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

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Abstract

This randomized trial compared the sequential use of letrozole and gonadotrophin (HMG) with HMG only in poor ovarian responders undergoing IVF. Patients with < four oocytes retrieved in previous IVF cycles or < 5 antral follicles were randomized to either letrozole for 5 days followed by HMG or HMG alone. 53 women were recruited. The letrozole group had significantly lower dosage of HMG ($P < 0.001$), shorter duration of HMG ($P < 0.001$) and fewer oocytes ($P = 0.001$) when compared to the control group. Live-birth rate was comparable with a significantly lower miscarriage rate in the letrozole group (($P = 0.038$). Serum FSH concentrations were comparable in both groups except on Day 8, while estradiol concentrations were all significantly lower in the letrozole group from day 4. Follicular fluid concentrations of testosterone, androstenedione, FSH and AMH were significantly higher in the letrozole group ($P = 0.009$, $P = 0.001$, $P = 0.046$ and $P = 0.034$ respectively). When compared to 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 but a more favourable hormonal environment of follicular fluid.

Keywords: follicular fluid; *in-vitro* fertilization; letrozole; live-birth rate; poor ovarian responders

Introduction

In vitro fertilization (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 (Keay et al. 1997; Karande and Gleicher 1999; Fasouliotis et al. 2000; Surrey and Schoolcraft 2000; Mahutte and Arici 2002; 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 estrogen, which in turn reduces the negative feedback resulting in an increase in gonadotrophins, that 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 (Goswami et al. 2004; Garcia-Velasco et al. 2005; Verpoest et al. 2006; Schoolcraft et al. 2008; Ozmen et al. 2009; Yarali et al. 2009; Davar et al. 2010) on the use of letrozole in poor ovarian responders undergoing IVF treatment have been reported but their results are not consistent.

We conducted this randomized trial to compare the sequential use of letrozole and gonadotrophin with gonadotrophin only in poor ovarian responders or women with poor ovarian reserve undergoing IVF treatment.

Materials and Methods

Study population

Subfertile patients attending 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 two 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 counseled 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 on the

HKClinicalTrials.com with the trial 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 the second day 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 randomization list in sealed, opaque envelopes into two groups: the letrozole and control groups. The sequence of allocation was concealed until 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 human menopausal gonadotrophin (HMG, Menogon, Ferring GmbH, Kiel, Germany) until the day of human chorionic gonadotrophin (hCG) administration. In the control group, they 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 0.25mg (Orgalutran®, Organon) was administered when the leading follicle was ≥ 12mm in diameter. Human chorionic gonadotrophin (hCG) at a dose of 10000 IU (Profasi, Serono, Geneva, Switzerland) was given if at least one follicle was ≥ 18 mm in diameter. Oocyte retrieval was scheduled 36 hours 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 aspiration and flushing was examined by an embryologist. The retrieved oocytes were inseminated conventionally or by intracytoplasmic sperm injection (ICSI) as indicated. Fertilization was checked 14-18 hours 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 hours and examined again. A maximum of two normally cleaving embryos were transferred to the uterine cavity two days after the retrieval.

Blood was taken for determination of serum estradiol (E2), progesterone (P), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations on alternate days from day 2 until the day of oocyte retrieval (hCG+2). Serum E2 and P concentrations were checked on hCG+10 day. Follicular fluid was assayed for E2, P4, 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 were summarized in Table 1. Urine pregnancy test was performed 20 days after the ovulatory dose of 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 live-birth after 24 gestational weeks.

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

We had shown previously that when the AFC was less than 5, the mean number of oocytes 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 can be assumed for the number of oocytes, then co-administration of letrozole will increase the mean number of oocytes in our 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. We aimed to recruit 35 patients in each group to allow for patients who might dropout 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 (SPSS Inc., Version 17.0, Chicago, USA). The two-tailed value of $P < 0.05$ was considered statistically significant.

Results

Study population

During the study period between 1ˢᵗ September 2005 and 30ᵗʰ 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 were 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.

