Infertility is a fairly prevalent disorder that affects one in seven couples in the reproductive age. Commonly encountered problems include anovulatory disorders (such as anovulation and oligo-ovulation) and infertility that is categorised as unexplained, because conventional evaluation has failed to find a specific cause to explain why a pregnancy has not occurred in the couple presenting for treatment. These problems are usually addressed by treatment regimens involving fertility drugs, either to induce ovulation in women who do not ovulate, or to stimulate the ovaries to produce multiple follicles (the so-called superovulation approach) in regularly ovulating women.

The more commonly encountered ovulation disorder classified by the World Health Organisation (WHO) is group II that includes women with normal levels of FSH and estrogen. They usually have oligomenorrhoea or amenorrhoea, but respond to a progesterone challenge with menstrual bleeding. Women in this group generally have oligo-ovulation or anovulation and are often diagnosed with polycystic ovary syndrome (PCOS). The traditional treatment for these women has been to induce ovulation with clomiphene citrate (CC).

Structurally, CC is a triphenylethylene derivative that exhibits properties of both estrogen agonist and estrogen antagonist. It is a racemic mixture of enclomiphene and zucomiphene, two distinct stereoisomers with different properties. The commercially available preparations of CC have a predominance of enclomiphene, the levels of which increase rapidly after oral administration and decrease to undetectable levels within a few days. In contrast, because of the slower clearance rate, the levels of the less active zuclomiphene remain detectable in the circulation for more than one month after administration and may accumulate if CC is administered over several consecutive cycles (1). The structural similarity of CC to estrogen enables it to bind to estrogen receptors, but unlike estrogen that binds for a relatively short duration, CC remains bound to the receptors for prolonged periods of time resulting in insufficient replenishment of receptors. At the hypothalamic level, this non-replenishment of estrogen receptors creates a false perception that the concentration of circulating estrogen is low, leading to the estrogen-induced negative feedback on GnRH production being lowered thereby increasing the production of gonadotropins that stimulate follicular growth.

The standard regimen for CC use is to begin treatment from day 2-5 of the menstrual cycle at a dose of 50 mg per day for five consecutive days, the dose being increased by 50 mg increments (up to a daily maximum dose of 200 mg) in subsequent cycles if ovulation has not occurred. This approach is generally effective, with rates of ovulation ranging between 50 to 90 percent, the vast majority of patients responding to daily doses of 50 to 100 mg. Among those who ovulated, the majority of pregnancies occurred within the first three cycles (2). However, the overall pregnancy rates are disappointingly low, ranging from 20 to 40 percent (3-5), and the likelihood of miscarriage is higher than would be expected (6). This discrepancy between relatively high rates of ovulation and low rates of pregnancy is believed to be the consequence of the antiestrogenic effect of CC on peripheral targets such as the endometrium (affecting its thickness and maturation) and the endocervix (affecting the production and quality of cervical mucus). The problem is compounded by the long half-life of CC and the persistence of zuclomiphene, both of which lead to an accumulation of the antiestrogenic effect over consecutive cycles of treatment. In addition, because of the prolonged depletion of estrogen receptors in the brain, the circulating levels of estrogen may be quite high as a result of the ongoing stimulation of the follicles by FSH. Such supraphysiological levels of estrogen may be deleterious to the developing oocyte, sperm and embryo and may provide additional explanations for the adverse effects on the outcome of infertility treatment with CC (7).

