shown in 3 RCTs and a retrospective cohort study (10, 29–31). This endogenous production includes the acidic isoform of FSH, absent in the exogenous recombinant FSH often used (9). These mechanisms provide a rationale for adding LZ as an alternative to increasing FSH doses in poor responders (29). In our recent systematic review and meta-analysis of 20 studies (11 RCTs) in poor responders, which included 2,596 women, evidence emerged to support this notion (32). Although the included studies varied in the specific protocol applied, cotreatment with LZ was associated with a significant increase in the live birth rate (LBR) (7%; 95% confidence interval, 1%–13%) (Fig. 2) (32). There was no difference in the number of oocytes retrieved or cycle cancellations; however, there was a significant reduction in FSH consumption and stimulation days (32). A further meta-analysis found slightly improved reproductive outcomes with an increased clinical pregnancy rate with an odds ratio of 1.57 (95% confidence interval, 1.00–2.44) and reduced FSH consumption (33). A large retrospective study reported an 11% increase in LBR in younger low responders (<35 years) treated with adjuvant LZ but no difference in older women (34). To obtain a consistent suppression of the E2 levels, LZ (5 mg daily) concomitantly with FSH for at least 5 days or continued until the day of triggering final oocyte maturation can be recommended with gonadotropin release hormone (GnRH) antagonist cotreatment.

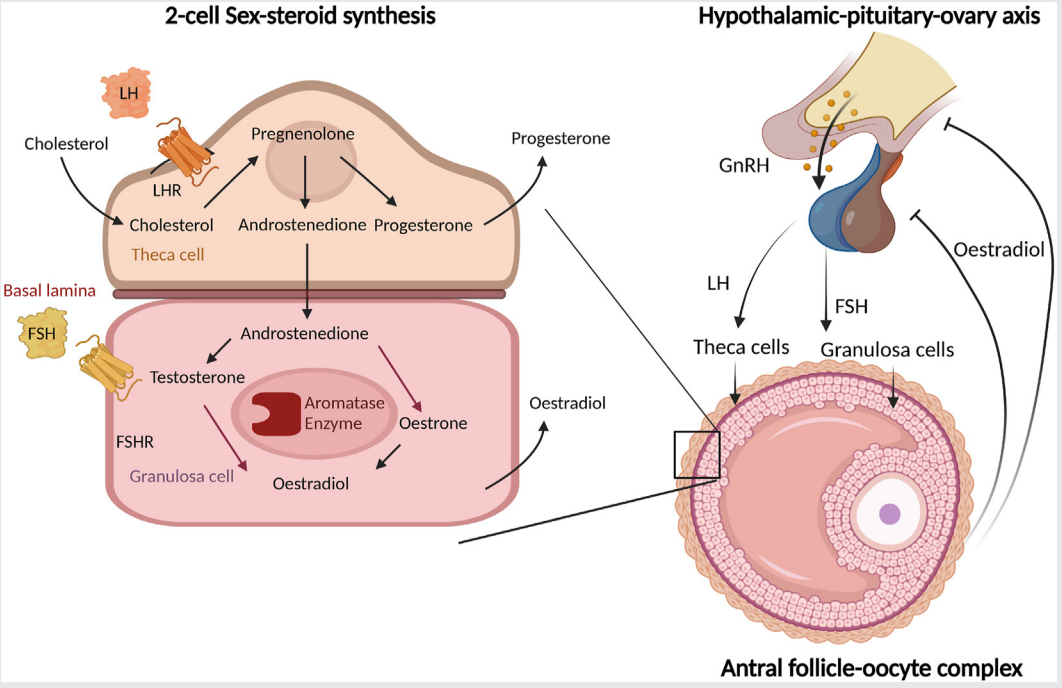
CAN LZ COTREATMENT ENHANCE THE FOLLICULAR PHASE OF EXPECTED NORMAL AND HIGH RESPONDERS?

