

The potential of letrozole use for priming in vitro maturation cycles

B. I. ROSE

Infertility Solutions, P. C., 1275 South Cedar Crest Boulevard, Allentown, PA 18103, USA.

Department of Obstetrics and Gynecology, St. Luke's University Health Network, 801 Ostrum Street, Bethlehem, PA 18015, USA.

Correspondence at: zygote@ptd.net

Abstract

Objective: To compare the pregnancy rates with two different methods of priming for in vitro maturation cycles (IVM). Cycles using letrozole together with hCG were compared to cycles using FSH together with hCG in 75 cycles in patients being treated for infertility by IVM.

Study design: Retrospective cohort non-inferiority trial

Results: Using letrozole for priming was found to not be inferior to using FSH for priming cycles. The live birth rate per transfer was 31.6% in letrozole primed cycles compared to 20.8% in FSH primed cycles (not significant). However, FSH priming produced more oocytes that were mature on the day of retrieval ($p = 0.004$) and also resulted in more high quality embryos than did letrozole priming ($p = 0.004$).

Conclusion: Letrozole may be used for priming in IVM cycles in which the clinician views the characteristics of letrozole as more desirable than FSH.

Key words: In vitro maturation, IVM, letrozole, IVM priming, FSH priming, letrozole priming.

Introduction

The purpose of this study was to evaluate a novel approach to priming in an in vitro maturation (IVM) program using letrozole. Priming is an adjunct used with IVM to improve the potential for a patient to achieve pregnancy. Priming utilizes low dose FSH several days before oocyte retrieval and/or uses hCG 36 to 38 hours before retrieval to maximize the potential of oocytes to mature in vitro. Fadini et al. (2009) undertook a randomized trial comparing no priming, to priming with hCG alone, to priming with FSH alone, and to priming using both FSH and hCG. The best results were obtained by priming with both FSH and hCG. With this priming approach, there was an increase in the number of mature oocytes, an increase in the number of high quality embryos and an increased clinical pregnancy rate. This approach also produced the highest number of oocytes that were mature on the day of retrieval (Fadini et al., 2009).

A major advantage of IVM compared to conventional IVF is its patient friendliness. Even with combined FSH and hCG priming the number of injections that a patient needs to administer is reduced by 89% compared to conventional IVF. The amount of medications is significantly reduced so that the cost of medications decreases by 91% compared to conventional IVF (Rose et al., 2014). Low doses of FSH stimulate cell division of granulosa cells of antral follicles and thus make small antral follicles larger (Wynn et al., 1998). This makes oocyte retrieval technically less difficult.

Letrozole blocks production of estradiol by granulosa cells. This interrupts the normal negative feedback of estrogen production on pituitary production of FSH. Thus over the course of a few days, increased endogenous FSH is produced compared to what would have occurred naturally (Casper, 2003). Letrozole has been used to treat anovulation in polycystic ovarian syndrome (PCOS) patients (Begum et al., 2009).

By using endogenous rather than exogenous FSH and oral medications rather than injectable FSH, IVM cycles could be made even more patient friendly by eliminating both the complication and cost of injectable FSH provided that the endogenous FSH was adequate to replicate the benefit of priming with FSH on an IVM cycle. This approach would make an IVM cycle require no more from patients than a typical clomiphene-IUI cycle in many specialty practices (Lewis and Guzick, 2002). This paper is a retrospective evaluation to determine the non-inferiority of letrozole and hCG priming for IVM compared to FSH and hCG priming for IVM.

Methods

All patients undertaking IVM either using letrozole for priming or low dose FSH for priming from the start of the program at Infertility Solutions, P. C. in December of 2009 through June of 2014 were included in this review. Based on physician recommendations, patients for this study either met standard criteria for PCOS (Rotterdam consensus group, 2004) or had a total antral follicle count greater than 20 and elected IVM rather than conventional IVF. As a new IVM program, Infertility Solutions used several approaches to priming in an attempt to optimize the program's pregnancy rate. Patients were not selected for a particular priming approach, but choose to utilize IVM during a time period that a particular priming approach was used.

The protocols used are summarized in Table I. All patients were treated with oral contraceptives and had a baseline ultrasound to exclude cysts on day 3 of bleeding or 6 days after stopping oral contraceptives. All patients with PCOS received metformin up to 1500 mg/day as tolerated. If FSH priming was used, it was initiated after the baseline

ultrasound. Most patients received 50 U FSH daily until they had two antral follicles 10 to 12 mm in diameter. At this time hCG was given and an oocyte retrieval was done 38 hours later. Patients having all antral follicles under 5 mm at the baseline ultrasound examination or who were very obese received 75 U FSH per day.

