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Pregnancy outcome after the use of an aromatase inhibitor for ovarian stimulation

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KEY WORDS
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Objective: This study was undertaken to report the outcome of pregnancies achieved after ovarian stimulation, including the use of the aromatase inhibitor, letrozole, for ovarian stimulation

Study design: A cohort study comparing the outcome of pregnancies achieved after letrozole and other ovarian stimulation treatments with a control group of pregnancies spontaneously conceived without ovarian stimulation.

Results: In 3 tertiary referral centers, there were 394 pregnancy cycles in 345 infertile couples (63 pregnancies with 2.5 mg of letrozole alone or with gonadotropins, 70 pregnancies with 5.0 mg of letrozole, 113 pregnancies with clomiphene alone or with gonadotropins, 110 pregnancies with gonadotropins alone, and 38 pregnancies achieved without ovarian stimulation). Pregnancies conceived after letrozole treatments were associated with similar miscarriage and ectopic pregnancy rates compared with all other groups. In addition, letrozole use was associated with a significantly lower rate of multiple gestation compared with clomiphene citrate.

Conclusion: The favorable pregnancy outcome and low multiple gestation rate of aromatase inhibitors for ovarian stimulation is encouraging for the development of these agents as first-line ovulation induction agents.

Recently, we reported that aromatase inhibitors can be used for ovulation induction or ovarian stimulation with higher pregnancy rates compared with clomiphene citrate (CC) and comparable pregnancy rates to gonadotropins.[1-6] Letrozole was successful in inducing ovulation in women with polycystic ovarian syndrome (PCOS).[1,2] The use of this aromatase inhibitor was associated with high pregnancy rates in women who failed to conceive with CC treatment.[2] Moreover, we found the combined use of an aromatase inhibitor with gonadotropin injection was associated with improved ovarian response and a significant reduction in the gonadotropin dose required for optimum-controlled

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ovarian hyperstimulation.[3,4] This improvement in response to gonadotropin stimulation was not associated the antiestrogenic effects often observed when CC was used in combination with gonadotropins.[3] In addition, we reported that the use of an aromatase inhibitor might benefit women with a poor response to ovarian stimulation with gonadotropins.[4] During these studies, letrozole, was given once a day from day 3 to 7 of the menstrual cycle. In our recent reviews on aromatase inhibitors, we discussed the future avenues for their use for infertility management.[5-7] Recently, others have reported comparable success rates using aromatase inhibitors for ovarian stimulation.[8-11]

A healthy infant after an uneventful singleton pregnancy is the goal of treatment for infertile couples. In this study, we report data on the early outcome of pregnancies that were achieved with aromatase inhibitors for ovarian stimulation compared with the outcome of pregnancies achieved after other ovarian stimulation, including CC and gonadotropins, or with pregnancies conceived without ovarian stimulation.

Material and methods

This was a cohort study that looked at early outcome of pregnancies achieved after treatment with letrozole compared with a control group that included pregnancies achieved spontaneously or after other ovarian stimulation protocols. The treatment groups and the control group were similar in age, duration of infertility, and infertility diagnosis. The study was conducted in 3 tertiary referral academic centers: the Reproductive Biology Unit of Mount Sinai Hospital, the Toronto Center for Advanced Reproductive Technology, and the Montreal Fertility Centre. The first 2 of these clinics were affiliated with the Division of Reproductive Sciences, Department of Obstetrics and Gynecology, the University of Toronto, Canada, whereas the latter clinic was associated with McGill University. Approval for the use of letrozole and for the follow-up of pregnancies achieved after such treatment was obtained from the Institutional Research Board of Mount Sinai Hospital and The Ethics Committee of Montreal Fertility Centre.

We looked at the early pregnancies rates (all positive pregnancy tests) in these infertile couples undergoing cycle monitoring with ovarian stimulation in conjunction with timed intercourse and intrauterine insemination (IUI) during the period from August 1999 until July 2001. The follow-up continued from clinical pregnancy confirmed by ultrasonography until delivery or pregnancy loss.

During the study period, 1650 infertile couples (unexplained infertility, PCOS, or mild male factor) had completed 3045 treatment cycles. The study included the outcome of 394 treatment cycles in 345 patients in which pregnancy was diagnosed by positive pregnancy test (quantitative assay of serum level of beta human chorionic gonadotropin [hCG] >5 U/mL 2 weeks after timed intercourse or IUI). Pregnancy was achieved after ovarian stimulation in 356 cycles, whereas in 38 cycles pregnancies were achieved spontaneously without ovarian stimulation. Treatment cycles included timed intercourse or IUI while undergoing follicular monitoring with the use of serial measurements of serum estradiol and luteinizing hormone (LH) and transvaginal ultrasonography.