Our recent RCT of applying adjuvant LZ to gonadotropin ovarian stimulation revealed certain novel insights into the endocrine effects of LZ cotreatment. Those randomized to receive the intervention in this double-blind placebo-controlled trial revealed higher androgen levels and endogenous FSH production during the follicular phase (Fig. 3) (10). In a subcohort, we also observed an enhanced follicular recruitment in those cotreated with LZ but no significant improvement in reproductive outcomes, although the study was underpowered for this endpoint (10, 15). This finding was consistent with our systematic review showing that the addition of LZ in normal responders resulted in nearly two more oocytes per cycle but did not significantly affect other outcomes (32). This meta-analysis, which included a limited number of studies on high responders, showed no significant effect of LZ on LBR or miscarriage rates and provided no data on other outcomes. On the basis of current evidence, there is insufficient evidence to recommend adjuvant LZ in IVF for expected normal or high responders to improve clinical outcomes (32).

CAN FOLLICULAR PHASE LZ TREATMENT ACT TO SUPPORT THE LUTEAL PHASE?

The impact of ovarian stimulation on the luteal phase has been a subject of much conjecture and research since the early days of IVF (35). Proposed mechanisms for the "inadequate luteal phase" and the need for support in IVF included prolonged suppression of the pituitary gland by GnRH agonists and the removal of granulosa cells at oocyte retrieval. However, in an intervention study published some years ago, Beckers et al. (36) provided compelling evidence to implicate high late follicular phase E2 levels as the primary disruptor. In this study, the duration of the unsupported luteal phase was found to be negatively correlated to the late follicular E2 blood level. Our group hypothesized that by preventing the supraphysiological estrogen levels from increasing, this may avoid suppression of corpus luteal function and, hence, limit the detrimental effect on luteal phase endocrinology. In turn, cotreatment with the LZ in the follicular phase may reduce the need for aggressive luteal phase support. Initial evidence supporting this hypothesis was provided by our RCT of LZ as an adjuvant for women undergoing IVF with luteal support (10, 15). Those who had received LZ cotreatment throughout the follicular phase were found to maintain high blood LH, P4, and androgen levels in the midluteal phase (Fig. 3). We then tested this hypothesis in a placebo-controlled RCT in oocyte donors who were triggered with GnRH agonist after antagonist cotreatment but received no luteal support. This study showed that when a GnRH agonist is employed to trigger final oocyte maturation, follicular phase cotreatment with LZ resulted in an extended duration of the unsupported luteal phase and a marked and significant increase in the midluteal phase P4 levels (Fig. 4) (14). Indeed, this increase was observed

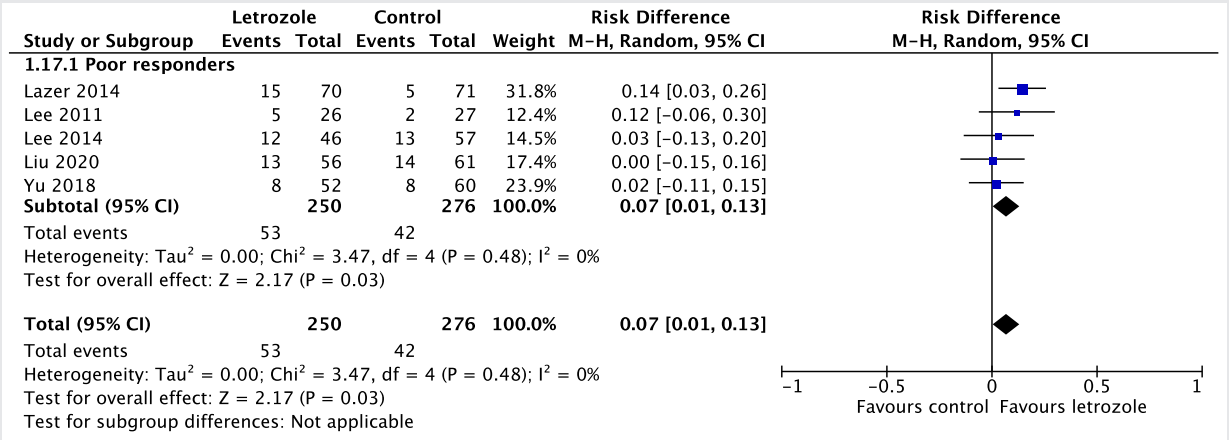
FIGURE 1



Letrozole mechanism of action. Created with BioRender. FSH = follicle-stimulating hormone; FSHR = follicle-stimulating hormone receptor; GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone; LHR = luteinizing hormone receptor.

