Section 1 Mild Forms of Ovarian Stimulation

Chapter 1

Oral Agents for Ovarian Stimulation

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Ovarian stimulation aims at the development of one or more of the ovarian follicles to reach the stage of maturity culminating in the release of one or more mature oocytes ready for fertilization. Ovarian follicular development is under the control of local factors inside the ovaries (most of it is poorly understood), as well as hormones produced from extraovarian sources, mainly pituitary gonadotropins. Other hormones may play a role in ovarian follicular development; the extent and details of such a role are not fully understood.

There are two mechanisms for ovarian stimulation: the first involves applying pharmacological agents that mimic endogenous gonadotropins (injectable gonadotropins) that directly stimulate ovarian follicular development through gonadotropin receptors. The second involves pharmacological agents that manipulate and moderate endogenous gonadotropin production. Those agents are oral ovulation induction agents that are believed to stimulate ovulation through moderating estrogen action, a major regulator of endogenous gonadotropin production. This chapter reviews those agents with a focus on the clinical aspects of their use.

Oral agents modulate estrogen action, and hence endogenous gonadotropin production through a direct effect on estrogen receptors, that is, selective estrogen receptor modulators (SERMs), or through modulation of estrogen production (inhibition), that is, aromatase inhibitors, or inhibitors of the estrogen synthesis enzyme (the aromatase enzyme). Clomiphene (clomifene) citrate (CC) is the most commonly used and known SERM and letrozole is the most commonly used and known aromatase inhibitor.

The first successful ovarian stimulation case was reported by Gemzell and his coworkers using human pituitary gonadotropins in 1958, and the first pregnancy was reported two years later [1;2]. One year later, in 1961, Bettendorf and his group reported a similar experience [3]. In the same year, Greenblatt and his coworkers published the first results of ovarian stimulation by an oral agent called at that time MRL/41, later known as CC [4]. Over the last two decades, insulin sensitizers have been introduced into clinical practice for ovulation induction in polycystic ovary syndrome (PCOS) patients with significant insulin resistance. The last decade introduced the success of a new group of oral agents for ovarian stimulation, the aromatase inhibitors. The aromatase inhibitor letrozole has been suggested as an alternative to CC as an agent for ovulation induction and to improve the outcome of controlled ovarian stimulation with gonadotropins. In 2000, we presented the first report in the literature on the success of letrozole in inducing ovulation in anovulatory women with PCOS [5].

Clomiphene Citrate

For more than half a century, CC has been the most commonly used agent for ovarian stimulation. Interestingly, since first reports in the early 1960s, results of CC treatment (ovulation and pregnancy rates) have not changed appreciably, despite the advent of modern immunoassays for steroid hormones, advances in ultrasound technology for cycle monitoring, and the introduction of commercial ovulation predictor kits that allow accurate identification of the midcycle luteinizing hormone (LH) surge. It has been puzzling that CC use has continued all those years as an ovarian stimulation agent despite the fact that CC is known as a pregnancy risk category X. This is particularly important when considering the relatively long half-life of about 5–21 days (depending on the isomer).

Moreover, CC can be stored in body fat. Those facts allow CC to accumulate in the body around crucial times of implantation, organogenesis, and embryogenesis [6–8].

Chemical Structure and Pharmacokinetics

Clomiphene citrate is a non-steroidal triphenylethylene derivative that exhibits both estrogen agonist and antagonist properties, that is, selective estrogen receptor modulator. Estrogen agonist properties are manifest only when endogenous estrogen levels are extremely low. Otherwise, CC acts mainly as an antiestrogen [6]. Clomiphene citrate is a racemic mixture of two distinct stereoisomers, enclomiphene and zuclomiphene, having different properties. Enclomiphene is the more potent antiestrogenic isomer and the one primarily responsible for the ovulation-stimulation actions of CC [6–8]. Enclomiphene has a half-life of few days, while the other isomer, zuclomiphene, is cleared far more slowly with levels detectable in the circulation for more than one month after treatment and may actually accumulate over consecutive treatment cycles [8]. Clomiphene citrate is cleared through the liver and excreted in the stool. About 85 percent of an administered dose is eliminated after approximately six days, although traces may remain in the circulation for much longer [7].

