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Sequential use of letrozole and gonadotrophin in women with poor ovarian reserve: a randomized controlled trial

Vivian Chi Yan Lee *, Carina Chi Wai Chan, Ernest Hung Yu Ng, William Shu Biu Yeung, Pak Chung Ho

Department of Obstetrics and Gynecology, The University of Hong Kong, Hong Kong Special Administrative Region, Queen Mary Hospital, Pokfulam Road, People's Republic of China
* Corresponding author. *E-mail address:* v200lee@hku.hk (VCY Lee).

Dr Vivian Lee is a resident specialist in the Reproductive Medicine team in the Department of Obstetrics and Gynaecology, Queen Mary Hospital. Her main research interest includes the use of letrozole in assisted reproduction technology and medical abortion.

Abstract Sequential use of letrozole and human menopausal gonadotrophin (HMG) was compared with HMG only in poor ovarian responders undergoing IVF. Patients ($n = 53$) with less than four oocytes retrieved in previous IVF cycles or less than five antral follicles were randomized to either letrozole for 5 days followed by HMG or HMG alone. The letrozole group had lower dosage of HMG ($P < 0.001$), shorter duration of HMG ($P < 0.001$) and fewer oocytes ($P = 0.001$) when compared with controls. Live-birth rate was comparable with a lower miscarriage rate in the letrozole group ($P = 0.038$). Serum FSH concentrations were comparable in both groups except on day 8, while oestradiol concentrations were all lower in the letrozole group from day 4 (all $P < 0.001$). Follicular fluid concentrations of testosterone, androstenedione, FSH and anti-Müllerian hormone were higher in the letrozole group ($P = 0.009$, $P = 0.001$, $P = 0.046$ and $P = 0.034$, respectively). Compared with HMG alone, sequential use of letrozole and HMG in poor responders resulted in significantly lower total dosage and shorter duration of HMG, a comparable live-birth rate, a significantly lower miscarriage rate and a more favourable hormonal environment of follicular fluid.

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Introduction

IVF is an effective treatment for various causes of subfertility. It involves ovarian stimulation for multiple follicular development, oocyte retrieval and embryo transfer after fertilization. Multiple embryos are usually transferred to compensate for their low implantation potential, which has remained steady at 20–25% despite recent advances in

ovarian stimulation, gamete handling, assisted fertilization and embryo culture. The success of embryo cryopreservation also makes it desirable to obtain multiple embryos to allow an increased number of embryo transfers, thus increasing the cumulative pregnancy rates (Wang et al., 1994).

Therefore, the development of multiple follicles in response to gonadotrophin stimulation is usually considered as one of the key factors leading to a successful outcome. Poor ovarian response has usually been associated with low pregnancy rates and many of these cycles are cancelled without proceeding to oocyte retrieval (Keay et al., 1997). The management of poor ovarian responders has been extensively reviewed (Fasouliotis et al., 2000; Karande and Gleicher, 1999; Keay et al., 1997; Mahutte and Arici, 2002; Surrey and Schoolcraft, 2000; Tarlatzis et al., 2003) but remains a great challenge in assisted reproduction technology.

Letrozole, a third-generation reversible aromatase inhibitor, has been tried in poor responders undergoing IVF treatment. It inhibits the aromatization of androgen into oestrogen, which in turn reduces the negative feedback and results in an increase in gonadotrophins, which is the underlying mechanism of its use in ovulation induction (Mitwally and Casper, 2001). The sequential use of letrozole and gonadotrophins was reported to induce more mature follicles in intrauterine insemination cycles (Mitwally and Casper, 2002). A few studies (Davar et al., 2010; Garcia-Velasco et al., 2005; Goswami et al., 2004; Ozmen et al., 2009; Schoolcraft et al., 2008; Verpoest et al., 2006; Yarali et al., 2009) on the use of letrozole in poor ovarian responders undergoing IVF treatment have been reported but their results are not consistent.

This randomized trial was conducted to compare the sequential use of letrozole and human menopausal gonadotrophin (HMG) with HMG only in poor ovarian responders or women with poor ovarian reserve undergoing IVF treatment.