Women who fail to ovulate or fail to conceive with treatment are categorised as being resistant to CC. The standard approach to their management has been to use gonadotropin preparations (such as human menopausal gonadotropin (HMG) or FSH) to stimulate ovarian follicles to develop. This approach in women with PCOS has met with success, but is more expensive than CC and is associated with a significantly higher risk of multiple pregnancy and ovarian hyperstimulation syndrome, owing to the high sensitivity of the ovary in these women to gonadotropin stimulation. Consequently, alternative methods for inducing ovulation in women with PCOS are desirable.
Studies in animals suggested that the aromatase enzyme, which is necessary for the conversion of androgens to estrogen, could be a potential target for the inhibition of estrogen production; when treated with aromatase inhibitors, both gonadotropin levels and ovarian weight were increased in female rats (8), and multiple ovarian follicles were observed in female primates (9). Aromatase inhibitors are either structurally related to androstenedione and are categorised as steroidal (Type I) inhibitors that act as false substrates and bind irreversibly (suicide inhibition) to the androgen-binding site of the enzyme, or are triazole derivatives categorised as non-steroidal (Type II) inhibitors that bind reversibly and competitively to the heme moiety of the enzyme. The development of inhibitors with more specific action, fewer side effects and lower toxicity has resulted in the current availability, for clinical use in the treatment of breast cancer in postmenopausal women, of three non-steroidal inhibitors (anastrozole, letrozole and vorozole) that represent the third generation of aromatase inhibitors. The drugs are very potent and at doses of 1-2.5 mg/day are able to inhibit over 90% of total body aromatase inhibition, and hence estrogen production (7). Their complete absorption after oral administration and rapid clearance, with a terminal half-life of approximately 45 hours, are attractive properties of the drugs.

Although aromatase activity is present in many tissues, the fact that the ovary is the main source of estrogen production in premenopausal women suggests that aromatase inhibition in humans is likely to be effective in inducing ovulation by releasing the hypothalamus from the negative feedback effect of estrogen. Further, because aromatase inhibitors (in contrast to CC) do not bind to estrogen receptors, the central feedback mechanism remains intact, and production of FSH can be regulated normally by the resulting production of estrogen. Thus, supraphysiological levels of estrogen can be prevented and peripheral targets for estrogen action can respond appropriately and avoid any of the adverse antiestrogenic peripheral effects noted with the use of CC. This theory was tested in a proof of principle study in 12 women with PCOS who, in response to CC, had either failed to ovulate (four women) or had ovulated, but in whom the endometrial thickness was suboptimal at <6 mm (eight women) (10). These women were treated with letrozole in a dose of 2.5 mg/day from days three to seven of the menstrual cycle, followed by administration of hCG (10,000 IU) subcutaneously to trigger ovulation and timed intercourse or intrauterine insemination. Ovulation was confirmed in 3/4 women who previously had not ovulated with CC and in 6/8 women who previously had ovulated with CC but had had thin endometrial development. Two clinical pregnancies (16.7%) were registered, but there is no indication in which group the pregnancies had occurred. The mean endometrial thickness in the group of 12 women was significantly greater, despite the fact that the mean estradiol level on the day hCG was administered was significantly lower, than when CC was used in a previous cycle. The rapid elimination and reversibility of letrozole and the lack of any antiestrogenic effect on the endometrium are believed to account for the observed endometrial response (10). Similar results (ovulation in 71.4% and clinical pregnancy in 42.9%) were observed in another small study using the same protocol for letrozole in up to two treatment cycles in 14 women with PCOS resistant to CC (11). In contrast, in a larger case series of 44 women with CC-resistant PCOS, treated with the same regimen of letrozole, the results were not as encouraging with ovulation in only 54.6% and clinical pregnancy in 13.6% (12). Further, no significant predictors of outcome could be identified among the many clinical variables examined.

It is well known that assessing the efficacy of any new treatment ideally should be undertaken within a randomised controlled trial with sufficient power. Unfortunately, such a study evaluating the efficacy of aromatase inhibitors in women with CC-resistant PCOS has not been performed, probably because an adequate comparator is not available and the use of placebo would be considered unethical. Further, any attempts to determine whether Recently, a randomised trial comparing the daily administration, in up to three cycles, of CC (100 mg) with letrozole (2.5 g), each administered for five days from cycle day 3, followed by hCG to trigger ovulation and timed intercourse, was undertaken in 60 women with CC-resistant PCOS who had commenced treatment with metformin (1500 mg/day in three divided doses) for 6-8 weeks prior to receiving the ovulation inducing drugs (13). The mean endometrial thickness was higher and the mean estradiol level on the day hCG was administered was significantly lower in the group treated with letrozole. Using an intention-to-treat analysis, the clinical pregnancy rate was higher with letrozole compared to CC (36.7% versus 16.7% respectively, odds ratio 2.89, 95% confidence interval 0.75 to 12.31). After excluding the miscarriages that occurred only in the CC group, the ongoing pregnancy rate
was statistically significantly higher with letrozole (36.7% versus 10%, odds ratio 4.82, 95% confidence interval 1.04 to 29.88).