If letrozole was used, patients started the medication on the same day they would have started FSH. Letrozole, 2.5 mg, was taken for five days. HCG was administered when two follicles were in the 10 to 12 mm range or the largest antral follicles stopped growing on daily or alternate day ultrasound examinations. Oocyte retrieval was again performed 38 hours after hCG.

Initially all retrievals in this program were done using a 19-gauge needle (K-OPS-7035-RWH-ET, Cook Medical, Spencer IN) without flushing and with heparin in the collection medium. The oocyte identification technique used employed a cell strainer (BD Falcon 352350, Franklin Lakes, NJ). Subsequently, all retrievals used a 21-gauge Steiner-Tan needle (www.ivfexflex.com, Graz, Austria) with flushing using non-heparinized PBS (Dulbecco's phosphate buffered saline, Gibco 14287-080, Grand Island, NY). This approach to oocyte collection enabled use of the same oocyte identification approach used with conventional IVF (Rose and Laky, 2013).

Oocytes were evaluated at multiple times during the standard workday for up to 48 hours after retrieval and if a polar body could be seen, then granulosa cells were removed from the oocyte and ICSI was performed. Immature oocytes were incubated in Sage maturation media (ART-1600B, Sage, Trumbull, CT, US) with 10% complement inactivated maternal serum. Generally, a transfer was performed three days after the first oocyte fertilized (for a few patients, 2 or 5 days after the

Table I. — Summary of protocols used.

Cycle day	-3	2 or 3	3	4	5	6	7	0 to 5 days			HCG +0	HCG +2	ICSI	Vaginal progesterone start	Possible lowered estradiol dose ¹
Letrozole protocol	Last day OC	Baseline US	2.5 mg Let				10K U	Ret	After hCG day 2, 3, or 4	Evening of first day of ICSI	Day after first day of ICSI				
FSH protocol	Last day OC	Baseline US	50 U FSH	50 U FSH	50 U FSH	10K U	Ret	After hCG day 2, 3, or 4	Evening of first day of ICSI	Day after first day of ICSI					

Abbreviations: OC – oral contraceptives, US – ultrasound, Let – letrozole, Ret – oocyte retrieval.

¹4 mg/day. May have started on higher dose of estradiol earlier in cycle depending on endometrial lining thickness.

Table II. — Demographic information.

	Letrozole only (SD)	Gonadotropins only (SD)	Significance testing ¹
Patients	21	42	
Cases (oocyte retrievals)	21	54	
Transfers	19	53	
Cancellation rate (%)	2/21 (9.5%)	1/54 (1.9%)	0.188
Age	30.9 (4.0)	32.0 (3.5)	0.267
BMI	24.2 (4.7)	26.9 (6.6)	0.1
Years infertile	2.5 (1.9)	3.0 (2.0)	0.346
# PCOS (%)	17/21 (80.9%)	29/42 (69%)	0.38
# male factor (%)	12/21 (57.1%)	28/42 (66.7%)	0.58

¹Using the unpaired Fisher exact test and the unpaired t-test, as appropriate.

first fertilization). Assisted hatching was rarely done.

“High quality embryos” had at most 10% fragmentation and were 7 to 9 cells if they fertilized three days earlier and 4 cells if they fertilized two days earlier. They were devoid of multi-nucleation or large vacuoles and had blastomeres of normal size. “High quality embryos” for a cycle were tallied on the day of transfer.

The thickness of the endometrial lining was actively managed. All patients received 4 mg of oral micronized estradiol following the day of retrieval. Prior to the day of aspiration, some patients received 4 to 12 mg micronized estradiol depending on the thickness of their endometrial lining and how close they were to the day of their oocyte retrieval. Doses greater than 6 mg were administered vaginally

(same oral micronized estradiol). To minimize the negative feedback of estradiol on endogenous FSH production, estradiol was not started until antral follicle growth was close to the desired target sizes. The higher doses of estradiol were used for those patients with very thin endometrial line thickness. All patients received at least 4 mg estradiol on the day of retrieval. All patients received vaginal progesterone starting on the day that ICSI was first performed.