Materials and methods

Study population

Subfertile patients attending the Centre of Assisted Reproduction and Embryology, The University of Hong Kong—Queen Mary Hospital were recruited if they had less than four oocytes retrieved in their previous failed IVF cycles or were found to have less than five antral follicles as assessed during early follicular phase within 2 months preceding the IVF treatment cycle. Those aged above 40 or having major medical illness, such as severe hepatic or renal impairments, were excluded.

Consecutive women who fulfilled the selection criteria and were willing to participate were counselled and recruited. They gave an informed written consent prior to participating in the study, which was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. The trial was registered with HKClinicalTrials.com (number HKCTR-1158). Subjects were recruited to join the study only once and did not receive any monetary compensation for participation in the study.

Randomization and treatment regimen

Eligible women were recruited on day 2 of the treatment cycle and underwent an ultrasound scan to exclude the presence of ovarian cysts. They were then randomized according to a computer-generated list in sealed, opaque envelopes into two groups: the letrozole and control groups. The sequence of allocation was concealed until the interventions were assigned by the research nurse after the patients were recruited by an investigator. In the letrozole group, women received letrozole (Femara; Norvatis, East Hanover, NJ, USA) 2.5 mg daily from day 2 to day 6 for 5 days. This was followed by a fixed daily dose of intramuscular injection of 225 IU HMG (Menogon; Ferring, Kiel, Germany) until the day of human chorionic gonadotrophin (HCG) administration. In the control group, women were given a fixed daily dose of 225 IU HMG from day 3 onwards. Ovarian response was monitored by serial transvaginal scanning and hormonal assays from day 6 onwards. Ganirelix 0.25 mg (Orgalutran; Organon Laboratories Ltd., United Kingdom) was administered when the leading follicle was $\geq$12 mm in diameter. HCG at a dose of 10,000 IU (Profasi; Serono, Geneva, Switzerland) was given if at least one follicle was $\geq$18 mm in diameter. Oocyte retrieval was scheduled 36 h after the HCG injection.

Oocyte retrieval was performed using a 16-gauge double-channel needle (Cook IVF, Cook, Australia) under ultrasound guidance with a 5 MHz vaginal probe fitted with a needle guide. The double-channelled needle allowed aspiration and flushing of follicles >10 mm from both ovaries. Follicular fluid of the first mature follicle on both sides not contaminated with blood was collected for hormonal analysis using medium-free collection tubes. Each follicle was flushed once with culture media and the fluid obtained from the aspriation. Flushing was examined by an embryologist. The retrieved oocytes were inseminated conventionally or by intracytoplasmic sperm injection as indicated. Fertilization was checked 14—18 h after the insemination. An oocyte was considered to be normally fertilized when two pronuclei were visible. When no pronucleus or only one pronucleus was visible, the oocyte was cultured for another 3—4 h and examined again. A maximum of two normally cleaving embryos were transferred to the uterine cavity 2 days after the retrieval.

Blood was taken for determination of serum oestradiol, progesterone, FSH and LH concentrations on alternate days from 2 days after HCG to the day of oocyte retrieval. Serum oestradiol and progesterone concentrations were checked 10 days after HCG. Follicular fluid was assayed for oestradiol, progesterone, FSH, LH, anti-Müllerian hormone (AMH), total testosterone, androstenedione and inhibin B concentrations. The sensitivity, intra-assay and inter-assay coefficients of variation for hormonal assays are summarized in Table 1. Urine pregnancy test was performed 20 days after HCG.

Ongoing pregnancies were those pregnancies beyond 10—12 weeks of gestation, at which stage the patients were referred out for antenatal care. Live birth was defined as the delivery of a live newborn after 24 gestational weeks.

Table 1 Details of hormone assays.

Laboratory tests	Limit of detection	Intra-assay CV (%)	Inter-assay CV (%)	Manufacturer
Oestradiol (pmol/l)	73	4.0	5.0	Beckman Coulter
Progesterone (nmol/l)	0.25	4.4	3.6	Beckman Coulter
FSH (IU/l)	0.2	3.1	5.4	Beckman Coulter
LH (IU/l)	0.2	3.6	4.3	Beckman Coulter
Testosterone (ng/ml)	0.04	4.8—6.8	2.8—4.9	Diagnostic Systems Laboratories
Androstenedione (ng/ml)	0.03	7.8—10.3	6.3—9.2	Diagnostic Systems Laboratories
AMH (pmol/l)	1	0—11.4	0—14	Immunotech Developments
Inhibin B (pg/ml)	7	3.5—5.6	6.2—7.6	Diagnostic Systems Laboratories

AMH = anti-Müllerian hormone; CV = coefficient of variation.

