The role of ketoconazole in the prevention of ovarian hyperstimulation syndrome in patients with polycystic ovary syndrome during assisted reproductive technology cycles

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ABSTRACT

Objective: To assess the clinical efficacy of ketoconazole (KCZ) as an inhibitory enzyme on ovarian steroidogenesis, in prevention of ovarian hyperstimulation syndrome (OHSS) during assisted reproductive technology (ART) in patients with polycystic ovarian syndrome (PCOS).

Methods: We included 58 PCOS patients, enrolled for in-vitro fertilization or intracytoplasmic sperm injection in a randomized clinical trial between November 2000 and October 2001 in the Royan Institute, Tehran, Iran. Twenty-eight patients received KCZ during the stimulation phase and 30 patients were controls. We compared serum E2 levels, number of lead follicles, number of retrieved oocytes, fertilization rate, occurrence of OHSS and cancellation rate using student's t-test, Chi-square and Fisher exact test.

Results: We found no significant differences between the 2 groups in the peak serum estradiol level, the number of lead follicles, oocytes, and fertilization rate. One patient in the control group had clinical symptoms of severe ovarian hyperstimulation, and none from the treated group.

Conclusion: This study reveals no significant effect of KCZ on the incidence of OHSS during ART cycles; however, also no significant negative effects of KCZ on the number and maturity of oocytes or on fertilization rate.

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Ovarian hyperstimulation syndrome (OHSS) is one of the most serious complications of assisted reproductive technology (ART) especially in patients with polycystic ovarian syndrome (PCOS).[1] When subjected to ART, PCOS patients, compared with controls, usually have significantly higher serum E2 levels on the day of human chorionic gonadotropin (HCG) administration, develop more follicles and produce more oocytes with the result that there is a higher incidence of OHSS.[2] Approaches to reducing this risk and the effect on ART outcome have included lowered dose of gonadotropin,[3] delaying, reducing or cancelling administration of HCG,[4] unilateral follicular aspiration,[5] administration of gonadotropin-releasing hormone (GnRH)-antagonist for luteinizing hormone (LH) surge suppression and GnRH agonist for triggering ovulation, and in vitro maturation of

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oocytes.[6] Ketoconazole (KCZ), an anti-mycotic drug with a short half-life is a known inhibitor of steroidogenic enzymes of the p450 family.[7] This inhibitory effect of KCZ has been confirmed in some human organs such as testis, adrenal, liver, kidney, and ovary. In the ovary, KCZ inhibits the key steroidogenic enzymes both in the theca and granulosa cell and leads to reduce E2 production.[8,9] Clinically, high doses of KCZ (600-1000 mg) in long-term therapy reduce ovarian hyperandrogenism.[10] Within the stimulation phase of ovulation induction, KCZ at a very low dose (50 mg every 48 hours) reversibly inhibits ovarian steroidogenesis in patients with PCOS.[11] There have been no reports of the use of KCZ in ART cycles, other studies such as the Parsanezhad et al[12] study used this drug during ovarian stimulation but not during ART cycles. We therefore conducted this single-blind randomized study to investigate the clinical efficacy of KCZ on ovarian steroidogenesis and the cycle outcome in PCOS patients undergoing ART cycles.

Methods.

Selection of patients. A total of 58 patients, <36-years-old were enrolled for in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) in a randomized clinical trial between November 2000 and October 2001 in Royan Institute, Tehran, Iran. The Institutional Review Board and University of Jahad Ethics Committee approved this study. All patients signed the informed consent forms. All patients had PCOS as defined by clinical, ultrasound and laboratory criteria,[13] and in all of them OHSS either had occurred or there was the risk of its occurrence (>20 follicles in each ovary or E2 >3000 pg/ml) in a previous ART cycle. Women with a history of liver disease or any abnormal liver function tests were excluded.