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 GnRH antagonist was comparable in both groups. The number of oocytes retrieved was significantly lower in the letrozole group than that in the control group ($P = 0.001$) but the number of embryos transferred 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) vs 7.4% (2/27), p 0.111) were comparable for both groups. A significantly lower miscarriage rate was noted in the letrozole group than the control group (0% vs 60%, respectively, $p=0.038$).

Serum and follicular fluid hormonal profiles

Serum E2 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 D8. (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 similar between the two groups.

Discussion

Results of this randomized study confirmed that sequential use of letrozole and HMG in poor ovarian responders significantly 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 concentrations were comparable in both groups on the majority of the days during ovarian stimulation. Follicular fluid concentrations 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 *in-vitro* fertilization treatment in several studies (Land et al. 1996; Klinkert et al. 2005; Lekamge et al. 2008). Letrozole has been used in ovulation induction and ovarian stimulation (Mitwally and Casper 2001; Mitwally and Casper 2004; Badawy et al. 2009a; Badawy et al. 2009b). Mitwally and Casper (2002) in an observational study reported that a sequential regimen of letrozole and gonadotrophin in intrauterine insemination cycles was associated with a significantly lower gonadotrophin dosage but a significantly higher number of mature follicles when compared with those of previous failed cycles using gonadotrophin 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) 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.

Our data confirmed the results of an early RCT with the significantly lower serum estradiol concentration and lower dosage of gonadotrophin usage (Goswami et al. 2004). We failed to demonstrate the improved implantation rate following sequential use of letrozole as shown in the prospective observational study by Garcia-Velasco et al. (2005), probably due to our small sample size. A favourable hormonal environment of the follicular fluid was reported in the same study; while our 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, our 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 RCT 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 our data, using letrozole in poor ovarian responders would reduce the HMG dosage by 915 IU for each patient on average. This would save 140 US dollars (about 25% reduction) in the Hong Kong setting, including the cost required for the purified urinary gonadotrophin, letrozole tablets and GnRH antagonist. If using recombinant products instead of the urinary products, the cost would drop by 32.2% (1,331 dollars to 902 dollars). 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 (Rockville 2003; Gill et al. 2008). 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 hours. 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 our 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.

Hormone concentrations

Serum E2 concentrations were significantly lower in women receiving letrozole together with gonadotrophin during ovarian stimulation in all trials (Goswami et al. 2004; Verpoest et al. 2006; Schoolcraft et al. 2008; Ozmen et al. 2009; Yarali et al. 2009; Davar et al., 2010). Our 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 we used the sequential regimen of letrozole and gonadotrophin, i.e. we did not give gonadotrophin 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 estrogen 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 we suggested that it may not be necessary to use gonadotrophin concomitantly with letrozole from day 2 to day 7, as used in previous studies.

Androgen was 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 estrogen synthesis (Tetsuka and Hillier 1997). Animal studies also revealed that testosterone

augmented follicular FSH receptor expression in granulose cells suggesting that androgens promoted follicular growth and estrogen 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 intra-ovarian environment, except one small prospective study (Garcia-Velasco et al. 2005). As letrozole selectively blocks the conversion from androgen to estrogen by suppressing the action of aromastase, the androgen concentration in the follicular fluid would be increased. Follicular fluid testosterone concentration was reported to be lower in the 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). Our data confirmed follicular fluid androgen concentrations were significantly higher in the letrozole group than that in the control group, which was 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 levels were positively correlated with the FSH sensitivity (Dumesic et al. 2009), and the fertilization rate (Takahashi et al. 2008). Our data showed that the 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 levels of inhibin B and estradiol 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, in our study, we failed to demonstrate the difference in estradiol and inhibin B level in follicular fluid between the two groups.

Limitation

One of the limitations of the present study was that we included both poor ovarian responders and those with a poor ovarian reserve as shown by a low AFC. 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.

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 to gonadotrophin alone. Further larger trials are required to confirm the findings.

References

Al-Fozan H, Al-Khadouri M, Tan SL, Tulandi T, 2004. A randomized trial of letrozole versus clomiphene citrate in women undergoing superovulation. Fertil Steril. 82,1561-3.

Badawy A, Abdel Aal I, Abulatta M, 2009a. Clomiphene citrate or letrozole for ovulation induction in women with polycystic ovarian syndrome: a prospective randomized trial. Fertil Steril. 92,849-52.