Mechanism of Action

Clomiphene citrate's structural similarity to estrogen allows it to bind to estrogen receptors (ER) throughout the body. Such binding lasts for an extended period of time, up to weeks rather than hours as is the case with natural estrogen. Such extended binding ultimately depletes ER concentrations by interfering with the normal process of ER replenishment [4].

It is believed that the hypothalamus is the main site of action because in normally ovulatory women, CC treatment was found to increase gonadotropin-releasing hormone (GnRH) pulse frequency [9]. However, actions at the pituitary level may also be involved since CC treatment increased pulse amplitude, but not frequency, in anovulatory women with polycystic ovarian syndrome, in whom the GnRH pulse frequency is already abnormally high [10]. The antiestrogenic effect on the hypothalamus, and possibly the pituitary, is believed to be the main mechanism of action for ovarian stimulation. Depletion of hypothalamic ER prevents correct interpretation of circulating estrogen levels, that is estrogen concentrations are falsely perceived as low leading to reduced estrogen negative feedback on GnRH production by the hypothalamus and gonadotropins (follicle-stimulating hormone [FSH] and LH) by the pituitary. During CC treatment, levels of both LH and FSH rise, then fall again after the typical five-day course of therapy is completed. In successful treatment cycles, one or more dominant follicles emerge and mature, generating a rising tide of estrogen that ultimately triggers the midcycle LH surge and ovulation [9;10]. It is important to stress the two main prerequisites for the success of CC ovarian stimulation: presence of reasonable estrogen levels in the body and an intact hypothalamic/pituitary axis capable of producing endogenous gonadotropins.

Regimens of Clomiphene Citrate Administration for Ovarian Stimulation

Clomiphene citrate regimens for ovarian stimulation usually start on the second to fifth day after the onset of spontaneous or progestin-induced menses. Treatment typically begins with a single 50 mg tablet daily for five consecutive days, increasing by 50 mg increments in subsequent cycles until ovulation is induced. Once the effective dose of CC for ovarian stimulation is established, there is no indication for further increments unless the ovulatory response is lost, that is, higher doses will not improve the probability of pregnancy. The day of starting CC treatment has not been shown to affect the ovulation rates, conception rates, or pregnancy outcome in anovulatory women.

The dose required for achieving ovulation is correlated with body weight. However, there is no reliable way to predict what dose will be required in an individual woman. Although the effective dose of CC ranges from 50 to 250 mg/day, lower doses (e.g., 12.5 to 25 mg/day) may be tried in some women who are very sensitive to CC. Most women respond to treatment with 50 mg (52%) or 100 mg (22%). Although higher doses are sometimes required, the success rates are usually very low (150 mg, 12%; 200 mg, 7%; 250 mg, 5%). Most women who fail to respond to 150 mg of CC will ultimately require alternative or combination treatments [11;12].

Pregnancy rates are highest in the early cycles of CC treatment (first three cycles) with a significant decline in the chance of achieving pregnancy beyond the third treatment cycle down to a very low chance beyond the sixth treatment

cycle. For that reason, it is not advisable to continue CC treatment beyond six treatment cycles [11]. It is important to mention here that the abovementioned data come from studies in anovulatory women when CC was used to induce ovulation. On the other hand, the value of CC treatment in enhancing the chance of achieving pregnancy in cases with ovulatory infertility has been questioned [12].

Outcome of Clomiphene Citrate Ovarian Stimulation

In anovulatory women with WHO Type II anovulation, CC has been reported to induce ovulation in 60–80 percent of patients with almost two-thirds responding to 50 mg or 100 mg dosage levels. After up to three ovulatory cycles, cumulative conception was encountered in a little less than two-thirds of patients (about 60 percent). Up to 85 percent pregnancy rate has been reported after five ovulatory cycles with fecundity of about 15 percent in ovulatory cycles [11]. It is important to realize that these figures were reported in anovulatory, young women in whom anovulation was the sole infertility factor. Interestingly, amenorrheic women are more likely to conceive than oligomenorrheic women after CC ovarian stimulation. This is probably because those who already ovulate, albeit inconsistently (oligomenorrheic), are more likely to have other coexisting infertility factors. Generally speaking, failure to conceive within six ovulatory cycles of CC treatment should be regarded as a clear indication to expand the diagnostic evaluation to exclude other infertility factors or to change the overall treatment strategy when evaluation is already complete [13].