Given that letrozole may have benefit in women with CC-resistant PCOS, it is appropriate to determine whether aromatase inhibitors are equivalent to CC as first-line treatment for ovulation induction in women with PCOS. However, such a study of equivalence would require a sample size so large that it would be financially challenging to organise. Consequently, smaller studies comparing aromatase inhibitors with CC are likely to be carried out. To date, the results of two randomised trials comparing CC (100 mg per day) and letrozole (2.5 mg per day) as first-line treatment for five days from day 3 onwards have been published (14,15). In the first trial involving 106 women with PCOS, the mean endometrial thickness and ovulation rate were significantly higher, and the numbers of follicles >17 mm on the day of hCG administration were significantly lower, with letrozole (14). Although the clinical pregnancy rate per patient was higher with letrozole (21.6% versus 9.1%), the difference was not statistically significant. In the second trial involving 80 women with PCOS, similar (although slightly higher) rate of ovulation and median endometrial thickness were observed with CC, and the numbers of follicles >15 mm in diameter on the day of HCG was significantly lower with letrozole (15). The clinical pregnancy rate was non-significantly higher with letrozole (22.5% versus 17.5%). When the data from these two trials were pooled using meta-analysis with a fixed effects model, the clinical pregnancy rate was not statistically significantly different (odds ratio 1.94, 95% confidence interval 0.88 to 4.23). Although the trend in clinical pregnancy rates was higher for letrozole (crude combined proportions of 22% for letrozole versus 12.6% for CC), the total sample size of 186 from these two trials does not provide sufficient power to detect a clinically meaningful difference between the two preparations.

Collectively, these observations of a trend towards a possible higher efficacy of letrozole when used in women with infertility due to PCOS, either as a first-line treatment or after adding metformin in those resistant to CC, suggest that letrozole may be an effective and better alternative to CC in the management of these patients, but further trials are required to adequately test these hypotheses.

In addition to the use of CC for ovulation induction, it is now well accepted that CC can be used for superovulation in regularly ovulating women (with unexplained infertility) to stimulate the development of more than one follicle so that the likelihood of pregnancy can be increased. Failure to conceive with this approach is believed to be the result of some of the adverse peripheral effects of CC. Although the next step generally is to stimulate multiple follicle development with gonadotropins, the use of aromatase inhibitors may be worthy of consideration. A study was undertaken to evaluate the proof of principle of using aromatase inhibitors in women with unexplained infertility that failed to have the desired outcome with CC (10). In seven women with unexplained infertility, the prior use of CC resulted in ovulation but the endometrial development was poor (<6 mm thickness). These women all ovulated after receiving letrozole in a subsequent cycle, using the same regimen as discussed above, and one clinical pregnancy (14.3%) was achieved. The endometrium was significantly thicker and the estrogen level was significantly lower than when CC had been used in previous cycles.

A search for randomised trials comparing CC with letrozole in women with unexplained infertility identified one double-blind (16), two randomised with computer generated lists (17,18) and one quasi-randomised (based on attendance order) (19) trials. The pregnancy outcome data in the double-blind trial were only provided in percentages, but the actual numbers could be estimated from the information provided. In all trials letrozole was administered for five days from day 3 of the cycle, but in three of the trials (16, 17, 19) the dose of letrozole was 2.5 mg/day, whereas in the fourth trial (18), the dose was 7.5 mg/day. CC in all trials was administered at a dose of 100 mg/day for five days from day 3 of the cycle.