Outcome measures

The primary outcome measure of the study was the number of oocytes retrieved. The secondary outcome measures were serum and follicular fluid hormone concentrations, ongoing pregnancy rate, live-birth rate, cumulative live-birth rate, including the live births resulting from the transfer of frozen—thawed embryos, and total dosage and duration of stimulation with gonadotrophins.

Statistical analysis

It has been shown previously that when the antral follicle count was less than 5, the mean number of oocytes
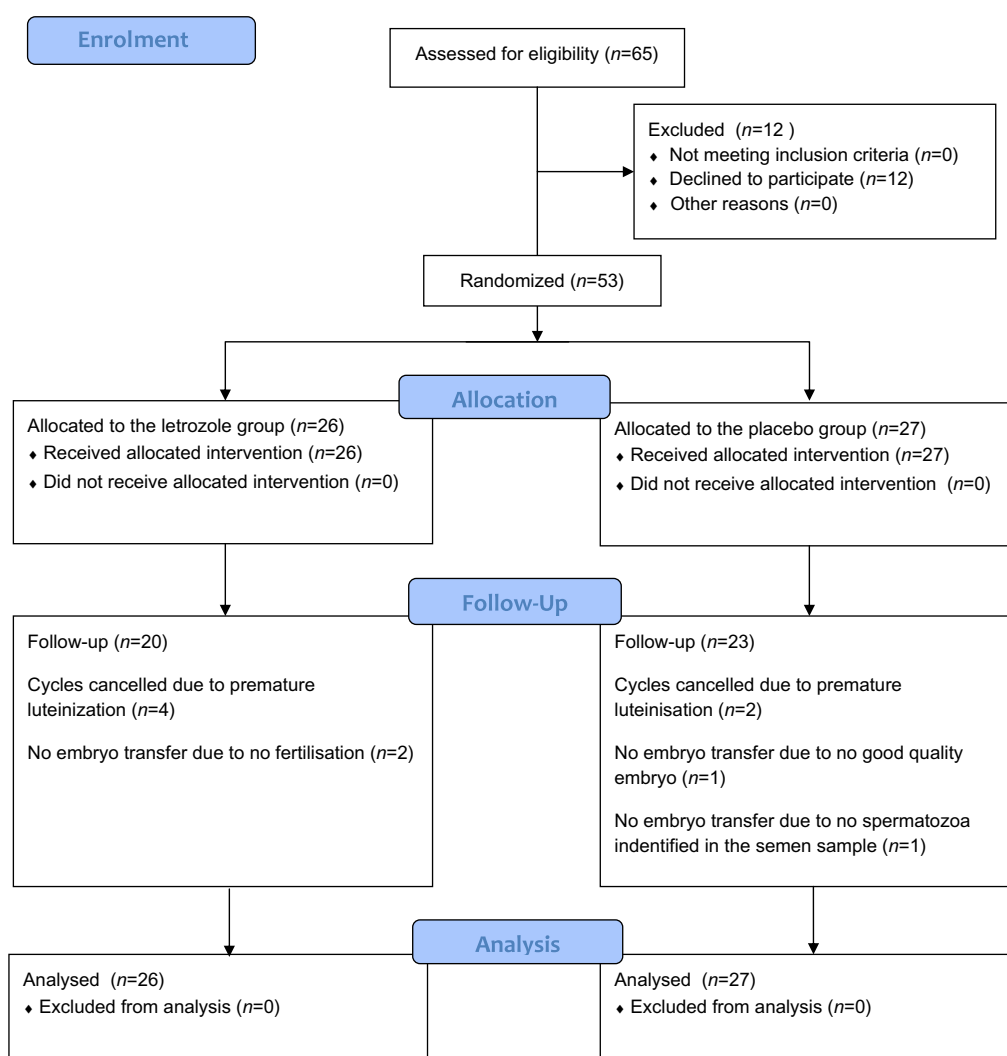

Figure 1 Flow diagram of patient participation in the trial.

Table 2   Demographic data.