Statistics

Statistical computation utilized statistical calculators from GraphPad Software, Inc. (www.graphpad.com). A probability less than 5% was considered statistically significant. Standard statistical tests

Table III. — Clinical information.

	Letrozole only (SD)	Gonadotropins only (SD)	Significance testing ³
Units of FSH used	0	344.5 (177.5)	p <0.0001 ⁴
Lead follicle day of hCG (mm)	13.6 (1.9)	13.4 (4.0)	0.83
Sum of 3 largest follicles (Day of hCG)	35.2 (3.4)	34.9 (6.9)	0.667
Endometrial line thickness on day of hCG	6.1 (1.3)	7.2 (2.4)	0.051
Endometrial line thickness on day of transfer	9.1 (2.7) ¹	10.6 (2.1) ²	0.055
# Receiving early estrogen (%)	10/21 (47.6%)	17/54 (31.5%)	0.284
Oocytes retrieved	11.6 (5.5)	12.3 (5.1)	0.603
Mature oocytes on day 0	1.7 (1.7)	3.1 (1.9)	0.004 ⁴
Total mature oocytes	6.3 (3.1)	8.6 (3.5)	0.103
Total fertilizations	4.9 (2.5)	5.8 (2.8)	0.203
Total high quality oocytes	1.4 (1.4)	2.6 (1.6)	0.004 ⁴
# Transferred	3 (0.8)	2.8 (0.7)	0.307

¹Missing data- 10 observations.

²Missing data- 49 observations.

³Using the unpaired Fisher exact test and the unpaired t-test, as appropriate.

⁴Statistically significant.

Table IV. — Pregnancy information.

	Letrozole only (SD)	Gonadotropins only (SD)	Significance testing ¹
Number transferred	3 (0.8)	2.8 (0.7)	0.307
Implantation rate	7/19 (12.3%)	24/149 (18.1%)	0.053
Elevated hCG (%) /retrieval (>5)	7/21 (33.3)	22/54 (40.7)	0.607
Clinical pregnancy (%) /retrieval	7/21 (33.3%)	20/54 (37.0%)	0.591
Ongoing or delivered (%) /retrieval	6/21 (28.6)	11/54 (20.4)	0.541
Elevated hCG (%) /transfer	7/19 (36.8)	22/53 (41.5)	0.79
Clinical pregnancy (%) /transfer	7/19 (36.8)	20/53 (37.7)	1.0
Ongoing or delivered (%) /transfer	6/19 (31.6%)	11/53 (20.8%)	0.359
Loss of clinical pregnancy (%)	1/7 (14.3%)	9/20 (45.0%)	0.204

¹Using the unpaired Fisher exact test and the unpaired t-test, as appropriate.

used were Fisher's exact test (two-tailed) and the unpaired t-test (two-tailed).

Results

Table II lists summaries demographic characteristics of the groups studied. There were no statistical differences in any of the parameters evaluated. Two cycles (9.5%) were cancelled in the letrozole group. One other of these was for failure to retrieve oocytes. The other was for cleavage arrest. One cycle (1.9%) was cancelled in the FSH group for cleavage arrest.

Table III describes clinical cycle parameters including the size of the largest antral follicles when the decision was made to give hCG, measurements of endometrial lining thickness, the number of oocytes recovered, the number of mature oocytes, the number of oocytes that fertilized, the number of embryos that were high quality at the time of transfer, and the number of embryos transferred. There were more mature oocytes on the day of aspiration ($p = 0.004$) and more high quality embryos on the day of transfer ($p = 0.004$) in the FSH group than in the letrozole group. All other differences were not statistically significant. There was a trend toward a thicker endometrial lining both on the day of hCG and on the day of transfer in the FSH group, but data collection on the endometrial line thickness was incomplete. In the letrozole group 23.8% of cases had an endometrial thickness less than 6 mm compared to 20.3% of the FSH group. All patients in both groups had an endometrial thickness greater than 6 mm on the day of transfer (no missing data).

Table IV compares pregnancy data for these two groups. There were no statistical differences. The implantation rate was almost 50% higher for the FSH group compared to the letrozole group ($p = 0.053$). The live birth rate for the letrozole

group was 31.6%. For the FSH group it was 20.8% (not significant; $p = 0.36$). Both groups had similar clinical pregnancy rates, but the miscarriage rate was higher in the FSH group (45% versus 14.3%, not significant; $p = 0.2$).