Badawy A, Elnashar A, Totongy M, 2009b. Clomiphene citrate or aromatase inhibitors for superovulation in women with unexplained infertility undergoing intrauterine insemination: a prospective randomized trial. Fertil Steril. 92,1355-9.

Bahceci M, Ulug U, Turan E, Akman MA, 2007. Comparisons of follicular levels of sex steroids, gonadotropins and insulin like growth factor-1 (IGF-1) and epidermal growth factor (EGF) in poor responder and normoresponder patients undergoing ovarian stimulation with GnRH antagonist. Eur J Obstet Gynecol Reprod Biol. 130,93–8.

Balasch J, Fabregues F, Penarrubia J, Carmona F, Casamitjana R, Creus M, et al, 2006. Pretreatment with transdermal testosterone may improve ovarian response to gonadotrophins in poor-responder IVF patients with normal basal concentrations of FSH. Hum Reprod. 21,1884-93.

Biljan MM, Hcmmings R, Brassard N, 2005. The outcome of 150 babies following the treatment wilh letrozole or lelrozole and gonadotrophins. Fertil Steril. 84(supp),1033.

Davar R, Oskouian H, Ahmadi S, Firouzabadi R, 2010. GnRH antagonist/letrozole versus microdose GnRH agonist flare protocol in poor responders undergoing in vitro fertilization. Taiwan J Obstet Gynecol. 49,297-301.

Devroey P, Bourgain C, Macklon NS, Fauser BC, 2004. Reproductive biology and IVF: ovarian stimulation and endometrial receptivity. Trends Endocrinol Metab. 15,84-90.

Dumesic DA, Lesnick TG, Stassart JP, Ball GD, Wong A, Abbott DH, 2009. Intrafollicular antimullerian hormone levels predict follicle responsiveness to follicle-stimulating hormone (FSH) in normoandrogenic ovulatory women undergoing gonadotropin releasing-hormone analog/ recombinant human FSH therapy for in vitro fertilization and embryo transfer. Fertil Steril. 92,217-21.

Fasouliotis SJ, Simon A, Laufer N, 2000. Evaluation and treatment of low responders in assisted reproductive technology: a challenge to meet. J Assist Reprod Genet. 17,357-73.

Garcia-Velasco JA, Moreno L, Pacheco A, Guillen A, Duque L, Requena A, et al., 2005. The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study. Fertil Steril. 84,82-7.

Gill SK, Moretti M, Koren G, 2008. Is the use of letrozole to induce ovulation teratogenic? Cana Fam Physician. 54,353-4.

Goswami SK, Das T, Chattopadhyay R, Sawhney V, Kumar J, Chaudhury K, et al., 2004. A randomized single-blind controlled trial of letrozole as a low-cost IVF protocol in women with poor ovarian response: a preliminary report. Hum Reprod. 19,2031-5.

Karande V, Gleicher N, 1999. A rational approach to the management of low responders in in-vitro fertilization. Hum Reprod. 14,1744-8.

Keay SD, Liversedge NH, Mathur RS, Jenkins JM, 1997. Assisted conception following poor ovarian response to gonadotrophin stimulation. Br J Obstet Gynecol. 104,521-7.

Klinkert ER, Broekmans FJ, Looman CWN, Habbema JDF, Te Velde ER, 2005. Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: a randomized controlled trial. Hum Reprod. 20,611-5.

Land JA, Yarmolinskaya MI, Dumoulin JCM, Evers JLH, 1996. High-dose human menopausal gonadotropin stimulation in poor responders does not improve in vitro fertilization outcome. Fertil Steril. 65,961-5.

Lekamge DN, Lane M, Gilchrist RB, Tremellen KP, 2008. Increased gonadotrophin stimulation does not improve IVF outcomes in patients with predicted poor ovarian reserve. J Assist Reprod Genet. 25,515-21.

Mahutte NG, Arici A, 2002. Poor responders: does the protocol make a difference? Curr Opin Obstet Gynecol. 14,275-81.

Mitwally MFM, Casper RF, 2001. Aromatase inhibition: A novel method of ovulation induction in women with polycystic ovary syndrome. Reprod Technol. 10,244-7.