	Letrozole (n = 26)	Control (n = 27)
Age (years)	37.3 ± 2.4	36.2 ± 2.2
Duration of subfertility (years)	5.3 ± 2.5	4.7 ± 2.0
Body mass index (kg/m^2)	21.0 ± 2.3	20.7 ± 2.1
Primary subfertility	18 (69.2)	22 (81.5)
Cause of subfertility		
Tubal	6 (23.0)	4 (14.8)
Endometriosis	3 (11.5)	8 (29.6)
Male	10 (38.5)	11 (40.7)
Unexplained	3 (11.5)	2 (7.4)
Mixed	4 (15.4)	2 (7.4)
Smoking status	1 (3.8)	1 (3.7)

Values are mean ± standard deviation or n (%). There were no statistically significant differences between the two groups.

retrieved was 4.3 (Ng et al., 2000). Co-administration of letrozole improved the ovarian response, from a mean number of follicles from 1.9 to 3.3 (Mitwally and Casper, 2002). If the same improvement is assumed for the number of oocytes, then co-administration of letrozole would increase the mean number of oocytes in this study's patients from 4.3 to 7.5. In order to detect such a difference with a power of 80% at 5% significance level, 33 patients would be needed in each group. This study aimed to recruit 35 patients in each group to allow for patients who might drop out after recruitment.

The Kolmogorov–Smirnov test was used to test the normal distribution of continuous variables. Results of continuous variables were given as mean ± standard deviation (SD) if normally distributed and as median (range) if not normally distributed. Statistical comparison was carried out by Student's t-test, Mann–Whitney U-test, Wilcoxon signed ranks test for continuous variables and chi-squared test or Fisher's Exact test for categorical variables, where appropriate. Statistical analysis was performed using the Statistical Package for Social Sciences version 17.0 (SPSS, Chicago,

USA). The two-tailed P-value <0.05 was considered statistically significant.

Results

Study population

During the study period between 1 September 2005 and 30 September 2009, 65 consecutive eligible patients were approached and 53 patients were recruited. The study was prematurely terminated before reaching the pre-defined sample size due to recruitment difficulties. Twenty-six subjects were randomized to the letrozole group while 27 subjects were in the control group (Figure 1). There was no adverse event in both groups leading to cessation of treatment cycles.

The demographic data are shown in Table 2. There were no significant differences in age of women, duration of subfertility, body mass index, the percentage of primary subfertility, the cause of subfertility and the smoking status between the letrozole and control groups.

Table 3   Comparison of outcomes.

	Letrozole (n = 26)	Control (n = 27)	P-value
Dosage of gonadotrophins (IU)	1507.5 ± 570.0	2422.5 ± 435.0	<0.001
Duration of stimulation (days)	6.4 ± 2.5	10.2 ± 2.6	<0.001
Antagonist dosage (IU)	4.7 ± 1.8	4.9 ± 2.1	NS
Total follicles aspirated	3.0 (0–11)	5.0 (0–26)	0.001
Oocytes retrieved	2.0 (0–4)	4.0 (0–19)	0.001
Embryos cleaved	1.0 (0–3)	2.0 (0–15)	0.020
Embryos transferred	1.0 (0–2)	2.0 (0–3)	NS
Cancellation rate (due to premature luteinization)	4 (15.0)	2 (7.4)	NS
Cancellation rate (all causes)	6 (23.1)	4 (14.8)	NS
Implantation rate	5/23 (21.7)	5/36 (13.9)	NS
Ongoing pregnancy rate	5 (19.2)	2 (7.4)	NS
Live-birth rate	5 (19.2)	2 (7.4)	NS
Miscarriage rate	0 (0)	3/5 (60.0)	0.038

