Clomiphene citrate versus letrozole for ovarian stimulation: a pilot study

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Abstract

The purpose of this pilot study was to compare the endocrinological environment of cycles stimulated with clomiphene citrate (CC) or letrozole. Fifteen patients undergoing intrauterine insemination (IUI) received from day 3 to day 7 of the cycle either letrozole 2.5 mg/day (n = 7) or clomiphene citrate 100 mg/day (n = 8). IUI was performed one day after the detection of LH peak. No luteal support was administered. Significantly lower serum oestradiol concentrations were present in the follicular phase on days 9, 13 and 15 of the cycle and in the luteal phase on days 3 and 6 post-IUI in the letrozole group compared with those in the CC group. Progesterone concentrations and oestradiol concentrations were significantly lower in the letrozole group than in the CC group on the day of LH peak. Significantly more follicles developed in patients in the CC group compared with those in the letrozole group. In conclusion, significantly lower oestradiol concentrations and fewer follicles are observed in cycles stimulated with 2.5 mg letrozole compared with cycles stimulated with 100 mg CC from day 3 to day 7 of the cycle.

Keywords: aromatase inhibitor, clomiphene citrate, letrozole, normo-ovulatory, ovarian stimulation

Introduction

Clomiphene citrate (CC) is a safe and cost-effective medication, used as a first choice treatment in World Health Organization (WHO) group II patients (Kolibianakis and Devroey, 2002). It exerts its ovulation-inducing effect by affecting both the gonadotrophin releasing system and the follicular apparatus of the ovary (Cohen, 2003). As an alternative to CC, the aromatase inhibitor letrozole appears to be promising for ovarian stimulation. Letrozole has so far been used in patients with an inadequate response to CC (Mitwally and Casper, 2001) and has been shown to improve the ovarian response to FSH in poor responders (Mitwally and Casper, 2002).

Currently no data exist on the endocrine environment of the follicular phase following letrozole administration from day 3 to day 7 following menstruation. This information is necessary before adopting letrozole for widespread use in ovarian stimulation (Mitwally and Casper, 2003). The purpose of the present study was to compare the endocrinological environment of cycles stimulated from day 3 to day 7 with either 100 mg CC or 2.5 mg letrozole.

Materials and methods

Patient population

Fifteen couples presenting with primary or secondary infertility at the Centre for Reproductive Medicine of the Dutch-Speaking Free University Brussels between September 2001 and August 2002 were included in the study.

Inclusion criteria were: age ≤39 years; body mass index (BMI) between 18 and 29 kg/m²; presence of ovulatory cycles with duration between 24 and 35 days; day 3 FSH concentrations ≤12 IU/l; normal liver and kidney function; negative history for tubal pathology; and normal semen analysis of the partner. Ovulation was confirmed in the cycle preceding stimulation by measuring progesterone on day 21 and by performing ultrasound in the midluteal phase.
Patients were randomized by a computer-generated list to receive either letrozole (Femara®; Novartis Pharmaceuticals, Brussels, Belgium) \((n = 7)\) or clomiphene citrate (Clomid®; Hoechst Marion Roussel, Belgium) \((n = 8)\) for ovarian stimulation. Patients could enter the study only once. The research project was approved by the Institutional Review Board at the centre. The median age of the patients in the letrozole and the CC group was 28.9 years (interquartile range 8.6 years) and 28.2 years (interquartile range 10.0 years), respectively.

**Ovarian stimulation and intrauterine insemination**

Patients received either 2.5 mg/day letrozole or 100 mg/day CC from day 3 to day 7 of the cycle. No ovulation triggering was used and intrauterine insemination (IUI) was performed one day following the detection of the LH peak. LH peak was considered to be present when LH concentrations became three times higher than the concentration observed in the previous 24 h (Frydman et al., 1982). Sperm preparation and IUI procedures have been described previously (Hoing et al., 1986). No luteal phase support was administered.