Adverse Effects and Drawbacks of Clomiphene Citrate Treatment

Clomiphene citrate is in general a safe medication and usually well tolerated, with most of the side effects being relatively mild. Side effects are rarely severe enough to prevent continuation of treatment. Side effects are generally divided into those related to medication itself and other side effects that are related to ovarian stimulation in general, such as ovarian hyperstimulation syndrome and multiple gestation. Other serious long-term adverse effects of CC treatment have been suggested, including increased risk of ovarian cancer.

Hot flashes, the most common side effect occurring in about 10 percent of all women, is due to the antiestrogenic property of CC and seems to be dose-dependent. They are transient, rarely severe, and typically resolve soon after treatment ends. Other important side effects include visual disturbances; for example, blurred or double vision, scotomata, and light sensitivity are generally uncommon (< 2% prevalence) and reversible. However, there are isolated reports of persistent symptoms long after treatment is discontinued, with more severe complications such as optic neuropathy. Those visual side effects are contraindication for the use of CC that warrants stopping treatment and considering alternative methods of ovarian stimulation. Other fairly common but less serious side effects include breast tenderness, pelvic discomfort, and nausea, all observed in 2–5 percent of CC-treated women [14]. In addition, we have noted relatively common reports of premenstrual syndrome-type symptoms in women on CC [15].

Multiple-Gestation Pregnancy

With CC, ovarian stimulation multifollicular development is relatively common, which increases the risk of multiple gestation, reported to be approximately 8 percent. However, the overwhelming majority of multiple gestations that result from CC treatment are twins. Triplet and higher-order pregnancies are rare [16]. Several studies have shown that the number of multiple-gestation pregnancies can be decreased by the more judicious use of ovarian stimulation agents and by increased monitoring [17;18].

Severe Ovarian Hyperstimulation Syndrome

The incidence of severe ovarian hyperstimulation syndrome (OHSS) after CC treatment is difficult to determine, as definitions of the syndrome vary widely among studies. Mild OHSS (moderate ovarian enlargement) is relatively common, but also does not require active management. When CC induction of ovulation proceeds in the recommended incremental fashion designed to establish the minimum effective dosage, the risk of severe OHSS is remote [13].

Ovarian Cancer

There is an uncertain association of ovarian cancer with CC treatment that has been suggested by two epidemiological studies published early in the last decade. The first was a case–control study concluding that ovarian cancer risk was increased nearly threefold overall in women receiving various infertility treatments including CC [19]. The study methodology had several problems. The study compared infertile treated women to fertile women rather than to infertile untreated women, even though infertility and nulliparity have long been recognized as risk factors for ovarian cancer. In addition, there was no apparent increase in ovarian cancer risk in treated women who conceived. The second study was a cohort study concluding that risk of ovarian tumors was increased in women treated with CC [20]. Comparisons within the CC ovarian stimulation cohort showed no increase in risk with fewer than 12 cycles of treatment. This study too was widely criticized, primarily because it included cancers of varying types and tumors of low malignant potential (e.g., epithelial, germ cell, stromal), where the pathophysiology of each is likely very different.

The results of subsequent studies have been reassuring, but the question of whether treatment with ovulation-inducing drugs increases risk of ovarian tumors or cancer remains unsettled and cannot be summarily dismissed [21–28].

Congenital Anomalies

There is no consensus about evidence that CC treatment increases the overall risk of birth defects or of any specific malformation.

In a review by Scaparrotta *et al.* about potential teratogenic effects of CC, the authors concluded that there was some evidence for increased risk of fetal malformations, particularly neural tube defects and hypospadias, associated with CC exposure. The authors recommended that further investigations are needed to allow safe use of the drug [29].

The National Birth Defect Study (1997–2005) mentioned that several associations have been observed between CC exposure and birth defects. However, the study concluded that we should be careful when interpreting those associations because of the small number of cases, inconsistency of some findings, and inability to separate the effect of CC from the effect of subfertility [30].

Several large series have examined the question and have drawn the same conclusion [31;32]. Earlier suggestions that the incidence of neural tube defects might be higher in pregnancies conceived during CC treatment have not been confirmed by more recent studies [33]. A small study of pregnancy outcome in women inadvertently exposed to CC during the first trimester also found no increase in the prevalence of congenital anomalies [34]. However, most recently, an increase in the risk of congenital malformations of the heart has been suggested, though the study was not designed or powered to answer that question and further studies are needed to confirm or negate such a finding [35].