CC, gonadotropins injections, and letrozole were used for ovarian stimulation either alone or in combination (CC plus gonadotropins or letrozole plus gonadotropins). CC (Serophene, Serono, Oakville, Ontario, Canada) was administered orally at a dose of 50 to 100 mg/d from day 3 to day 7 of the menstrual cycle. When combined with gonadotropins, gonadotropin injections (Gonal-F, Serono or Puregon, Organon, Scarborough, Ontario, Canada) were started on the last day of CC administration. Letrozole (Femara, Novartis, East Hanover, NJ), was given orally at a dose of 2.5 mg/d in the 2 Toronto clinics, and 5.0 mg/d in the Montreal clinic, from day 3 to day 7 of the menstrual cycle. When combined with gonadotropins, gonadotropin injections were started on the last day of letrozole administration. When given alone, gonadotropin injections started on day 3 of the menstrual cycle. Both highly purified and recombinant gonadotropins were used at a dose of 50 to 300 IU/d depending on the patient's clinical profile. HCG (Profasi, Serono, or Pregnyl, Organon) was given subcutaneously at a dose of 10,000 units to trigger ovulation in most of the cycles when an average of 1 to 2 mature follicles (mean follicular diameter >1.8 cm) were obtained. Pregnancy was diagnosed by beta hCG levels performed 2 weeks from the insemination or timed intercourse day, and pregnancy ultrasound was performed 2 to 4 weeks after a positive pregnancy test to confirm clinical pregnancy by cardiac activity and number of gestational sacs.

Study groups

Pregnancy cycles were grouped according to the treatment used for ovarian stimulation into: pregnancies after treatment with the aromatase inhibitor, letrozole 2.5 mg/d alone (33 pregnancies) or letrozole 2.5 mg with gonadotropins (30 pregnancies), 5.0 mg/d letrozole alone (70 pregnancies), pregnancies after CC alone (80 pregnancies) or CC with gonadotropins (33 pregnancies), pregnancies after gonadotropins alone (110 pregnancies), and pregnancies achieved without the use of ovarian stimulation (38 pregnancies).

The patients were not randomly assigned and the choice of receiving an aromatase inhibitor for ovarian

Table Pregnancy rates and the rates of pregnancy loss (chemical pregnancy, miscarriage, and total pregnancy loss) associated with various ovarian stimulation treatments

Treatment	All started cycles	All positive pregnancy test n (%)	Chemical pregnancy n (% from positive pregnancy test)	Miscarriage n (% from positive pregnancy test)	Total pregnancy loss n (% from positive pregnancy test)
CC	994	80 (8.0)	10 (12.5)	15 (18.8)	25 (31.3)
Gonadotropins alone	671	110 (16.4)	15 (13.6)	12 (10.9)	27 (24.6)
Letrozole (2.5 mg/d)	167	33 (19.8)	6 (18.2)	4 (12.1)	10 (30.3)
Letrozole (5.0 mg/d)	432	70 (16.2)	9 (12.8)	4 (5.7)	13 (18.2)
CC + gonadotropins	205	33 (16.1)	4 (12.1)	3 (9.1)	7 (21.2)
Letrozole + gonadotropins	153	30 (19.6)	2 (6.7)	6 (20.0)	8 (26.7)
Spontaneous (no ovarian stimulation)	423	38 (9.0)	4 (10.5)	7 (18.4)	11 (29.0)
All cycles	3045	394 (12.9)	50 (12.7)	51 (12.9)	101 (25.6)

stimulation was based on discussion between the patient and the prescribing physician, often after previously failing to conceive with another stimulation protocol. At the end of the study period, analysis of the patients' characteristics revealed no significant difference among the study groups on age, duration of infertility, or infertility diagnosis. The number of prior treatment cycles and type of insemination (timed intercourse or IUI) were also comparable (data not shown). The empiric use of stimulation treatments was based on decisions usually shared by the treating physician and patient. We did not use strict algorithms for ovarian stimulation in any of these cases and these factors explain, at least in part, the absence of significant differences in patients' characteristics between the various groups. However, this does not correct the nonrandomized design of this study, and a randomized design may result in more homogenous study and control groups. However, because pregnancy outcome and not the pregnancy rate was being examined, we believe that the results of this study provide useful clinical information.

Statistical analysis

The various outcome measures, including positive pregnancy tests, chemical pregnancies, clinical pregnancies, multiple gestations, and ectopic pregnancies, were expressed as numbers and rates (percentage per treatment cycle). The Student t test, χ^2 test, and Bonferroni t test as well as analysis of variance were used where appropriate to analyze the various data among the study groups. P value less than .05 was considered statistically significant. The statistical tests were performed with SigmaStat for Windows Version 1.0 software (SigmaStat Software HighEdit Professional copyright 1993, MicroHelp Inc, and HeilerSoftware GmbH, San Rafael, Calif).