Ovarian stimulation. All patients received subcutaneous Buserelin acetate (Suprefact, Hoechst, Frankfurt am main, Germany) 0.5 mg daily from the 21st day of the menstrual cycle. After 2 weeks, when suppression was achieved as defined by ultrasound (endometrial thickness <6 mm and inactive ovaries), 150-225 IU human menopausal gonadotropin (HMG) (Pergonal, Organon, Holland) daily was initiated. Ovarian monitoring by vaginal ultrasound, and serum E2 determinations was started on day 5 of stimulation and was repeated every 24-72 hours as required in each case. A dose of 10,000 IU of HCG (Profasi HP, Serono, Italy) was administered when at least one follicle ≥18 mm together with 2 follicles ≥16 mm were visualized and E2 was ≤3000 pg/ml. Oocyte aspiration was performed 35 hours after HCG administration under vaginal ultrasound guidance. Fertilization was achieved through IVF or ICSI and embryo transfer was performed 48 hours after oocyte retrieval. The luteal phase was supported by daily intramuscular injection of 100 mg progesterone (Daroupakhsh, Iran) beginning on the day after oocyte retrieval. Five to ten days after embryo transfer, the patients were interviewed, and physical examinations and pelvic ultrasonography were performed if needed. The degree of OHSS was defined according to the system of Golan et al.[14] In the present study, only moderate and severe forms of OHSS were considered as complications of ovarian hyperstimulation. Clinical pregnancy was diagnosed if a gestational sac with embryonic heart activity was seen by transvaginal ultrasound 4-5 weeks after embryo transfer.

Ketoconazole administration. Random assignment of patients into 2 groups was carried out by the consecutive number method. The patients of group I (n=28) received KCZ while those of group II (n=30) were to serve as controls. The drug was administered 50 mg/day starting on day 4 of stimulation until the day before of HCG injection. In order to evaluate the side effects of KCZ, liver function tests were performed before and after KCZ treatment (on initial and terminal day of KCZ administration).

Data analysis. Statistical evaluation was performed using student's t test, Fisher exact and χ^2 tests. Statistical significance was defined as $p<0.05$ and the results were expressed as means ± SD and percentages. We used Statistical Package for Social Sciences version 11 for data entry.

Results.

The mean ± SD of age was 28.5 ± 4.4 in the patients of group I (treated), and 28.5 ± 3.9 years in group II (control) (not significant). The causes of infertility leading to IVF or ICSI in group I were male factor (n=1), anovulation (n=1), tubal factor (n=3), unexplained (n=2), male and anovulation (n=21). The causes of infertility in group II were male factor (n=15), anovulation (n=4), tubal factor (n=4), unexplained (n=1), and male and anovulation (n=6). There were no significant differences between 2 groups in the total number of HMG ampules per patient treatment, the duration of stimulation, the number of follicles ≥16 mm or the serum E2 levels on the day of HCG administration **(Table 1).** The number of canceled cycles were one in the treated group and 2 in the control group, (not significant) also 2 patients in group I and 2 in group II were underwent coasting (not significant). The fertilization rate and pregnancy rate per cycle were not significantly different between the 2 groups. **Table 1** shows that the number of cases with serum estradiol >3000 pg/ml (4 in group I versus 9 in group II), and also the number of patients that yielded more than 10 oocytes (8 in group I versus 14 in group II) was

Table 1 - The ovarian response to stimulation and the assisted reproductive technology cycle outcome in patients of Group I (ketoconazole [KCZ]), and Group II (control).