Pregnancy Loss

A fairly large study reviewed outcomes of 1744 CC pregnancies compared with outcomes of 3245 spontaneous pregnancies. Pregnancy loss was defined as clinical if a sac was seen on ultrasound or if it occurred after six weeks' gestation, and as preclinical if a quantitative human chorionic gonadotropin (hCG) was ≥ 25 IU/L and no sac was seen or pregnancy loss occurred earlier. The overall incidence of pregnancy loss was slightly higher, but not significant, for CC pregnancies (23.7%), compared with spontaneous pregnancies (20.4%). Preclinical pregnancy losses were increased by CC treatment (5.8% vs. 3.9%, $p < 0.01$) and for age ≥ 30 years (8.0% vs. 4.9%, $p < 0.001$), but not for age < 30 years (3.7% vs. 3.0%). Clinical miscarriages were increased by CC for women younger than 30 years (15.9% vs. 11.2%, $p < 0.01$), but not for age ≥ 30 years (20.1% vs. 22.3%) or overall (18.0% vs. 16.4%) [36].

A more recent study looking at rates of spontaneous miscarriage in 62 228 clinical pregnancies resulting from assisted reproductive technology procedures initiated in 1996–8 in US clinics also found that spontaneous miscarriage risk was increased among women who used CC [37]. However, the results of these studies are not definitive. Pregnancy loss after infertility treatment is a complex matter, influenced by several significant confounding factors such as insulin resistance and other genetic factors related to PCOS, the presence of endometriosis or unexplained infertility, and advancing maternal age [38].

Failure of Clomiphene Citrate Treatment

In anovulatory infertility, CC treatment failure is defined into two groups. The first group, ovulation failure (clomiphene resistance), includes patients who fail to ovulate in response to CC ovarian stimulation. The second group, clomiphene pregnancy failure, includes patients who ovulate in response to CC ovarian stimulation but fail to achieve pregnancy. This second group also includes women with ovulatory infertility who failed to achieve pregnancy after CC treatment.

Clomiphene citrate resistance (failure to achieve ovulation) is believed to be due to one of two main reasons: insulin resistance (women with PCOS) and inappropriate indication for CC treatment, for example, use in women with WHO Type I or III anovulation or women with ovulatory dysfunction due to medical disorders that require specific treatments such as thyroid disorders, congenital adrenal hyperplasia, and hyperprolactinemia.

The reasons for clomiphene pregnancy failure (women who ovulate in response to CC ovarian stimulation but do not achieve pregnancy) may be related to a wide variety of underlying infertility factors such as male factor, endometriosis, undiagnosed tubal factor, or endometrial receptivity factors. However, the success of many of these women in achieving pregnancy with alternative ovarian stimulation protocols using injectable gonadotropins or aromatase inhibitors supports the hypothesis that persistent antiestrogenic effects associated with CC might play a major role in the discrepancy between ovulatory rates and pregnancy rates [39–41].

Alternative Approaches for Clomiphene Resistance (Failure to Ovulate)

Longer duration or higher doses of CC treatment have been suggested, such as an eight-day treatment regimen or doses of 200 to 250 mg/day that can be effective when shorter courses of therapy fail. However, longer treatment and higher doses are expected to be associated with more antiestrogenic effects and reduced chances for achieving pregnancy even though ovulation is achieved [13]. Other suggestions included adjuvant treatments including the use of "insulin-sensitizing" agents (e.g., metformin and glitazones), exogenous hCG and combinations (sequential treatment with CC and exogenous gonadotropins) and laparoscopic ovarian drilling, as well as corticosteroids to suppress adrenal androgens. The choice of adjuvant treatment should be based on the patient's history and the results of laboratory evaluation.