Mitwally MFM, Casper RF, 2002. Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders. Fertil Steril. 77,776-80.

Mitwally MFM, Casper RF, 2004. Aromatase inhibitors in ovulation induction. Semin Reprod Med. 22,61-78.

Ng EHY, Tang OS, Ho PC, 2000. The significance of the number of antral follicles prior to stimulation in predicting ovarian responses in an IVF programme. Hum Reprod. 15,1937-42.

Ocal P, Aydin S, Cepni I, Idil S, Idil M, Uzun H, et al., 2004. Follicular fluid concentrations of vascular endothelial growth factor, inhibin A and inhibin B in IVF cycles: are they markers for ovarian response and pregnancy outcome? Eur J Obstet Gynecol Reprod Biol. 115,194-9.

Ozmen B, Sonmezer M, Atabekoglu CS, Olmu H, 2009. Use of aromatase inhibitors in poor-responder patients receiving GnRH antagonist protocols. Reprod Biomed Online. 19,478-85.

Requena A, Herrero J, Landeras J, Navarro E, Neyro JL, Salvador C, et al., 2008. Use of letrozole in assisted reproduction: a systematic review and meta-analysis. Hum Reprod Update. 14,571-82.

Rockville, M. D. (2003). "FDA oncology tools product label details for administration of letrozole.". Retrieved Feb 15, 2008, from http://www.accessdata.fda.gov/scripts/cder/onctools/administer.cfm?GN=letrozole.

Schoolcraft WB, Surrey ES, Minjarez DA, Stevens JM, Gardner DK, 2008. Management of poor responders: can outcomes be improved with a novel gonadotropin-releasing hormone antagonist/letrozole protocol? Fertil Steril. 89,151-6.

Shanbhag, S., L. Aucott, et al. (2007). "Interventions for 'poor responders' to controlled ovarian hyperstimulation (COH) in in-vitro fertilisation (IVF)." Cochrane Database of Systematic Reviews(1): Art. No.: CD004379. DOI: 004310.001002/14651858.CD14004379.pub14651852.

Surrey, E. S. and W. B. Schoolcraft, 2000. Evaluating strategies for improving ovarian response of the poor responder undergoing assisted reproductive techniques. Fertil Steril 73,667-676.

Takahashi C, Fujito A, Kazuka M, Sugiyama R, Ito H, Isaka K, 2008. Anti-Mullerian hormone substance from follicular fluid is positively asooicated with success in oocyte fertilization during in vitro fertilization. Fertil Steril. 89,586-91.

Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J, 2003. Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update. 9,61-76.

Tetsuka M, Hillier SG, 1997. Differential regulation of aromatase and androgen receptor in granulosa cells. J Ster Biochem Mole Biol. 61,233-9.

Tulandi T, Martin J, Al-Fadhli R, Kabli N, Forman R, Hitkari J, et al., 2006. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. Fertil Steril. 85,1761-5.

Verpoest WMJA, Kolibianakis E, Papanikolaou E, Smitz J, Van Steirteghem A, Devroey P, 2006. Aromatase inhibitors in ovarian stimulation for IVF/ICSI: a pilot study. Reprod Biomed Online. 13,166-72.

Wang XJ, Ledger W, Payne D, Jeffrey R, Matthews CD, 1994. The contribution of embryo cryopreservation to in-vitro fertilization/gamete intra-fallopian transfer: 8 years experience. Hum Reprod 9,103-109.

Weil SJ, Vendola K, Zhou J, Adesanya OO, Wang J, Okafor J, et al., 1998. Androgen receptor gene expression in the primate ovary: cellular localization, regulation, and functional correlations. J Clin Endocrinol Metabol. 83,2479-85.

Weil S, Vendola K, Zhou J, Bondy CA, 1999. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. J Clin Endocrinol Metabol. 84,2951-6.

Wen X, Tozer AJ, Butler SA, Bell CM, Docherty SM, Iles RK, 2006. Follicular fluid levels of inhibin A, inhibin B, and activin A levels reflect changes in follicle size but are not independent markers of the oocyte's ability to fertilize. Fertil Steril. 85,1723-9.