Variables	Group I (KCZ) n=28	Group II (control) n=30	*p*-value
Duration of stimulation (days)	12.4 ± 3.1	11.8 ± 3.2	0.500
Human menopausal gonadotropin ampules (n)	26.2 ± 8.8	27.2 ± 11.6	0.703
Peak estradiol before human chorionic gonadotropin (pg/ml)	1801.3 ± 1093.0	2153.5 ± 1374.1	0.305
Follicles ≥16 mm (n)	9.1 ± 4.9	10.4 ± 4.4	0.287
Oocytes retrieved (n)	7.5 ± 5.3	10.0 ± 6.7	0.128
Metaphase II oocyte (n)	6.8 ± 3.5	7.5 ± 4.4	0.871
In-vitro fertilization rate (%)	78.1 ± 20.4	62.4 ± 26.1	0.277
Intracytoplasmic sperm injection rate (%)	58.3 ± 36.9	68.6 ± 32.1	0.359
Total embryos (n)	5.7 ± 4.9	4.1 ± 3.2	0.252
Cancellation rate % (n)	3.6 (1)	6.7 (2)	1
Ovarian hyper- stimulation syndrome rate % (n)	0 (0)	3.3 (1)	1
Pregnancy rate/cycle% (n)	14.3 (4)	10 (3)	0.701
Cases with estradiol >3000 pg/ml (n)	4	9	0.224
Cases with >10 oocytes (n)	8	14	0.123

considerably lower in group I than in group II but the difference was not statistically significant. No patient experienced any known side effect of KCZ treatment.

Discussion. Ketoconazole has been applied successfully to suppress clinical disorders associated with adrenal and testicular steroidogenesis including Cushing's syndrome, prostate cancer, and precocious male puberty. Also, ovarian hyperandrogenism and hirsutism in patients with PCOS has been shown to diminish with KCZ treatment. However, to achieve these effects, KCZ was administered long-term at high dose rates (400-1200 mg/day).[15,16] The present study showed that administration of KCZ (50 mg daily) has no preventive effect on the incidence of OHSS during ART cycles; however, there was no significant negative effect of KCZ on the number and maturity of oocytes or on fertilization rate. Parsanezhad et al[12] studied 109 patients with PCOs referred for treatment with gonadotrophin in a randomized, double-blind, placebo-controlled study and revealed that KCZ at a dose rate of 50 mg every 48 hours does not prevent OHSS,[12] which is similar to the present study. In their study, the number of HMG ampoules and duration of treatment were higher among the KCZ group, while in the present study, the number of HMG ampoules was lower in the KCZ group, but this difference was not significant. Gal et al,[11] reported that administration of KCZ at a very low dose rate during ovarian stimulation reduces the peak serum E2 level, and consequently diminishes the cancellation rate of hyperstimulated cycles, although the number of lead follicles does not change significantly[11] while in the present study we found no significant differences between the KCZ group, and the control group in E2 levels and the numbers of follicles (2 important indicators of OHSS). It is important to notice that Gal[11] used KCZ with protocol of ovulation induction while in the present study, we used KCZ for ovarian stimulation in a long protocol for IVF/ICSI cycles. The difference between these studies may be related to the higher level of stimulation and consequently higher E2 levels resulting from the long protocol compared to Gal's[11] induction ovulation protocol.

All previous studies on the KCZ effect were induction cycles, so that fertilization outcome cannot be evaluated while pregnancy rates were similar in both the KCZ and the control group cycles. Zelinski-Wooten et al[17] reported that Trilostane (3-b Hydrogenase inhibitor), another inhibitor of steroidogenesis, caused a profound reduction of estrogen level in monkeys, and had no effect on the number of oocytes retrieved or their maturity, but there was a reduction in fertilization rate[17] while the present study revealed no significant effect of KCZ on the fertilization rate. In order to reduce side effect, we chose an aromatase inhibitor with short half life, KCZ, dosing in minute amounts and withholding of drug administration 24 hours before triggering of ovulation.

In conclusion, this study showed a considerable but not significant effect of low dose KCZ in modulating peak estrogen levels and number of oocytes (2 important indicators of OHSS). However, it has no significant effect on incidence of

OHSS. Whether this idea, and which earlier and more intense administration of KCZ may reduce OHSS during ART cycles remains to be evaluated in future studies with more patients. Further studies should also assess the effect of KCZ on progesterone levels and endometrial thickness in the luteal phase.