In total, there were 264 subjects who received one or more cycles of treatment. Owing to significant statistical heterogeneity across the trials, the pregnancy outcome data were pooled using the random effects model. When CC was compared to letrozole, the common odds ratio for clinical pregnancy per patient was 0.89, 95% confidence interval 0.46 to 1.65. After excluding the trial with the higher dose of letrozole (18), the common odds ratio was 1.08, 95% confidence interval 0.34 to 3.47. Although the numbers of subjects studied are not large enough to make precise inferences, the limited data fail to demonstrate any preference for CC or letrozole in women with unexplained infertility. No differences were observed in endometrial thickness. These findings are in contrast to those in women with PCOS. Estradiol levels, as expected, were lower in the group receiving letrozole. Interestingly, a study
performed in sexually mature female rats that were 20 weeks of age demonstrated similar results with reduction in estradiol levels, but no difference in endometrial thickness (20), suggesting that the effect of CC on the endometrium in women with PCOS is different from that in women with unexplained infertility. Also, the higher rates of ovulation with letrozole in women with PCOS may have contributed to the improved pregnancy rates observed in this group, an effect not observed in women with unexplained infertility because they all had evidence of ovulation prior to treatment.

The experience with aromatase inhibitors in women with unexplained infertility led to the evaluation of its role when combined with gonadotropins. In a pilot study undertaken to obtain preliminary evidence using the combined approach, 36 women with unexplained infertility or whose partners had mild male factor infertility volunteered to receive letrozole 2.5 mg/day from days 3 to 7 followed by highly purified or recombinant FSH (50-150 IU/day) from day 7 until the day of hCG (21). Another group of 56 women with a similar diagnosis received FSH only (50-225 IU/day) from day 3 onwards until hCG. In a third similar group of 18 women, CC (100 mg/day) was administered from days 5-9 plus FSH (50-150 IU/day) from day 7 until hCG. Lower estrogen levels were observed when letrozole was used but pregnancy rates were similar to the FSH only group. The amount of FSH used was significantly lower in the combined regimens, regardless of whether letrozole or CC was used, but the pregnancy rate with CC was lower. Although the reduction in FSH dosage in the combined groups was by design (the FSH only groups starting treatment earlier in the cycle and with higher doses), the investigators believed the reduction in dose to be due to an enhancing effect of letrozole in two ways. First, by releasing the estrogenic effect on the hypothalamus and/or pituitary gland there is an increase in endogenous FSH production. Second, inhibition at the ovarian level of the conversion of androgens to estrogens may lead to temporary accumulation of androgens that increase follicular sensitivity to FSH via either amplification of the FSH gene receptor or mediators such as the insulin-like growth factor (21).

Another prospective study in women with unexplained infertility and no conception in previous cycles with CC, with or without gonadotropins, was undertaken using the same regimens for letrozole and CC (the choice of drug being left to the patient) (22). Human menopausal gonadotropin (in contrast to FSH) was administered at a fixed dose of 150 IU every alternate day from day 5 onwards (overlapping with CC or letrozole). Although total doses of HMG were similar, the number of mature follicles and serum levels of estradiol on the day of hCG administration were significantly lower in the letrozole group. Endometrial thickness was similar in the two groups. The clinical pregnancy rate was higher (though not statistically significantly) in the CC group (25.9% versus 18.2%).

Collectively, these studies have failed to demonstrate a clear difference in pregnancy probability with letrozole compared to CC, with or without supplementation with gonadotropins, in women with unexplained infertility.

The evidence indicates possible benefit for the use of aromatase inhibitors in women with PCOS, but in women with unexplained infertility there does not appear to be much difference when compared to using CC. There are several issues that require discussion.