Bülow. Letrozole in IVF. Fertil Steril 2025.

FIGURE 2



Forest plot of meta-analysis showing an increased live birth rate in poor responders after gonadotropin ovarian stimulation with adjuvant letrozole treatment. CI = confidence interval; M-H = The Mantel-Haenszel method. (Modified from Bülow et al. (32) with permission.).

Bülow. Letrozole in IVF. Fertil Steril 2025.

to be 30-fold compared with that in the group that did not receive LZ cotreatment (14). These striking findings suggest that adjuvant LZ mitigates the adverse effects of ovarian stimulation on the luteal phase and potentially decreases the necessity for supplementary luteal hormonal support in cases where a GnRH agonist is used for triggering. Clinical studies are now warranted to test this further.

OOCYTE AND EMBRYO QUALITY

It has been suggested that the relative decrease in the E2 level within the follicular compartment, which could arise in response to LZ cotreatment during ovarian stimulation, may be detrimental to oocyte development (37, 38). However, a number of studies challenge this (39, 40). Studies in fertility preservation using adjuvant LZ report not only more mature oocytes and frozen embryos but also higher abnormal fertilization rates (41, 42). A similar number and quality of oocytes and markers of embryo development were reported in both a recent systematic review and subsequent RCT (43, 44). Other studies have shown adjuvant LZ to be associated with an increase in oocytes obtained and the quality of the embryos (31, 45–48). Thus, the effect of adjuvant LZ on oocyte development in IVF treatment remains unclear. Our recent systematic review on adjuvant LZ found insufficient studies for a qualified comparison of embryo quality between LZ-exposed and nonexposed oocytes, indicating the need for further research (32).

DOES LZ OFFER A "BEST OF BOTH WORLDS" APPROACH TO ENDOMETRIAL PREPARATION FOR FROZEN-THAWED EMBRYO TRANSFER CYCLES?

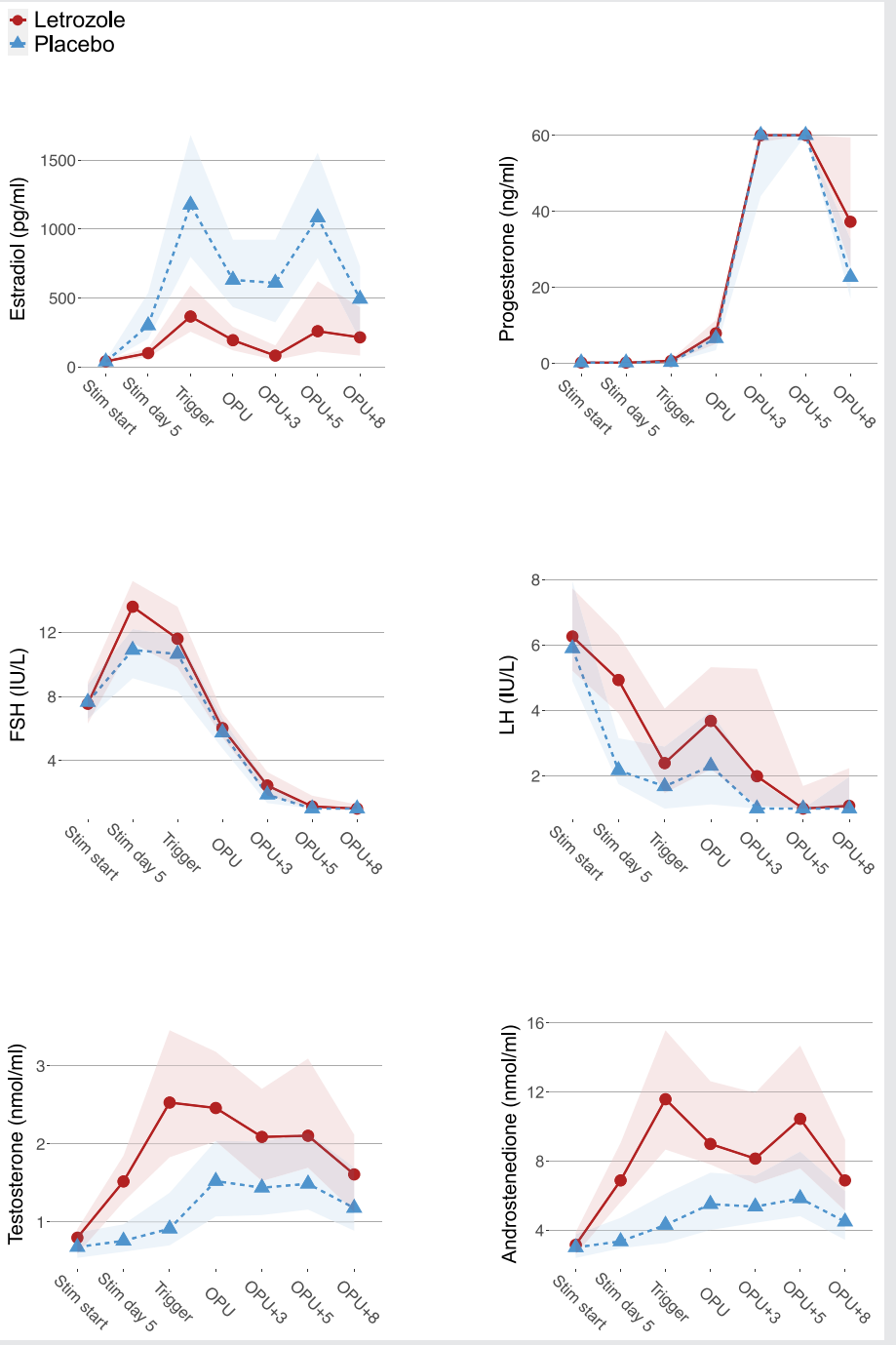
Endometrial preparation before frozen-thawed embryo transfer (FET) can be achieved through various approaches. In women with ovulatory cycles, a natural cycle FET can be employed, whereas stimulated FET using LZ or FSH/hMG can be used for women with oligoanovulation to induce follicular growth and ensure formation of a corpus luteum. Artificial cycle (AC) FET, involving E2 followed by combined E2 and P4 treatment, is widely used because of the convenience of scheduling (2, 49). However, recent studies have highlighted adverse obstetric and perinatal outcomes associated with AC FET in the absence of a corpus luteum (49, 50) suggesting that this approach should perhaps be restricted to women with premature ovarian failure undergoing oocyte donation.