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References

1. Rizk B, Smitz J. Ovarian hyperstimulation syndrome after super ovulation for IVF and related procedures. ***Hum Reprod*** 1999; 7: 320-327.
2. MacDougall MJ, Tan SL, Balen A, Jacobs HS. A controlled study comparing patients with and without polycystic ovaries undergoing in-vitro fertilization. ***Hum Reprod*** 1993; 8: 233-237.
3. Buvat J, Buvat Herbaut M, Marcolin G, Dehaence JL, Verbeeq P, Renouard O. Purified follicle stimulating hormone in polycystic ovary syndrome: slow administration is safer and more effective. ***Fertil Steril*** 1989; 52: 553-559.
4. Ron EL-R. Complications of ovulation induction. ***Baillieres Clin Obstet Gynecol*** 1993; 7: 735-753.
5. Egbase PE, Makhseed M, Al-Sharhan M, GrudZinskas JG. Timed unilateral ovarian follicular aspiration prior to administration of human chorionic gonadotrophin for the prevention of severe ovarian hyperstimulation syndrome in in-vitro fertilization: a prospective randomized study. ***Hum Reprod*** 1997; 12: 2603-2606.
6. Chian RC, Buckett W, Too L, Tan SL. Pregnancies resulting from in vitro matured oocytes retrieved from patients with polycystic ovary syndrome after priming with human chorionic gonadotropin. ***Fertil Steril*** 1999; 72: 639-642.
7. Smith EB, Henry JC. Ketoconazole: an orally effective antifungal agent. Mechanism of action, pharmacology, clinical efficacy and adverse effects. ***Pharmacotherapy*** 1984; 4: 199-204.
8. Weber MM, Will A, Adelmann B, Engelhardt D. Effect of ketoconazole on human ovarian C17, 20-desmolase and aromatase. ***J Steroid Biochem Mol Biol*** 1991; 38: 213-218.
9. Gal M, Orly J, Barr I, Algur N, Boldes R, Diaman TYZ. Low dose ketoconazole attenuates serum androgen levels in patients with polycystic ovary syndrome and inhibits ovarian steroidogenesis in vitro. ***Fertil Steril*** 1994; 61: 823-832.
10. Pepper G, Brenner SH, Gabrilover JL. Ketoconazol use in the treatment of ovarian hyperandrogenism. ***Fertil Steril*** 1990; 54: 438-444.
11. Gal M, Eldar Geva T, Margalioth EJ, Barr I, Orly J, Diamanty Z. Attenuation of ovarian response by low dose ketoconazole during superovulation in patients with polycystic ovary syndrome. ***Fertil Steril*** 1999; 72: 26-31.
12. Parsanezhad ME, Alborzi S, Pakniat M, Schmidt EH. A double blind, randomized, placebo controlled study to assess the efficacy of Ketoconazole for reducing the risk of ovarian hyperstimulation syndrome. ***Fertil Steril*** 2003; 80: 1151-1155.
13. Homburg R, Neiman E. Polycystic Ovary Syndrome United Kingdom: Martin Dunitz; 2001.
14. Golan A, Ron EL-R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. ***Obstet Gynecol Surv*** 1989; 44: 430-440.
15. Engelhardt D, Weber MM. Therapy of Cushing's syndrome with steroid biosynthesis inhibitors. ***J Steroid Biochem Mol Biol*** 1994; 49: 261-267.
16. Mahler C, Verhelst J, Denis L. Ketoconazole and Liarozole in the treatment of advanced prostatic cancer. ***Cancer*** 1993; 71: 1068-1073.
17. Zelinski-Wooten MB, Hess DL, Wolf DP, Stouffer RL. Steroid reduction during ovarian stimulation impairs oocyte fertilization, but not folliculogenesis, in rhesus monkeys. ***Fertil Steril*** 1994; 61: 1147-1155.