**Hormonal analysis and ultrasound monitoring**

Endocrine screening included serum assay of FSH, LH, progesterone and oestradiol on day 3 and on day 9 of the cycle. From day 9 onwards, and until the LH peak, daily blood sampling was performed. In order to confirm pregnancy, serum human chorionic gonadotrophin (HCG) was assessed on days 12 and 16 post-IUI.

Serum LH, FSH, HCG, oestradiol and progesterone were measured with the automated Eleeys immunoanalyser (Roche Diagnostics, Mannheim, Germany). Intra-assay and inter-assay coefficients of variation (CV) were <3 and <4% for LH, <3 and <6% for FSH, <5 and <7% for HCG, <5 and <10% for oestradiol and <3 and <5% for progesterone, respectively.

Ultrasound assessment of endometrium and follicular development was performed on day 9. Thereafter, ultrasound was performed on alternate days. Ultrasound was in addition performed in the midluteal phase to confirm ovulation. Endometrial thickness was measured at the thickest longitudinal plane.

**Statistical analysis**

Continuous variables were analysed with the Mann–Whitney U-test. A \(P\)-value of <0.05 is considered significant. Values are expressed as median plus interquartile range (IQT range).

**Results**

**Follicular phase**

A tendency for shorter duration of follicular phase was present in the letrozole group than in the CC group (13.0 days–IQT range 5.0 versus 15.0 days–IQT range 7.0, respectively). Similar FSH concentrations were present between the two groups compared (Figure 1), while oestradiol concentrations were significantly lower in the letrozole group compared with those in the CC group on cycle days 9, 13 and 15 (Figure 2).

**Day of LH surge**

An LH surge was observed in all patients in both groups. Median LH peak concentration was 43.0 IU/l in the letrozole group (IQT range 24.0) and 23.0 IU/l in the CC group (IQT range 11.9). Moreover, on the day of LH peak, median FSH concentrations were 10.0 IU/l in the letrozole group (IQT range 13.0) and 8.4 IU/l in the CC group (IQT range 6.5).

Oestradiol concentrations on the day of LH peak were significantly higher in the CC group compared with those in the letrozole group (255 pg/ml–IQT range 218 versus 768 pg/ml–IQT range 1179 respectively; \(P < 0.05\)).

In addition, on the same day progesterone concentrations were

![Figure 1. Box plot of FSH concentrations in the letrozole and clomiphene citrate (CC) groups during the follicular phase (differences are not significant).](image1)

![Figure 2. Box plot of oestradiol concentrations in the letrozole and clomiphene citrate (CC) groups during the follicular phase.](image2)
significantly higher ($P < 0.04$) in the CC group (2.1 ng/ml–IQT range 1.3) than in the letrozole group (0.8 ng/ml–IQT range 1.0). A similar endometrial thickness was present in the CC group (8.3 mm–IQT range 2.6) and the letrozole group (8.0 mm–IQT range 1.4).

**Luteal phase**

Significantly lower oestradiol concentrations were present in the letrozole group than in the CC group on days 3 and 6 post-IUI (**Figure 3**). Progesterone concentrations during the same period in the letrozole group and CC group are shown in **Figure 4**.

**Cycle outcome**

Significantly more follicles with a diameter of ≥17 mm ($P < 0.03$) were present on the day of LH rise in the CC group (2.0–IQT range 1.7) compared with the letrozole group (1.0–IQT range 1.0). Two ongoing pregnancies were achieved in the letrozole group and three in the CC group.

**Discussion**

This pilot study investigated the endocrinological profile of normo-ovulatory women in cycles stimulated with 2.5 mg letrozole versus 100 mg CC administered from day 3 to day 7 following menstruation. It was shown that significantly lower oestradiol concentrations and fewer follicles could be expected in the letrozole group compared with the CC group.

In a randomized study, Fisher et al. (2002) compared the administration of CC and letrozole for IUI. Patients received either letrozole 2.5 mg/day or CC 50 mg/day from day 5 to day 9 of the cycle. In that study it was suggested that more follicles could be expected in the CC-stimulated cycles compared with the letrozole-stimulated cycles (2.2 versus 1.7, respectively), although by contrast to the present study the difference observed did not reach statistical significance. This could be attributed to the higher dose of CC used in the current study.