No twins were conceived in the letrozole group. Embryo cryopreservation was not done in the letrozole group although one patient had the potential of cryopreserving one high quality cleavage stage embryo that was not transferred. Two sets of twins were conceived in the FSH group. One patient delivered twins and the other a singleton. In the FSH group, one patient had embryos cryopreserved, but 13 patients had high quality cleavage stage embryos that were not transferred or cryopreserved. The choice of not cryopreserving embryos was made by individual patients usually based on the relative cost versus benefit of freezing one or two embryos, storing them and transferring them in another cycle compared to the cost of a fresh cycle.

Discussion

The pregnancy rate for the group of patients receiving letrozole priming was not inferior to the pregnancy rate for the group receiving low dose FSH. The fact that it was nominally higher reinforces the non-inferiority conclusion for letrozole priming which was the primary objective of this paper. The pregnancy rate for the letrozole group was not shown to be (statistically) higher than for the FSH group. Other data generated by this study may provide a basis for choosing FSH treatment as superior to letrozole therapy. In particular, embryo quality was better for patients in the FSH group. This was most clear in the statistically different findings of maturity on the day of retrieval and the number of high quality embryos available on the

day of transfer. Several other parameters reflecting better embryo quality and a better clinical setting (endometrial lining thickness) were also nominally better in the FSH group.

As a non-inferiority trial, this paper suffers from its lack of prospective randomization (Scott, 2009). However, both sample size calculations and the nominally higher pregnancy rate in the letrozole group support the validity of the primary conclusion. A total sample size of only 52 patients would be required to demonstrate that there was no difference between the two treatments with an 80% probability that the upper limit of a one-sided 95% confidence interval would exclude a difference in favor of the FSH group of more than 30% (<https://www.sealedenvelope.com/power/binary-noninferior/>). Post hoc analysis using the pregnancy rates obtained show that if approximately 200 patients in each group were treated and achieved the same pregnancy rates, letrozole would have been shown to be superior to FSH. In particular, with the assumption that pregnancy rates would remain stable for each group, no increase in patient numbers would be able to show that letrozole was inferior to FSH. Using a more negative assumption that if additional patients were recruited and if the pregnancy rate for the FSH doubled while the pregnancy rate for the letrozole group dropped by half, adding 100 additional patients to each group would not demonstrate a statistically significant difference (www.graphpad.com/quickcalcs/contingency2/).

Letrozole priming thus presents an option for use by the clinician who may have specific reasons for wishing to use endogenous FSH priming. Some of the settings in which this may occur are for oocyte or embryo banking (Chian et al., 2013) in patients with estrogen sensitive cancers, patients with coagulopathies, needle phobic patients, and patients who philosophically view this approach as more natural and thus more desirable. Some programs may also value the increased simplicity or lower cost of this approach for their patients as an adequate reason to use it as a primary or an initial approach for IVM therapy.

Another setting for which letrozole priming for IVM may be valuable would be to rescue a patient during a planned IUI cycle in which the antral follicles proliferate but don't sufficiently enlarge in response to letrozole. This was the setting in which we first tried this approach. The patient involved had received 50 mg of clomiphene citrate for 5 days and was given the option of a retrieval for IVM or cycle cancellation. She successfully conceived and delivered after IVM. This led us to further consider oral medications to stimulate endogenous FSH stimulation prior to IVM. We elected to use letrozole

rather than clomiphene citrate to avoid the theoretically negative impact of clomiphene citrate on the development of the endometrium (Young et al., 1999). Cortinez et al. looked at eight patients with unexplained infertility and compared their natural ovulatory cycles to cycles after taking 5 mg of letrozole using traditional parameters as well as electron microscopy of mid-luteal endometrial biopsies (Cortinez et al., 2005). Maximal endometrial thickness was identical (0.2 mm thicker with letrozole). Letrozole corrected the out of phase biopsies present in the natural cycles and electron microscopy showed endometrial apical surfaces covered by ciliated and microvillous cells with pinopodes present in all biopsy specimens. Letrozole has also been shown to more positively influence genetic markers associated with endometrial receptivity than clomiphene citrate (Wallace et al., 2011).