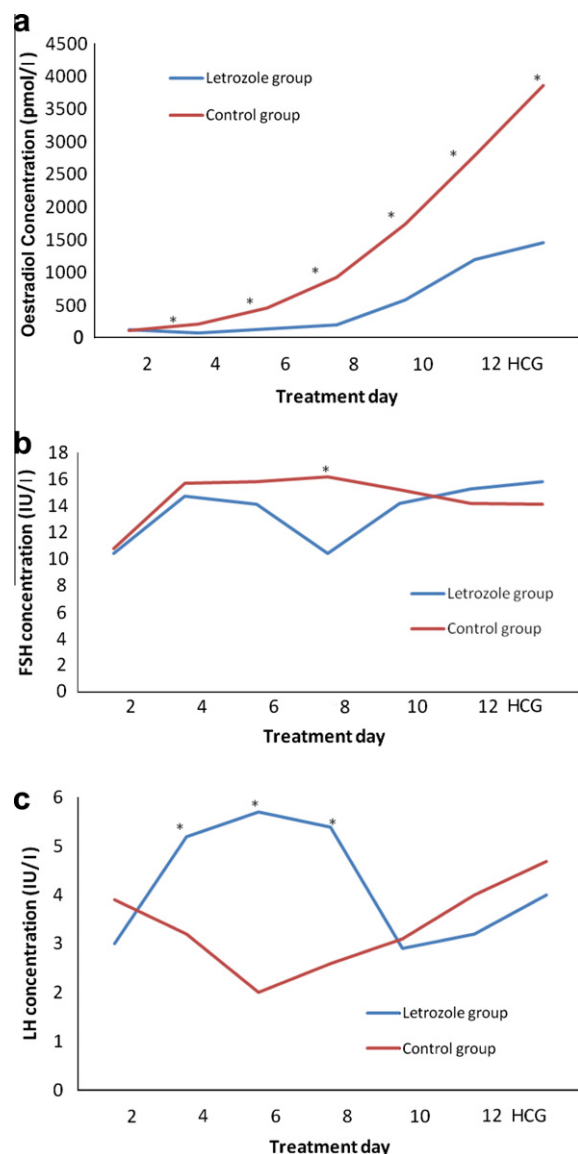Values are mean ± standard deviation, median (range) or n (%). NS = not statistically significant.

Figure 2 Serum hormone concentrations on various days of ovarian stimulation. (**A**) Oestradiol concentrations of the letrozole group were significantly lower than the control group from day 4 to the day of HCG (all *P*-values < 0.001). (**B**) FSH concentrations of the letrozole and control groups were comparable on most days, except on day 8 when FSH concentration of the letrozole group was significantly lower than that of the control group (*P* < 0.001). (**C**) LH concentrations of the letrozole group were significantly higher than the control groups on days 4, 6 and 8 (*P* = 0.018, *P* < 0.001 and *P* = 0.018, respectively). HCG = human chorionic gonadotrophin; *signifi-cantly different values.

Outcomes

The letrozole group used a significantly lower dose of HMG and had a significantly shorter duration of HMG when compared with the control group (Table 3). The duration of gonadotrophin-releasing hormone antagonist was compara-ble in both groups. The number of oocytes retrieved was sig-nificantly lower in the letrozole group than that in the

control group (*P* = 0.001) but the number of embryos trans-ferred was similar for the two groups. The cancellation rate, implantation rate, ongoing pregnancy rate, live-birth rate and cumulative live-birth rate (23.1% (6/26) versus 7.4% (2/27)) were comparable for both groups. A significantly lower miscarriage rate was noted in the letrozole group than the control group (0% versus 60%, respectively; *P* = 0.038).

Serum and follicular fluid hormonal profiles

Serum oestradiol concentration of the letrozole group was significantly lower than that of the control group from day 4 to the day of HCG administration. FSH concentrations of the letrozole and control groups were comparable on most of the days, except on day 8 when the FSH concentration of the letrozole group was significantly lower than that of the control group. Serum LH concentration was significantly higher in the letrozole group than that in the control group from day 4 to day 8 (Figure 2).

Follicular fluids were collected in 36 patients (18 in the letrozole group and 18 in the control group). Follicular FSH, testosterone, androstenedione and AMH concentrations were significantly higher in the letrozole group than those in the control group (*P* = 0.046, *P* = 0.009, *P* = 0.001 and *P* = 0.034, respectively; Table 4). Other hormones were sim-ilar between the two groups.

Discussion

Results of this randomized study confirmed that sequential use of letrozole and HMG in poor ovarian responders signif-icantly reduced the total dosage and duration of HMG used. Although the number of oocytes retrieved was significantly lower in the letrozole group, the live-birth rate was similar for both groups. It was also shown that serum FSH concen-trations were comparable in both groups on the majority of the days during ovarian stimulation. Follicular fluid con-centrations of testosterone, androstenedione, FSH and AMH were significantly higher in the letrozole group.