However, in agreement with the present study, endometrial thickness at midcycle was comparable between the two groups. Whether differences in endometrial thickness are present between the two groups if HCG is used to time IUI in the presence of a dominant follicle remains to be assessed. It should be noted, however, that the predictive value of endometrial thickness for pregnancy achievement in cycles stimulated with CC has recently been questioned (Kolibianakis et al., 2003b).

According to the two-cell two-gonadotrophin theory (Falck, 1959), stimulation of LH receptors in theca cells results in androgen production and, in turn, oestradiol synthesis by the granulosa cells via the action of FSH. Moreover, FSH is able to induce LH receptors in granulosa cells (Zeleznik et al., 1974), a process enhanced by oestrogen (Kessel et al., 1985). The stimulation of these receptors by LH results in an increase in steroidogenesis (Erickson et al., 1979), as in the developing follicle the aromatase system becomes directly responsive to FSH as well as to LH (Zeleznik and Hillier, 1984). The lower oestradiol concentrations observed during the follicular phase in the letrozole group compared with the CC group are probably attributed to the inhibitory effect of letrozole on aromatase activity. Letrozole is known to reduce the systemic production of oestrone from androstenedione by up to 80% (Sinha et al., 1998).

However, the significantly lower progesterone and oestradiol concentrations observed in the letrozole group than in the CC group at the time of LH peak are probably due to the decreased number of follicles present in the letrozole group on that day.

Similarly, the lower oestradiol concentrations observed in the luteal phase of letrozole cycles compared with those in the CC cycles might also be attributed to the smaller number of corpora lutea present in the letrozole group, as a consequence.
of the decreased number of follicles developed in the letrozole group.

Induction of ovulation by letrozole is thought to be mediated by an increased secretion of gonadotrophins due to inhibition of oestrogen negative feedback on the hypothalamus (Sinha et al., 1998; Shetty et al., 1997). Although in the current study FSH concentrations on days 3, 9 and 13 in the two groups were similar (Figure 1), a difference in FSH concentrations among the two treatment schemes between days 3 and 9 cannot be excluded, as no blood samples were taken during this period of the cycle.

Additional modes of letrozole action have been described. When administered to cycling bonnet monkeys, letrozole resulted in a suppression of oestriadiol concentrations, with a corresponding increase in serum androstenedione (Shetty et al., 1997). The increased androgens might stimulate FSH receptor gene expression (Mitwally and Casper, 2001; Shetty et al., 1997), which could in turn result in enhanced sensitivity of the developing follicles to serum FSH values.

The latter mechanism suggests a role of letrozole in ovarian stimulation for IVF/embryo transfer cycles. Letrozole could probably result in a decreased requirement for gonadotrophins. In addition, its use could be associated with reduced oestriadiol concentrations during the follicular phase. This might be important, as exposure to high concentrations of oestradiol in the early follicular phase of gonadotrophin-releasing hormone antagonist (GnRHa) cycles has been associated with a reduced chance of pregnancy (Kolibianakis et al., 2003a).

In conclusion, lower oestradiol concentrations and fewer follicles can be expected in cycles stimulated with 2.5 mg letrozole, compared with those stimulated with 100 mg CC, from day 3 to day 7 of the cycle in normo-ovulatory women. A comparative study between letrozole and CC in anovulatory women is necessary to further investigate the role of these two drugs.

Acknowledgements

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References


Kolibianakis EM, Albano C, Kahn J et al. 2003a Exposure to high levels of luteinizing hormone and estradiol in the early follicular phase of gonadotropin-releasing hormone antagonist cycles is associated with a reduced chance of pregnancy. Fertility and Sterility 79, 873–880.


Mitwally MF, Casper RF 2002 Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders. Fertility and Sterility 77, 776–780.


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