Antiestrogenic Effects: Probable Reason behind Clomiphene Citrate Treatment Failure

Clomiphene citrate exerts undesirable adverse antiestrogenic effects in the periphery (endocervix, endometrium, ovary, ovum, and embryo) that are unavoidable due to the long half-life of CC isomers. This could explain the "discrepancy" between the ovulation and conception rates observed in CC-treated patients, that is, explain the clomiphene treatment failure (ovulation but no pregnancy). Adverse effects on the quality or quantity of cervical mucus, endometrial growth and maturation, follicular or corpus luteum steroidogenesis, ovum fertilization, and embryo development have been reported by several studies [42–46]. The endometrium is believed to be one of the most important targets of the antiestrogenic effect of CC treatment. Successful implantation requires a receptive endometrium, with synchronous development of glands and stroma [47]. An interesting study has prospectively applied morphometric analysis of the endometrium, a quantitative and objective technique, to study the effect of CC on the endometrium in a group of normal women. In this study, CC caused a deleterious effect on the endometrium, demonstrated by a reduction in glandular density and an increase in the number of vacuolated cells [48]. In addition, a reduction in endometrial thickness below the level thought to be needed to sustain implantation was found in up to 30 percent of women receiving CC for ovulation induction or for unexplained infertility [44]. This observation has been confirmed by other studies [45;46].

Decreased uterine blood flow during the early luteal phase and the peri-implantation stage has been found with CC treatment [49]. Moreover, a direct negative effect of CC on fertilization and on early mouse and rabbit embryo development has been suggested [50].

Several investigators tried to reverse these antiestrogenic effects by administering estrogen

concomitantly during CC treatment. Some studies reported increased endometrial thickness and improved pregnancy rates with this approach [51;52], while others have reported no benefit [43] or even a deleterious effect of estrogen administration [42]. Another approach has been to administer CC earlier during the menstrual cycle rather than starting on day 5 [53], to allow the antiestrogenic effect to wear off to some extent prior to ovulation and implantation. A third method has been to combine another SERM such as tamoxifen, which has more estrogen agonistic effect on the endometrium with CC, or to use tamoxifen as an alternative to CC [54]. However, none of these strategies have proved to be completely successful in avoiding the peripheral antiestrogenic effects of CC. A more recent publication has suggested that high-dose soy isoflavones may be able to overcome the antiestrogenic effect of CC on the endometrium [55]. This report remains to be confirmed by other investigators.

Aromatase Inhibitors

The aromatase enzyme is a microsomal member of the cytochrome P450 hemoprotein-containing enzyme complex superfamily (P450arom, the product of the CYP19 gene). It catalyzes the rate-limiting step in the production of estrogens, that is, the conversion of androgens (androstenedione and testosterone) into estrogens (estrone and estradiol, respectively) [56;57]. Aromatase activity is present in many normal tissues, such as the ovaries, the brain, adipose tissue, muscle, liver, and breast tissue, as well as in pathological tissues such as malignant breast tumors. The main sources of circulating estrogens are the ovaries in premenopausal women and adipose tissue in postmenopausal women [57]. Three generations of aromatase inhibitors have been developed (Table 1.1). The disadvantages of early generations (Box 1.1) as well as the advantages of third-generation aromatase inhibitors (Box 1.2) are presented (Table 1.1).

The third-generation aromatase inhibitors that are commercially available include two non-steroidal preparations, anastrozole and letrozole, and a steroidal agent, exemestane [58–60]. Anastrozole, ZN 1033 (Arimidex), and letrozole, CGS 20267 (Femara) are the most commonly used aromatase inhibitors in North America, Europe, and other parts of the world for treatment of postmenopausal breast cancer. They are completely absorbed after oral administration, with mean terminal half-life of approximately 45 hours (range, 30–60 hours) and clearance from the systemic circulation mainly by the liver. Mild gastrointestinal disturbances account for most of the adverse events, although these have seldom limited continuation of clinical use. Other adverse effects are asthenia, hot flashes, headache, and back pain based on studies in postmenopausal women [58–60].

Along the last decade, the success of using aromatase inhibitors for ovarian stimulation has been reported, with letrozole the most commonly used aromatase inhibitor [61–68].

Hypotheses of the Mechanism of Ovarian Stimulation by Aromatase Inhibitors

Almost two decades now have passed since the first report of the use of aromatase inhibition for ovarian stimulation. Unfortunately, the underlying mechanisms behind the success of aromatase inhibition for ovarian stimulation have not been

Table 1.1 Different generations of aromatase inhibitors

Generation	Non-steroidal aromatase inhibitors; work by temporary (reversible) inactivation of the aromatase enzyme	Steroidal aromatase inhibitors (sometimes called suicidal inhibitors of the aromatase enzyme); work by permanent (irreversible) inactivation of the aromatase enzyme
First generation	Aminoglutethimide (Cytadren)	N/A
Second generation	Rogletimide Fadrozole (Afema)	Formestane
Third generation	Letrozole (Femara 2.5 mg/tablet) Anastrozole (Arimidex 1 mg/tablet) Vorozole (not marketed)	Exemestane (Aromasin 25 mg/tablet)