A popular approach to priming not addressed in this study is the use of hCG without FSH (Son and Tan, 2010; Gremeau et al., 2012). Priming with letrozole and hCG may or may not be inferior to the use of hCG alone. Priming with hCG alone was used at Infertility Solutions in only 10 cases with 8 having an embryo transfer. The parameters studied were not as good (nominally) as the results with letrozole (data not shown). Priming with hCG alone was not used for further cycles primarily because oocyte retrieval was believed to be more difficult without the addition of FSH (directly or indirectly) in some patients with small antral follicles.

It is difficult to interpret the nominally lower miscarriage rate in the letrozole group compared to the FSH group. There were more high quality embryos in the FSH group. The implantation rate and endometrial line thickness were nominally better in the FSH group and the proportion of PCOS patients was nominally lower. The high miscarriage rate seen in many reports for IVM patient series has in part been attributed to patient characteristics such as PCOS (Buckett et al., 2008). Our overall loss rate of 27% with patient subsets having loss rates of 14 and 45% is similar to other reports in the literature (Child et al., 2001).

It is important to emphasize that determining the value of letrozole in priming in vitro maturation cycles will require a larger prospective study. All conclusions of this study remain tentative until such a study is available.

Conclusion

Letrozole may be used to stimulate endogenous FSH prior to IVM. The impact of this form of priming was not inferior to low dose FSH treatment

in terms of clinical, live birth rates per aspiration procedure or per transfer. However, FSH priming appeared to produce more better quality embryos than cycles using letrozole priming.

References

- Begum MR, Ferdous J, Begum A et al. Comparison of efficacy of aromatase inhibitor and clomiphene citrate in induction of ovulation in polycystic ovarian syndrome. *Fertil Steril*. 2009;92:853-7.
- Buckett WM, Chian R-C, Dean NL et al. Pregnancy loss in pregnancies conceived after in vitro oocyte maturation, conventional in vitro fertilization, and intracytoplasmic sperm injection. *Fertil Steril*. 2008;90:546-50.
- Casper RF. Letrozole: ovulation or superovulation? *Fertil Steril*. 2003;80:1335-37.
- Chian R-C, Uzelac PS, Nargund G. In vitro maturation of human immature oocytes for fertility preservation. *Fertil Steril*. 2013;99:1173-81.
- Child TJ, Abdul-Jalil AK, Gulekli B et al. In vitro maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. *Fertil Steril*. 2001;76:936-42.
- Cortinez A, De Carvalho I, Vantman D et al. Hormonal profile and endometrial morphology in letrozole-controlled ovarian hyperstimulation in ovulatory infertile patients. *Fertil Steril*. 2005;83:110-5.
- Fadini R, Dal Canto MB, Renzini MM et al. Effect of different gonadotropin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study. *Reprod BioMed Online*. 2009;19:343-51.
- Gremeau A-S, Andreadis N, Fatum M et al. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. *Fertil Steril*. 2012;98:355-60.
- Lewis V, Guzik DS. Clomiphene and intrauterine insemination (IUI)- What is the best way to time insemination? *Fertil Steril*. 2002;73:S154.
- Rose BI, Laky DC, Miller B: The case for IVM: Lower cost and more patient friendly. *J Reprod Med*. 2014; in press.
- Rose BI, Laky DC. A comparison of the Cook single lumen immature ovum IVM needle to the Steiner-Tan pseudo double lumen flushing needle for oocyte retrieval for IVM. *J Assist Reprod Genet*. 2013;30:855-60.
- Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovarian syndrome (PCOS). *Hum Reprod*. 2004;19:41-7.
- Scott IA. Non-inferiority trials: determining whether alternative treatments are good enough. *Med J Aust*. 2009;190:326-30.
- Son W-Y, Tan SL. Laboratory and embryological aspects of hCG-primed in vitro maturation cycles for patients with polycystic ovaries. *Hum Reprod Update*. 2010;16:675-89.
- Wallace KL, Johnson V, Sopolak V et al. Clomiphene citrate versus letrozole: molecular analysis of the endometrium in women with polycystic ovary syndrome. *Fertil Steril*. 2011;96:1051-6.
- Wynn P, Picton HM, Krapez JA et al. Pretreatment with follicle stimulating hormone promotes the numbers of human oocytes reaching metaphase II by in-vitro maturation. *Hum Reprod*. 1998;13:3132-8.
- Young SL, Opsahl MS, Fritz MA. Serum concentrations of enclomiphene and zuclomiphene across consecutive cycles of clomiphene citrate therapy in anovulatory infertile women. *Fertil Steril*. 1999;71:639-44.