Wunder DM, Guibourdenche J, Birkhauser MH, Bersinger NA, 2008. Anti-Mullerian hormone and inhibin B as predictors of pregnancy after treatment by in vitro fertilization/ intracytoplasmic sperm injection. Fertil Steril. 90,2203–10.

Yarali H, Esinler I, Polat M, Bozdag G, Tiras B, 2009. Antagonist/letrozole protocol in poor ovarian responders for intracytoplasmic sperm injection: a comparative study with the microdose flare-up protocol. Fertil Steril. 92,231-5.

Table 1. Details of hormone assays

Laboratory tests	Limit of detection	Intra-assay CV (%)	Inter-assay CV (%)	Manufacturer
Estradiol (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	Immunoassay (DSL)
Androstenedione (ng/mL)	0.03	7.8-10.3	6.3-9.2	DSL
AMH (pM)	1	0-11.4	0-14	Immunotect (BC)
Inhibin B (pg/mL)	7	3.5-5.6	6.2-7.6	DSL

CV-coefficients of variation. FSH - follicle-stimulating hormone. LH – luteinizing hormone. AMH – antimullerian hormone.

Table 2. Demographic data.

	Letrozole group (n=26)	Control group (n=27)
Age (yrs)	37.3 ± 2.4	36.2 ± 2.2
Duration of subfertility (yrs)	5.3 ± 2.5	4.7 ± 2.0
Body mass index (kg/m^2)	21.0 ± 2.3	20.7 ± 2.1
Primary subfertility (%)[1]	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)

Data presented as mean ± standard deviation or number (percentage).
There were no statistically significant differences between the two groups.

Table 3. Comparison of outcomes.

	Letrozole group (n=26)	Control group (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 no of follicle aspirated[a]	3.0 (0-11)	5.0 (0-26)	0.001
No of oocytes retrieved[a]	2.0 (0-4)	4.0 (0-19)	0.001
No of embryos cleaved[a]	1.0 (0-3)	2.0 (0-15)	0.020
No of embryos transferred[a]	1.0 (0-2)	2.0 (0-3)	NS
Cancellation rate (due to premature luteinisation)[b]	4 (15.0%)	2 (7.4%)	NS
Cancellation rate (all causes)[b]	6 (23.1%)	4 (14.8%)	NS
Implantation rate[b]	5/23 (21.7%)	5/36 (13.9%)	NS
Ongoing pregnancy rate[b]	5 (19.2%)	2 (7.4%)	NS
Live birth rate[b]	5 (19.2%)	2 (7.4%)	NS
Miscarriage rate[b]	0 (0)	3/5 (60.0%)	0.038

Data were presented as mean ± standard deviation
[a]Data were given as median (range)
[b]Data presented as number (percentage).
NS = not statistically significant.

Table 4. Follicular fluid hormone concentrations.

	Letrozole group (n=18)	Control group (n=18)	p-value
Estradiol (pmol/L)	1186510 (330010-2484680)	1012030 (518-4799080)	NS
Progesterone (nmol/L)	45313 (9442-62351)	45230 (38-58016)	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 (pM)	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

Data presented as median (range).
AMH-anti-Müllerian hormone, NS = not statistically significant.

Table 5. Summary of studies using letrozole in poor ovarian responders during in-vitro fertilisation treatment.

	No of women	Days of FSH	FSH dosage (IU)	ET (mm)	E2 (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+FSH	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 +FSH	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

ET – endometrial thickness. E2 – estradiol. IR – implantation rate. PR – pregnancy rate. (/ET) – pregnancy rate per embryo transfer. Let – letrozole.
[a]$p<0.05$

Legends
Figure 1 Flow diagram of patient participation in the trial.
Figure 2. Serum hormone concentrations on various days of ovarian stimulation. Panel 2a, 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). Panel 2b, FSH concentrations of the letrozole and control groups were comparable on most the days, except on day 8 when FSH concentration of the letrozole group was significantly lower than that of the control group (p <0.001). Panel 2c, LH concentrations of the letrozole group were significantly higher than the control groups on days 4, 6 and 8 (p 0.018, <0.001 and 0.018 respectively).