Box 1.1 Problems associated with early-generation aromatase inhibitors

Pharmacological disadvantages:

1. Low potency in inhibiting the aromatase enzyme, particularly in premenopausal women (very low potency)
2. Lack of specificity in inhibiting the aromatase enzyme with significant inhibition of other steroidogenesis enzymes, leading to medical adrenalectomy
3. Not all members are available orally (some require parenteral administration)
4. Variable bioavailability after oral administration
5. Variable half-life that changes with the period of administration due to induction of its metabolism

Clinical disadvantages:

1. Poorly tolerated on daily administration, with more than a third of patients discontinuing treatment due to adverse effects
2. Significant side effects related to the aromatase inhibitors, for example, drowsiness, morbilliform skin rash, nausea and anorexia, and dizziness, and side effects secondary to the steroids used for replacement therapy, for example, glucocorticoids
3. Interaction with alcohol with significant potentiation of its action
4. Significant interactions with other medications, for example, coumarin and warfarin
5. Need for replacement therapy due to medical adrenalectomy, for example, glucocorticoid and mineralocorticoid replacement
6. Long-term possible carcinogenesis (at least in animals)

Box 1.2 Advantages of third-generation aromatase inhibitors

Pharmacological advantages:

1. Extreme potency in inhibiting the aromatase enzyme (up to a thousand times the potency of the first-generation aminoglutethimide)
2. Very specific in inhibiting the aromatase enzyme without significant inhibition of the other steroidogenesis enzymes. This is true even at high doses
3. Absence of estrogen receptor depletion
4. Orally administered (other routes of administration are also possible, e.g., vaginal and rectal)
5. Almost 100 percent bioavailability after oral administration
6. Rapid clearance from the body due to short half-life (~ 8 hours for exemestane [Aromasin] to ~ 45 hours for letrozole [Femara] and anastrozole [Arimidex])
7. Absence of tissue accumulation of the medications or any of their metabolites
8. No significant active metabolites

Clinical advantages:

1. Well tolerated on daily administration for up to several years (in postmenopausal women with breast cancer), with few adverse effects
2. Few mild side effects
3. Very safe without significant contraindications
4. Absence of significant interactions with other medications
5. Very wide safety margin (toxic dose is several thousand times higher than recommended efficacious therapeutic dose)
6. Relatively inexpensive

completely elucidated. We believe that there are several mechanisms both centrally (at the level of the brain) and peripherally (at the level of the ovaries and the uterus) that work together.

Central Mechanism

By blocking estrogen synthesis in the brain, and by lowering circulating estrogens by reducing whole body estrogen synthesis, letrozole counteracts the negative feedback effect of estrogen on endogenous gonadotropin production (without depletion of ER as occurs with antiestrogens, e.g., CC). The resulting increase in endogenous gonadotropin secretion will stimulate the growth of the ovarian follicles. Withdrawal of estrogen centrally also increases activins, which are produced by a wide variety of tissues including the pituitary gland [69], and will stimulate synthesis of FSH by a direct action on the gonadotrophs [70].

Peripheral Mechanism

Peripherally, blocking the conversion of androgen substrates to estrogens by aromatase inhibition may increase ovarian follicular sensitivity to FSH stimulation. This is possibly due to the temporary accumulation of intraovarian androgens. There are data showing a stimulatory role for androgens in early follicular growth in primates [71], mediated directly through testosterone augmentation of follicular FSH receptor expression [72;73] and indirectly through androgen stimulation of insulin-like growth factor 1 (IGF-1), which may synergize with FSH to promote folliculogenesis [74;75].

Role of Aromatase Inhibitors in Ovarian Stimulation

Aromatase inhibitors may be used alone for ovarian stimulation, or as an adjuvant in conjunction with injectable gonadotropins. A major advantage of an aromatase inhibitor used *alone* is the ability to achieve restoration of monofollicular ovulation in anovulatory infertility, e.g., PCOS. Both multiple [61;62;64] and single-dose [63] regimens of aromatase inhibitor administered early in the menstrual cycle have shown efficacy in restoring ovulation in anovulatory women. A single dose regimen has the benefit of convenience, but the potential disadvantage of increasing side effects from administration of a larger dose. However, single doses that have been well tolerated were larger than the doses reported for ovarian stimulation [63;76].