The management of poor ovarian responders remains a great challenge in assisted reproduction (Shanbhag et al., 2007). An increased dose of gonadotrophin did not improve the outcome of IVF treatment in several studies (Klinkert et al., 2005; Land et al., 1996; Lekamge et al., 2008). Letrozole has been used in ovulation induction and ovarian stimulation (Badawy et al., 2009a,b; Mitwally and Casper, 2001, 2004). Mitwally and Casper (2002) in an observational study reported that a sequential regimen of letrozole and gonadotrophin in intrauterine insemination cycles was asso-ciated with a significantly lower gonadotrophin dosage but a significantly higher number of mature follicles when com-pared with those of previous failed cycles using gonadotro-phin alone. The use of letrozole in poor ovarian responders during IVF treatment is summarized in Table 5, but results in terms of pregnancy outcomes are not consistent. Goswami et al. (2004), Garcia-Velasco et al. (2005), Ozmen et al. (2009), Verpoest et al. (2006), Yarali et al. (2009) and Davar et al. (2010) reported a comparable pregnancy rate in the letrozole group whereas Garcia-Velasco et al. (2005) and Yarali et al. (2009) showed a higher implantation rate.

Table 4 Follicular fluid hormone concentrations.

	Letrozole (n = 18)	Control (n = 18)	P-value
Oestradiol (pmol/l)	1,186,510 (330,010–2,484,680)	1,012,030 (518–4,799,080)	NS
Progesterone (nmol/l)	45,313 (9442–62,351)	45,230 (38–58,016)	NS
FSH (IU/l)	11.4 (6.0–16.2)	9.0 (5.3–121.5)	0.046
LH (IU/l)	1.0 (0.1–4.6)	0.4 (0.1–6.4)	NS
Testosterone (ng/ml)	19.2 (9.4–53.0)	9.0 (2.1–52.4)	0.009
Androstenedione (ng/ml)	10.8 (3.5–234.1)	5.0 (2.4–370.1)	0.001
AMH (pmol/l)	3.0 (1.2–45.8)	1.9 (0.8–5.1)	0.034
Inhibin B (pg/ml)	390.3 (31.3–2568.3)	321.4 (0–1114.6)	NS

Values are median (range). AMH = anti-Müllerian hormone; NS = not statistically significant.

The present data confirmed the results of an early randomized controlled trial with significantly lower serum oestradiol concentration and lower dosage of gonadotrophin usage (Goswami et al., 2004). However, the present study failed to demonstrate the improved implantation rate following sequential use of letrozole shown in the prospective observational study by Garcia-Velasco et al. (2005), probably due to the small sample size. A favourable hormonal environment of the follicular fluid was reported in the same study, while the current data further elaborated on the hormonal concentrations that letrozole may provide to give more favourable clinical outcomes. Although the majority of previous studies showed a comparable number of oocytes in the letrozole and control groups, the current data showed a significantly lower number of oocytes retrieved in the letrozole group. This is in line with the result of Davar et al. (2010). Despite a smaller number of oocytes obtained in the letrozole group, the live-birth rate was higher, although not statistically significantly, in the letrozole group than the control group. The required sample size with adequate power to show a statistically significant difference in live-birth rate would be 139 patients in each group, in total 278 women.

All the above trials used 2.5 mg letrozole daily for 5 days, either sequentially or concomitantly. A randomized controlled trial using 7.5 mg letrozole in intrauterine insemination showed a non-significantly higher number of follicles than using 100 mg clomiphene citrate in women with unexplained infertility (Al-Fozan et al., 2004). There are no studies on the use of 7.5 mg or even higher dosage of letrozole in IVF treatment. The optimal dosage or duration of letrozole treatment remains unclear and requires further studies to refine it.

Another advantage of the letrozole regimen is the lower cost as a result of the lower dose of FSH used (Goswami et al., 2004). Based on the current data, using letrozole in poor ovarian responders would reduce the HMG dosage by 915 IU for each patient on average. This would save US$140 (about 25% reduction) in the Hong Kong setting, including the cost required for the purified urinary gonadotrophin, letrozole tablets and gonadotrophin-releasing hormone antagonist. If using recombinant products instead of the urinary products, the cost would drop by 32.2% (US$1,331 to US$902). In view of the comparable live-birth rate, the use of letrozole would be a more favourable protocol in poor ovarian responders.