The comparison of LZ vs. AC FET for women with polycystic ovary syndrome or oligoanovulation has been studied extensively in cohort studies showing an increased LBR in most cases (51–54). Perinatal outcomes with hypertensive disorders of pregnancy are mostly found lower or similar (52–55), as well as the risk of having a newborn with birth weight larger for gestational age (52, 53, 55, 56). However, the improved LBRs or ongoing pregnancy rates are not confirmed in the only two RCTs, where the LBRs/ongoing pregnancy rates are similar in the two groups (57, 58). In conclusion, LZ FET shows potential benefits in reducing perinatal complications and may emerge as an optimal means of supporting the natural cycle in oligoanovulatory women while retaining corpus luteum function. Clinical studies addressing this possibility are ongoing.

DOES COTREATMENT WITH LZ REDUCE THE RISK OF OVARIAN HYPERSTIMULATION SYNDROME?

Restricting the supraphysiological estrogen levels with LZ has been proposed as a strategy to reduce the risk of clinical consequences of ovarian hyperstimulation syndrome (OHSS). In a systematic review of the incidence of OHSS with adjuvant LZ in a long GnRH agonist protocol that included eight studies, a significantly reduced overall incidence of early OHSS in two

FIGURE 3

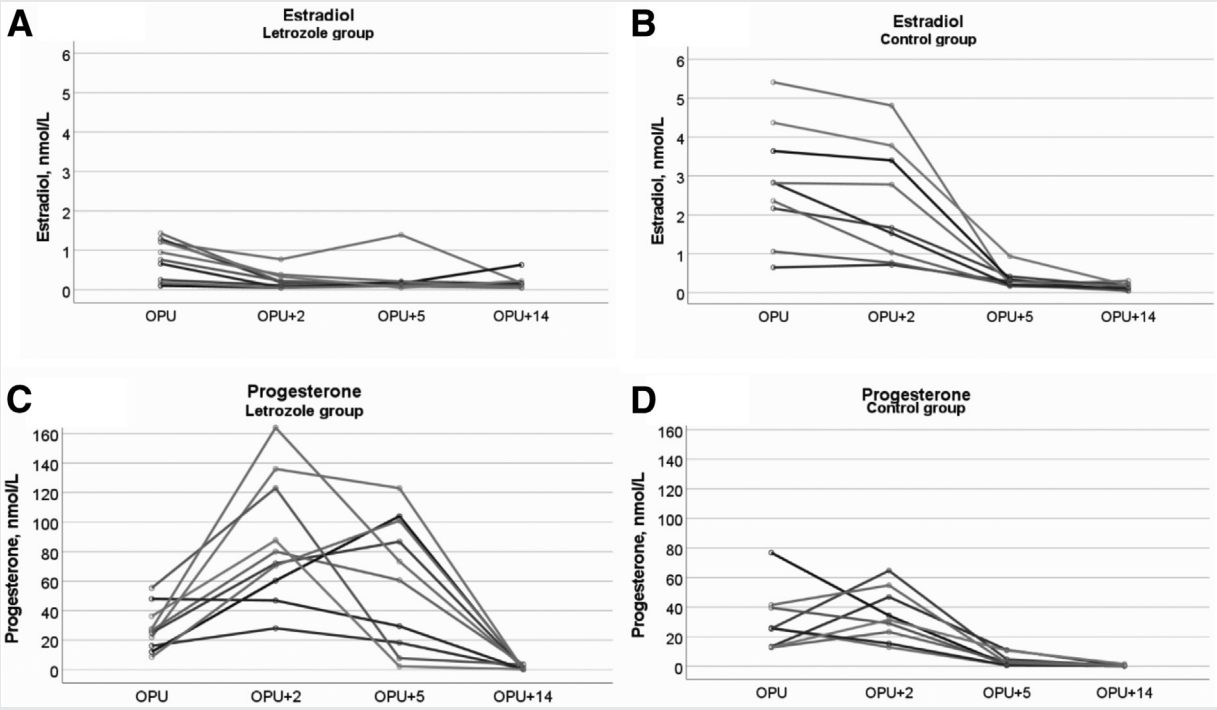


Follicular and supported luteal phase hormonal changes with adjuvant letrozole vs. placebo. Median and interquartile range. *P<0.05. FSH = follicle-stimulating hormone; LH = luteinizing hormone; OPU = oocyt pick up; stim = stimulation. (Modified from Bülow et al. (10) with permission.).

Bülow. Letrozole in IVF. Fertil Steril 2025.

studies of 566 women and pooled moderate and severe OHSS in eight studies of 1,551 women were reported. However, no difference was found when patients were categorized by mild, moderate, or severe OHSS. Because of significant heterogeneity among the included studies, LZ cotreatment for OHSS prevention is not recommended by recently updated OHSS guidelines for the American Society for Reproductive Medicine (59, 60). ClinicaTtrials.gov shows that an RCT of LZ's role in OHSS risk reduction is being performed, and the evidence base may, therefore, shift in the future.

FIGURE 4



Luteal phase estradiol and progesterone after ovarian stimulation with gonadotropins, with and without adjuvant letrozole after agonist trigger and an unsupported luteal phase. (**A**) Estradiol in the letrozole group. (**B**) Estradiol in the control group. (**C**) Progesterone in the letrozole group. (**D**) Progesterone in the control group. OPU = oocyt pick up. (Modified from Dreyer Holt et al. (14) with permission.)

Bülow. Letrozole in IVF. Fertil Steril 2025.

SIDE EFFECTS OF LZ

LZ is associated with mild and infrequent side effects, such as gastrointestinal issues, fatigue, headaches, and dizziness (61), and some reports suggest that it has a similar side effect profile to CC; however, CC is more commonly linked to a higher incidence of hot flushes (62, 63). The side effects listed in the product information for LZ primarily stem from its long-term use in postmenopausal women, making it challenging to directly apply these data to younger women who use the medication for shorter durations.