The concomitant use of an aromatase inhibitor with injectable gonadotropins has been shown to improve the treatment outcome by reducing the total dose of gonadotropins required for optimum stimulation [64] and to improve the response to gonadotropins stimulation in poor responders [65]. The additional effect of aromatase inhibitors to reduce the supraphysiological levels of estrogen seen with the development of multiple ovarian follicles may also improve treatment outcome [77].

Women Who Might Benefit Most from Use of Aromatase Inhibitors for Ovarian Stimulation

Ovarian stimulation by aromatase inhibitors is associated with significantly lower estrogen production per follicle, hence overall lower estrogen levels. With multiple follicular development, such low estrogen production per developing follicle prevents the achievement of supraphysiological estradiol levels that are inevitable during ovarian stimulation. There are certain groups of women who might benefit from reducing estrogen levels during ovarian stimulation and ameliorating the supraphysiological estrogen levels attained during multiple follicular development. Examples include women who have estrogen-dependent disorders such as endometriosis or breast cancer, or those with an inherent clotting abnormality.

Polycystic Ovarian Syndrome

This group of patients is at particular risk of severe OHSS, particularly during intense stimulation with gonadotropins in assisted reproduction. Aromatase inhibitors may reduce the risk of OHSS in those patients, as discussed earlier, by lowering estrogen levels [78]. In our experience along the last 12 years, combining the aromatase inhibitor, letrozole, with the insulin sensitizer, rosiglitazone, during ovarian stimulation for assisted reproduction in women with PCOS has not resulted in any case of severe OHSS. Rosiglitazone might help in two ways, one by further reduction of estrogen levels through a direct inhibitory effect on the adipose cells' aromatase activity [79], and the other through a direct modulating effect on ovarian steroidogenesis, in particular reducing androgen production [80].

Letrozole may play a role at the level of the endometrium of PCOS women. Estrogen decreases the level of its own receptor by stimulating ubiquitination of ER (ERα). This results in rapid degradation of those receptors. Low estrogen levels decrease ubiquitination, which allows upregulation of the ER and increasing sensitivity to subsequent estrogen rise [81]. This could increase endometrial response to estrogen, resulting in faster proliferation of endometrial epithelium and stroma and improved blood flow to the uterus and endometrium, which might have a positive effect on implantation [82]. This might explain the normal endometrial development during letrozole stimulation despite the observed lower estrogen concentrations in these treated cycles.

Endometriosis

The expression of the aromatase enzyme in endometriotic tissues highlights the possible role played by locally produced estrogen in endometriosis progression [82]. Hence, aromatase inhibitors could be used for treating endometriosis [83]. The inhibition of local estrogen production in endometrial implants, and the lower estrogen levels associated with aromatase inhibition by aromatase inhibitors during ovarian stimulation, could possibly protect against progression of endometriosis during ovarian stimulation. This may improve the outcome of infertility treatment in this group of women. However, this idea still awaits confirmation by clinical trials.

Survivors of Estrogen-Dependent Malignancies Desiring Fertility

Recent advances in oncology including early detection and newer treatments have resulted in increasing numbers of patients surviving cancer following successful treatment. A significant proportion of estrogen-sensitive malignancies, such as breast cancer, affect women in the reproductive age group. Unfortunately, despite successful treatment, the majority of those women usually suffer from ovarian failure following chemotherapy. With the recent success of different fertility preservation options such as embryo and oocyte cryopreservation, some women may opt to freeze embryos or oocytes for later use by themselves or a gestational carrier. Oktay *et al.* reported the success of ovarian stimulation by aromatase inhibitors, letrozole and anastrozole, without a dramatic increase in serum estrogen concentrations, in women undergoing assisted reproduction before receiving cancer treatment. Patients were followed for almost two years after receiving ovarian stimulation with an aromatase inhibitor. During this follow-up period, the cancer recurrence rate was similar to that in patients who had no ovarian stimulation (control patients) [84].