One major concern of using letrozole in ovulation induction or ovarian stimulation is its possible teratogenicity (Biljan et al., 2005). Animal data showed embryo and fetal death in both rats and rabbits, and congenital malformation of kidneys and ureters and altered sexual function in male offspring in rats (Gill et al., 2008; Rockville, 2003). The data from humans seem to be reassuring as no congenital abnormality has been linked to the use of letrozole (Tulandi et al., 2006) which has a short half-life of 45 h. There is a sufficient time interval for complete clearance after its administration in the early follicular phase, i.e. days 2–6 of the cycle in ovulation induction or ovarian stimulation, so the drug should not be present during the fertilization and implantation periods (Requena et al., 2008). In the current study, there were no congenital anomalies noted in the letrozole group, while in the control group, one patient had second-trimester termination of pregnancy for fetal body stalk syndrome after conceiving in the first frozen–thawed embryo transfer cycle.

Serum oestradiol concentrations have been shown to be significantly lower in women receiving letrozole together with gonadotrophin during ovarian stimulation in all trials (Davar et al., 2010; Goswami et al., 2004; Ozmen et al., 2009; Schoolcraft et al., 2008; Verpoest et al., 2006; Yarali et al., 2009) and the current data also confirmed this. It has been shown that both the embryo quality and endometrial receptivity would be improved in a more physiological hormonal environment following a mild ovarian stimulation protocol (Devroey et al., 2004), which may explain the comparable live-birth rate in the two groups with the significantly lower number of oocytes in the letrozole group.

The baseline serum FSH concentration was comparable in both groups. Although a sequential regimen of letrozole and gonadotrophin was used, i.e. gonadotrophin was not given during the first 5 days of stimulation, the serum FSH concentrations were comparable for the letrozole and control groups except on day 8, despite the fact that the control group received gonadotrophin from day 3 onwards. It is likely that the suppression of oestrogen production by letrozole releases the negative feedback mechanism causing a rise in the gonadotrophin concentration. The serum FSH concentration rose quickly from day 2 to day 4, then plateauing afterwards, so it may not be necessary to use gonadotrophin concomitantly with letrozole from day 2 to day 7, as used in previous studies.

Table 5 Summary of studies using letrozole in poor ovarian responders during IVF treatment.

	No. of women	Days of FSH	FSH dosage (IU)	ET (mm)	Oestradiol (pg/ml)	No. of eggs	IR (%)	PR (%)
GnRHa long protocol								
Goswami et al. (2004) (RCT)								
Let + rFSH	13	—	150 (0)[a]	8.5 (0.4)	227 (45)[a]	1.6 (0.8)	—	23
rFSH	25	—	2865 (228)[a]	7.4 (0.4)	380 (46)[a]	2.1 (0.7)	—	24
Antagonist protocol								
Garcia-Velasco et al. (2005) (prospective observational)								
Let + rFSH	71	9.3 (0.3)	3627 (116)	9.6 (0.5)	770 (67)	6.1 (0.4)[a]	25[a]	22.4
rFSH	76	8.9 (0.2)	3804 (127)	9.8 (0.3)	813 (60)	4.3 (0.3)[a]	9.4[a]	15.2
Verpoest et al. (2006) (RCT)								
Let + rFSH	10	—	—	10.3[a]	—	13.8 (9.2)	31.3	50
rFSH	10	—	—	8.1[a]	—	9.6 (7.7)	12.5	20
Ozmen et al. (2009) (RCT)								(/ET)
Let + rFSH	35	—	2980 (435)[a]	9.3 (2.6)	1870 (159)[a]	4.9 (1.6)	—	25.8
rFSH	35	—	3850 (580)[a]	9.7 (3.2)	2015 (175)[a]	4.8 (1.4)	—	20
Microflare protocol								
Schoolcraft et al. (2008) (prospective)								
Let + rFSH	179	9.9 (1.3)	4223 (743)	—	1403 (965)[a]	12 (6)	15	37[a]
GnRHa + rFSH	355	10.1 (1.6)	3938 (975)	—	3147 (1189)[a]	13 (5.3)	21	52[a]
Yarali et al. (2009) (retrospective)								(/ET)
Let + rFSH	212	9.0 (2.5)[a]	4020 (1178)[a]	9.7 (2.4)	794 (711)[a]	4.1 (3.4)[a]	14.5[a]	22.8
GnRHa + rFSH	673	10.2 (4.0)[a]	4538 (1493)[a]	10.1 (2.5)	1805 (1228)[a]	6.7 (4.5)[a]	9.8[a]	17.4
Davar et al. (2010) (RCT)								
Let + rFSH	45	8.5 (1.1)[a]	3158 (563)[a]	8.3 (1.3)	477 (54)[a]	2.8 (2.7)[a]	3.8	5.3
GnRHa + rFSH	49	9.2 (1.2)[a]	3458 (533)[a]	8.4 (1.2)	1065 (706)[a]	4.4 (2.7)[a]	7.7	14.3