First, the optimal dose of the aromatase inhibitor has not been identified. In the randomised trials comparing CC with letrozole in women with unexplained infertility, the use of letrozole at the higher dose (7.5 mg/day) compared to the lower dose (2.5 mg/day) was associated with an apparent change in the direction of the effect of treatment towards letrozole as far as pregnancy rates were concerned. It is possible that this effect is simply due to sampling error and no dose-response effect exists. However, a recent small, randomised trial comparing two daily doses of letrozole (2.5 mg and 5.0 mg) in women with unexplained infertility suggests
otherwise (24). The number of follicles on the day of hCG administration was significantly higher with the higher dose. Also, the clinical pregnancy rate was significantly higher with the higher dose; in part, the magnitude of this difference can be explained by the somewhat lower rate in the lower dose group when compared to the rates in randomised trials that have ranged from 16-25% (16,18,19). Nevertheless, the issue of the optimal dose of aromatase inhibitor needs to be determined by conducting dose-response studies that have sufficient numbers of subjects to permit precise estimates to be derived. The doses currently used are derived from the treatment doses used in postmenopausal women with breast cancer. In a phase I study of anastrozole in premenopausal female volunteers, there was an apparent dose-response effect on numbers of follicles that developed, with the highest mean number of follicles being observed in the group with the highest exposure to the study drug (25). Second, it is not clear whether the aromatase inhibitor should be administered in multiple daily doses for five days (as has been the case with CC administration) or in a single dose. The notion of administering a single dose on day 3 of the menstrual cycle was based on the short half-life of letrozole. It was estimated that a single dose of 20 mg should last about 5 days with complete clearance from the body by day 13, a time when ovulation is expected to occur (26). A small, nonrandomised study was conducted in women with unexplained infertility comparing a single dose (20 mg) of letrozole administered on day 3 (alone or in conjunction with FSH from day 7 at a dose of 50-150 IU/day) with a multiple-dose regimen of 2.5 mg/day administered from days 3-7 (alone or with FSH as described). From the limited data provided, no significant differences were observed between the single-dose and multiple-dose regimens with respect to endometrial thickness, estradiol levels, numbers of follicles and clinical pregnancy in the groups with and without FSH co-stimulation. This study suggested that the single-dose regimen might be an option that could be considered if randomised trials were able to confirm the findings.

Third, aromatase inhibitors were registered for use in postmenopausal women with breast cancer. The current use in premenopausal women with infertility represents 'off-label' use of the drug. Although, such use of drugs is not unusual, concerns about drug exposure in early pregnancy were raised recently (October 18, 2005) by a Canadian study a higher than expected rate of congenital anomalies was observed with letrozole use (27). Among 150 babies born to women who had been treated with letrozole (with or without co-treatment with gonadotropins) for unexplained infertility or PCOS, seven cases (4.7%) of serious malformations were observed, a rate that is much higher than that of 1.8% observed in a control group of 36,050 births in women considered to have a 'low-risk' pregnancy (odds ratio 2.9, 95% confidence interval 1.4 to 5.9) (28). Locomotor malformations and cardiac anomalies were more common in the exposed group compared to the control group (27). Following this report, Novartis, the manufacturer of letrozole (marketed under the name Femara) reviewed its safety database and found two reports of children with birth defects among 13 pregnant women who had received the drug (29). On November 17, 2005, after having had discussions with Health Canada (the health care agency for Canada), officials from Novartis Pharmaceuticals Canada Inc. issued a letter advising Canadian health care professionals that, “Femara (letrozole) is contraindicated in women with premenopausal; endocrine status, in pregnancy, and/or lactation due to the potential for maternal and fetal toxicity and fetal malformations”. On November 28, 2005, The Health Products and Food Branch posted a safety alert on the Health Canada web site alerting all stakeholders of the concerns about the use of letrozole in premenopausal women by publishing the letter that had been issued from Novartis Pharmaceuticals Canada Inc. to Canadian health professionals (30). A spokeswoman from Novartis (Switzerland) had indicated that letters were going to be sent to fertility doctors worldwide to reiterate this warning about the use of letrozole (29). Although the U.S. Food and Drug Administration had not taken any action at that time, the matter was being reviewed (29). Interestingly, this issue had surfaced much earlier in India, where it was reported in 2003 that the Drug Controller-General had commissioned an inquiry into media reports that a Mumbai-based pharmaceutical company had been promoting and marketing letrozole for treating infertility in women without the required regulatory approval (31).

The concern about congenital anomalies was refuted by the findings from a recent observational study undertaken to evaluate outcomes among infertile women receiving CC or letrozole, rather than comparing outcomes in treated infertile women with those of women in the general population conceiving spontaneously. No differences in the overall rate of major and minor congenital anomalies were observed (32). However, it should be noted that these
rates are generally higher than those expected in the general population indicating that it remains to be ascertained whether higher rates are due to the treatment or the disorder. Conclusive evidence of safety or risk of the use of aromatase inhibitors requires studies with much larger sample sizes than those available to date. Resolution of this issue is of utmost importance given the relatively low event rates for congenital anomalies, the limited data from controlled trials on the superior efficacy (or lack thereof) of letrozole compared with CC, and the warning issued by the manufacturer of letrozole regarding off-label use of the drug.

References
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