Patients at High Risk of Coagulation Disorders

High estrogen states, both physiological such as during pregnancy or iatrogenic, for example, during estrogen treatment (hormone therapy or estrogen-containing contraceptives) and ovarian stimulation for fertility treatment, have been found to be associated with increased risk of thrombosis. This is particularly significant in women at high risk such as carriers of thrombophilia gene mutations, for example, antithrombin factors II and V [85]. Although it seems logical that those patients might benefit from lower estradiol levels when an aromatase inhibitor is used during ovarian stimulation, there are no data in the literature in support of this hypothesis.

Safety of Aromatase Inhibitors for Ovarian Stimulation

Almost all the data in the literature regarding pregnancy outcomes following the use of aromatase inhibitors for ovarian stimulation relate to the use of the aromatase inhibitor, letrozole. The accumulating data on outcome of babies delivered following letrozole use for ovarian stimulation support its safety. However, because of the short period of clinical experience with letrozole use for infertility treatment, patient understanding of the experimental use of letrozole for such indication is necessary.

Adverse Effects

Most of the data about clinical safety and adverse effects associated with the aromatase inhibitors come from clinical application in postmenopausal women with breast cancer. In this group of patients third-generation aromatase inhibitors were well tolerated, with most of the reported side effects being mild ones, including hot flashes, gastrointestinal events (nausea and vomiting), and leg cramps. Very few patients had to

discontinue aromatase inhibitors due to drug-related adverse events, confirming the high clinical tolerability of aromatase inhibitors [86;87]. It is important to mention here that those reported adverse effects were observed in older women with advanced breast cancer who had received aromatase inhibitors daily over long periods of time, up to several years. Such treatment was obviously for much longer treatment periods than used for ovarian stimulation. In our clinical experience with letrozole use for ovarian stimulation, we have observed few adverse effects such as hot flashes and premenstrual syndrome-type symptoms. Interestingly, most of the patients who had a history of treatment with CC found letrozole better tolerated with fewer side effects. However, there are no clinical trials that have specifically looked at the adverse effects associated with the use of letrozole for ovarian stimulation.

Outcome of Pregnancies Achieved after Ovarian Stimulation with Letrozole

Although animal embryonic safety studies have found the aromatase inhibitor anastrozole to have no teratogenic or clastogenic effects, we do not have clinical data on the safety in babies delivered after its use for ovarian stimulation. On the other hand, there are reassuring data confirming the safety of the pregnancies achieved following the use of letrozole for ovarian stimulation.

We reported early pregnancy outcomes achieved after the use of letrozole for ovarian stimulation [88] compared with the outcome of pregnancies achieved with other ovarian stimulation treatments (gonadotropins and CC), as well as a control group of pregnancies spontaneously conceived without ovarian stimulation. Pregnancies conceived after letrozole treatment were associated with comparable miscarriage and ectopic pregnancy rates compared with all other groups, including the spontaneous conceptions. Later, a large multicenter study [89] that included 911 babies, 514 born after letrozole treatment and 397 after CC treatment, did not find any increase in the rates of major and minor malformations in babies conceived after letrozole treatment [90]. A more recent study that compared babies delivered after letrozole or CC stimulation protocols found a possible risk for low birth weight in the CC group. The babies in the letrozole group were the same percentile of birth weights as the spontaneous conception controls [91]. The short half-life of letrozole and absence of ER antagonism result in a very favorable profile for infertility treatment compared with CC.

A recent Cochrane Database review about the use of aromatase inhibitors in subfertile women with PCOS concluded that the aromatase inhibitor letrozole appeared to improve live birth compared with CC [92].

A landmark multicenter, randomized trial comparing letrozole with CC for ovulation induction in 750 women with PCOS was published in 2014 by the Reproductive Medicine Network funded by the National Institutes of Health (NIH) in the United States [93]. The usual starting doses of letrozole (2.5 mg daily) or CC (50 mg daily) were given from day 3 to day 7 of the cycle for five days for up to five treatment cycles with the dose increased to a maximum of 7.5 mg for letrozole or 150 mg for CC if no ovulation occurred. The ovulation rate was higher in the letrozole group compared with the CC group (61.7% vs. 48.3%, respectively). The cumulative live birth rate was significantly higher in the letrozole group (25.5%) than in the CC group (19.1%; 95% CI 1.1–1.87) with no impact of body mass index on the results. In addition, the twin pregnancy rate was 3.4% in the letrozole group and 7.4% in the CC group [93].

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