[a] $P < 0.05$. ET = endometrial thickness; GnRHa = gonadotrophin-releasing hormone antagonist; IR = implantation rate; Let = letrozole; PR = pregnancy rate; RCT = randomized controlled trial; rFSH = recombinant FSH; (/ET) = pregnancy rate per embryo transfer.

Androgen has been shown to be pivotal in folliculogenesis (Weil et al., 1998). Other than its action in the early stage of follicular differentiation, focusing on the enhancement of FSH-stimulated follicular differentiation, a gradual transition to a later stage of folliculogenesis would result in an increase of the stimulation of FSH and LH and androgen mainly becomes a substrate for oestrogen synthesis (Tetsuka and Hillier, 1997). Animal studies have also revealed that testosterone augmented follicular FSH receptor expression in granulose cells, suggesting that androgens promote follicular growth and oestrogen biosynthesis indirectly by amplification of the effect of FSH (Weil et al., 1999). However, there are scarce data on the effect of letrozole use on the intraovarian environment, except one small prospective study (Garcia-Velasco et al., 2005). As letrozole selectively blocks the conversion from androgen to oestrogen by

suppressing the action of aromastase, the androgen concentration in the follicular fluid would be increased. Follicular fluid testosterone concentration has been reported to be lower in poor ovarian responders (Bahceci et al., 2007) and serum androstenedione in cycles not cancelled was found to be significantly higher than that in cancelled cycles in poor ovarian responders (Balasch et al., 2006). The current data confirmed that follicular fluid androgen concentrations were significantly higher in the letrozole group than that in the control group, which is consistent with those of the previous study (Garcia-Velasco et al., 2005).

One longitudinal study reported a positive correlation between the follicular fluid AMH concentration and pregnancy rate and also with the number of oocytes retrieved (Wunder et al., 2008). Follicular fluid AMH concentrations were positively correlated with the FSH sensitivity (Dumesic et al., 2009) and the fertilization rate (Takahashi et al., 2008). In the current study, follicular fluid AMH concentrations were significantly higher after the use of letrozole, which further supports the favourable hormonal profiles especially in cases of poor ovarian responders. However, the mechanism of the increase in AMH concentrations after letrozole treatment and its effect on growing follicles are still unclear.

Elevated follicular fluid concentrations of inhibin B and oestradiol have been reported to be related to the oocyte retrieval rate, better ovarian response and higher pregnancy rate (Ocal et al., 2004; Wen et al., 2006). However, the current study failed to demonstrate the difference in oestradiol and inhibin B concentration in follicular fluid between the two groups.

One of the limitations of the present study was that it included both poor ovarian responders and those with a poor ovarian reserve as shown by a low antral follicle count. The primary outcome was the number of oocytes obtained rather than the live-birth rate. The sample size was not large enough to show any statistical significance in the live-birth rate. The study was prematurely terminated because of recruitment difficulties. The significant difference in the miscarriage rate should be interpreted with caution as it could be a chance event.

In conclusion, sequential use of letrozole and gonadotrophin in poor responders resulted in a significantly lower total dosage and shorter duration of gonadotrophin administration, significantly fewer oocytes retrieved but a comparable live-birth rate and a significantly lower miscarriage rate, when compared with gonadotrophin alone. Further larger trials are required to confirm the findings.

Acknowledgements

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