NEONATAL SAFETY

The safety of LZ treatment concerning neonatal outcomes has been a topic of concern since 2005 when a study of 150 newborns indicated an increased risk of locomotor disabilities and congenital cardiovascular malformations, despite no overall increase in malformations (64). This study faced significant criticism because of its high-risk of bias: the control group was from a tertiary hospital and excluded high-risk births, and the women were spontaneously pregnant and significantly younger and included only one twin pregnancy compared with 19 twin pregnancies in the LZ group. This led to widespread abandonment of LZ use by health authorities, restricting its use to controlled trials.

Subsequent research provided more reassuring results. A large retrospective study involving 911 children and a systematic review of 4,697 children born after LZ treatment for IUI found no increase in major congenital malformations compared with other fertility treatments (65, 66). Furthermore, published data of FET cycles using LZ show no increase in the proportion of congenital malformations, with a total of 5 of 1,181 newborns after LZ treatment, which was not significantly different from the AC preparation before FET, although the numbers in both groups are suspected to be underreported compared with the congenital malformation rates in naturally conceived pregnancies (52, 54, 56, 67). Despite the first study of LZ in IVF being published in 2004, only one retrospective study of adjuvant LZ in IVF in obese women has specifically addressed neonatal malformations and found no malformations in either group (36 LZ-treated women vs. 21 controls) (68). This forces clinicians to rely on IUI and FET data for safety extrapolations. Although these findings are promising, the dose and timing of adjuvant LZ in IVF differ, underscoring the need for further research on neonatal outcomes in this specific context.

Participants in our recent RCT (10) consented to the screening of their resulting offspring's medical histories after birth. The findings showed that all children had a normal weight for gestational age, and no neonatal malformations were recorded within 1 month after birth, although these data remain unpublished. This provides preliminary evidence supporting the safety of adjuvant LZ in IVF but highlights the necessity for more comprehensive studies to confirm these outcomes.

TABLE 1

Potential roles of letrozole in assisted reproductive technology.

Patient groups	Insemination	FET cycles
Ovulatory women	Higher LBR with LZ than with CC, equal to FSH	No benefit compared with natural cycles
Oligoanovulatory women	Higher LBR with LZ than with CC, equal to FSH	Higher LBR with LZ than with AC FET Lower HDP and lower risk of LGA children with LZ than with AC FET
	Adjuvant letrozole to FSH for ovarian stimulation for IVF	
Poor responders	Higher LBR, the highest benefit in younger women	
Normal responders	Similar LBR Evidence for improved luteal phase	
High responders	Similar LBR Awaiting a larger study of OHSS risk reduction	

Note: AC = artificial cycle; CC = clomiphene citrate; FET = frozen-thawed embryo transfer; FSH = follicle-stimulating hormone; HDP = hypertensive disorders of pregnancy; IVF = in vitro fertilization; LBR = live birth rate; LGA = large for gestational age; LH = luteinizing hormone; LZ = letrozole; OHSS = ovarian hyperstimulation syndrome.

Bülow. Letrozole in IVF. Fertil Steril 2025.

COST-EFFECTIVENESS OF LZ

Fertility treatments can be financially burdensome, and the cost of LZ is significantly lower than that of other fertility medications; hence, LZ offers a more affordable alternative for OI. Additionally, the observed increase in FSH levels and decreased FSH consumption with adjuvant LZ have the potential to reduce costs in IVF. This could be particularly advantageous for patients with a poor ovarian response (13). The considered indications and current evidence for the comparative efficacy of LZ are briefly summarized in Table 1.

CONCLUSION

Aromatase inhibitors, particularly LZ, represent an important therapeutic option in the management of ovulatory disorders and unexplained infertility with insemination. In addition, LZ can induce follicular growth, producing E2 for endometrial preparation before FET and after ovulation, securing a corpus luteum during pregnancy with a similar LBR and lower perinatal complication. Much of this review has focused on the potential advantages of incorporating adjuvant LZ into ovarian stimulation regimens for IVF. Recently published evidence indicates that it may have a more central place in treatment than hitherto recognized. Benefits appear to include an increased LBR in poor responders and a means of normalizing the luteal phase after ovarian stimulation without the need for burdensome luteal support. Further clinical studies will elucidate whether this is a mirage or, indeed, a new remedy.

CRediT Authorship Contribution Statement

Nathalie Bülow: Software; Investigation, Writing – original draft. **Nick Macklon:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Interests

N.B. has nothing to disclose. N.M. has nothing to disclose.

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