Results

The Table shows pregnancy rates per cycle and rates of pregnancy loss (chemical pregnancy, miscarriage, and total pregnancy loss) associated with the various ovarian stimulation treatments. Pregnancies achieved after letrozole use were not associated with increased risk for chemical pregnancies or miscarriage. The rates of total pregnancy loss ranged from 18.2% to 31.3% (5.0 mg/d letrozole treatment and CC treatment, respectively). The rate of total pregnancy loss in spontaneous conceptions without ovarian stimulation was 29%. There was no statistically significant difference in rate of pregnancy loss between letrozole alone (2.5 or 5 mg) or with gonadotropins and other treatment protocols. P values were .94, .52, .61, and .93 comparing letrozole 2.5 mg with CC, CC plus gonadotropins, gonadotropins, or spontaneous cycles, respectively. P values were .17, .81, .45, and .33 for letrozole 5 mg and .73, .72, .88, and .88 for letrozole plus gonadotropins for the same comparisons.

The rate of multiple gestation (all multiple gestation cases were twins) was significantly lower with 2.5 mg (P values were .03, .04, .05, and .05) or 5.0 mg/d (P values were .02, .03, .04, and .05) letrozole treatment (alone) when compared with all other ovarian stimulation treatments (CC, CC plus gonadotropins, gonadotropins alone, and letrozole plus gonadotropins respectively) as shown in the Figure. CC treatment (alone or with gonadotropins) was associated with the highest multiple gestation rate when compared with the other ovarian stimulation treatments (letrozole alone or with gonadotropins) and gonadotropins alone. There was no significant difference between the rate of multiple gestation after gonadotropin only treatment and letrozole plus gonadotropins treatment. There were no multiple pregnancies achieved without ovarian stimulation.

There were 9 cases of ectopic pregnancies (all tubal ectopic pregnancies), 1 case after CC plus gonadotropin

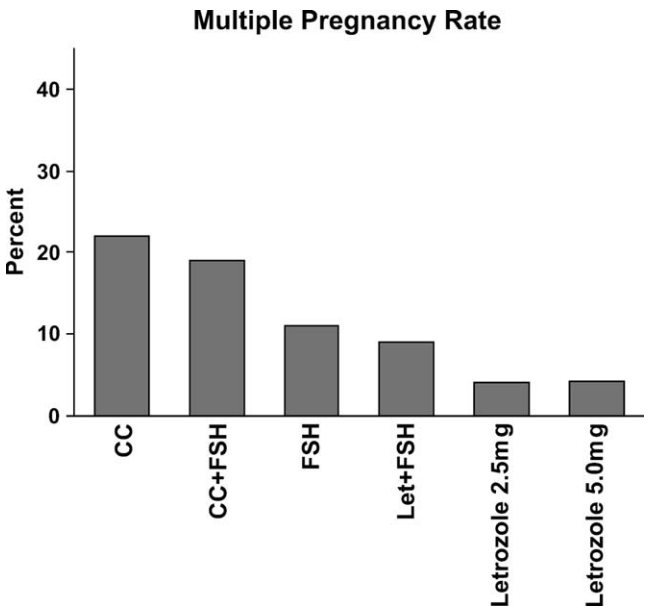

Figure Multiple gestation rates associated with the different ovarian stimulation treatments. There were no multiple gestation cases among pregnancies that occurred spontaneously. Letrozole treatment, at both the 2.5 mg/d and the 5.0 mg/d doses, was associated with significantly lower multiple gestation rates compared with all other methods of ovarian stimulation. Clomiphene treatment (alone or with gonadotropins) was associated with significantly higher multiple gestation rates compared with all other ovarian stimulation treatments.

treatment, one case in the spontaneous pregnancy group (no ovarian stimulation), 2 after CC treatment, 2 after gonadotropins alone treatment, and 3 after letrozole plus gonadotropins treatment. There were no cases of ectopic pregnancy after letrozole only treatment regardless of the dose used. There was no statistically significant difference in the rates of ectopic pregnancies among the various ovarian stimulation treatments. The overall low rate of ectopic pregnancy (2.3%) in these patients may be explained by the absence of any demonstrable tubal damage as a criterion for selection for IUI. It is possible that 1 or more cases of ectopic pregnancy could have occurred in the letrozole groups but spontaneously resolved before clinical detection and were counted as cases of chemical pregnancy (total of 21 cases).

Comment

This study reports the outcome of pregnancies conceived after use of an aromatase inhibitor for ovarian stimulation. Pregnancies achieved after treatment with letrozole for ovarian stimulation were associated with comparable rates of pregnancy loss (chemical pregnancy and miscarriage) but with noticeably lower rates of multiple gestations. Interestingly, no ectopic pregnancies were recorded among 103 patients who achieved a pregnancy with either 2.5 mg or 5.0 mg/d of letrozole.

The success of aromatase inhibitors in inducing ovulation may be based on multiple mechanisms of action. We hypothesized that aromatase inhibition results in a temporary reduction of estrogen production, early in the menstrual cycle resulting in the release of the pituitary and/or the hypothalamus from estrogen negative feedback. This interruption of negative feedback results in an increase in endogenous gonadotropin production that stimulates the development of ovarian follicles. Peripherally, at the level of the ovaries, we also postulated that a possible temporary accumulation of intraovarian androgens, as a result of preventing their conversion into estrogens, would enhance follicle-stimulating hormone (FSH) receptor gene expression[12,13] leading to an increase in the sensitivity of the ovarian follicles to gonadotropins stimulation.

We believe that the short half-life of the new aromatase inhibitors (2 days)[14,15] as well as the reversibility of the aromatase enzyme inhibition results in production of estrogen at more physiologic concentrations during the later part of the follicular phase. This normal increase in estrogen allows healthy development of the follicles as well as the peripheral estrogen-sensitive genital tissues (the endometrium and cervix) and may avoid any undesirable antiestrogenic effects associated with CC treatment. The peripheral estrogen receptor (ER) depletion associated with CC is believed to be responsible for the decreased pregnancy rates, despite high ovulation rates with CC treatment.[16,17]

It was previously suggested that 5.0 mg/d of letrozole may lead to better follicular development and perhaps higher pregnancy rates.[10] In the current study, we failed to observe significant differences in pregnancy rates or outcome between 2.5 mg/d and 5.0 mg/d of letrozole. This finding suggests that 2.5 mg/d of letrozole is sufficient to induce competent ovulation. A large prospective study is required to determine whether 2.5 mg/d or 5.0 mg/d is the optimal dose for ovulation induction.

The rates of pregnancy loss after ovarian stimulation in our series are in concordance with what has previously been reported for infertile patients.[18-21] However, the rates of preclinical pregnancy loss (chemical pregnancy) are slightly higher in our series. We believe that this may be due to our study design in which we measured beta hCG levels very early (2 weeks after timed intercourse or IUI). Together with the higher sensitivity of the currently available bioassays for beta hCG, we would anticipate the detection of more early pregnancy losses. A significant number of early pregnancy losses may be missed when hCG is assayed later or when applying a less sensitive hormonal assay as reported in earlier studies.[22,23]

In this study, CC treatment with or without FSH addition was associated with the highest rate of multiple gestation. The lowest multiple gestation rate was associated with letrozole treatment and is an exciting potential advantage of aromatase inhibitor treatment. In our studies of letrozole for ovarian stimulation, we found CC treatment to be consistently associated with development of more ovarian follicles than with aromatase inhibitor treatment.[1,2] We believe this observation is consistent with the long tissue half-life of CC[24] resulting in long-lasting ER depletion centrally. ER depletion centrally prevents negative feedback suppression of FSH levels by rising E2 from growing follicles and results in multiple follicle development on ovulation. A major advantage of applying aromatase inhibitors for ovulation induction is mono-ovulation, particularly in patients with PCOS. This advantage likely arises from the preservation of the physiologic estrogen feedback mechanisms in the hypothalamus and pituitary caused by the absence of ER depletion with aromatase inhibitor treatment as discussed recently.[25]

Clomiphene treatment was associated with the highest rate of multiple gestations, whereas gonadotropins treatment was associated with much lower multiple gestation rates than expected. We believe that in addition to the nonrandomization design of the study, 2 reasons might also explain the high multiple gestation rate associated with CC treatments: first, the rate of multiple gestation has been calculated per clinical pregnancy rather than per positive pregnancy test. This would increase the rate by decreasing the denominator. Second, multiple gestation was diagnosed on the basis of an early ultrasound (2-4 weeks after a positive pregnancy test). It is known that the rate of multiple gestation decreases with advancing gestational age as sometimes 1 or more of the embryos may vanish away as pregnancy progresses.[26-28] This known phenomenon may lead to a higher rate of multiple gestation when an ultrasound is performed early in pregnancy to determine the number of gestational sacs. On the other hand, the lower than expected multiple gestation rate with gonadotropin treatment may be a function of liberal cycle conversion to in vitro fertilization when stimulation with gonadotropins resulted in a high risk for multiple pregnancy, or to cycle cancellation.

In the current trial, we demonstrate promising clinical pregnancy rates after using an aromatase inhibitor for ovarian stimulation, similar pregnancy outcome compared with other stimulation regimens, and a reduced risk of a multiple gestation. These results suggest that aromatase inhibitors are successful for ovarian stimulation and could possibly be applied in the future as a first-line treatment for World Health Organization (WHO) type 2 anovulation. Moreover, the oral administration of an aromatase inhibitor without the need for close cycle monitoring would enhance the clinical application of these agents for ovarian stimulation in the absence of sophisticated and expensive monitoring for infertility management.

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