Oral agents for ovulation induction: Old drugs revisited and new drugs re-evaluated

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Oral agents for ovulation induction: Old drugs revisited and new drugs re-evaluated

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(met een samenvatting in het Nederlands)

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Chapter 1

Introduction

Current epidemiological evidence suggests that 15% of couples will experience infertility. Background prevalence rates now appear to be reasonably stable, but there is evidence of an upcoming increase in the rate of couples suffering from the problem (1, 2). Farley and Belsey, 1988 (3), have reported estimates of the prevalence of primary infertility by region and country. They estimated 6% for North America, 5.4% for Europe, 3% for the Middle East, 10.1% for Africa, 4.8% for Asia and Oceania, 3.1% for Latin America and 6.5% for the Caribbean. The American Society for Reproductive Medicine (ASRM) estimated that 5 million American heterosexual couples report difficulties in achieving a viable pregnancy, of which 1.3 million seek advice for the problem (4). Absence of ovulation (anovulation) or infrequent ovulation (oligo-ovulation) is seen in a fifth of all women presenting with infertility.

The most widely used treatments for infertility are ovulation induction and superovulation (5). In anovulatory women, the purpose of treatment is to induce the development of at least one follicle, whereas in other causes of infertility ovarian stimulation is used to increase the number of preovulatory follicles; an approach known as superovulation or controlled ovarian hyperstimulation (6). These approaches result in a pregnancy rate of around 8%–15% per cycle depending on the method of ovulation induction and the characteristics of the couple, such as the woman's age and the presence or absence of a male factor. The practice of ovarian stimulation interferes with physiological mechanisms underlying single dominant follicle selection applied in normo-ovulatory women.

Physiology of ovulation

Initiation of growth of primordial follicles, referred to as "primary recruitment", occurs continuously and in a random fashion. Follicle development from the primordial to the preovulatory stage usually takes several months (7). The great majority of primordial follicles that enter this development phase undergo atresia before reaching the antral follicle stage through a process of apoptosis. The degree to which early stages of follicle developments are influenced by FSH remains unclear. Studies in hypophysectomized and transgenic mice suggest that gonadotropins may be involved in the activation of resting follicles (8, 9). However, human FSH receptor mRNA is only expressed from the primary follicle onward. Studies in women with a mutated FSH β-subunit have shown follicular growth to occur up to the stage of secondary recruitment (10).

In addition, exogenous FSH can stimulate follicle growth up to the preovulatory stage in hypophysectomized women (11). Factors such as TGF-α from theca cells, growth differentiation factor 9, and bone morphogenetic protein 15 produced by the oocyte may limit the effects of FSH on granulosa cell differentiation and follicle development at this early stage (12). Only at more advanced stages of development do follicles become responsive to FSH and obtain the capacity to convert the theca-cell derived androstenedione to estradiol (E2) by the induction of the aromatase enzyme activity (13,14). Due to the demise of the corpus luteum during the late luteal phase of the menstrual cycle, E2, inhibin A, and progesterone (P) levels fall. This results in an increased frequency of pulsatile GnRH secretion, inducing rising FSH levels at the end of the luteal phase (15, 16). Although each growing follicle may initially have an equal potential to reach full maturation, only those antral follicles that happen to be at a more advanced stage of maturation during this intercycle rise in FSH (levels surpassing the so-called threshold for ovarian stimulation) gain gonadotropin dependence and continue to grow (17). This process is referred to as cyclic, gonadotropin-dependent or “secondary” recruitment, as opposed to the initial gonadotropin-independent “primary” recruitment of primordial follicles (18).

In the subsequent follicular phase, FSH levels plateau during the initial days (18, 19) and are gradually suppressed thereafter by ovarian inhibin B (20) and E2 (21) negative feedback.

Gonadotropin withdrawal studies have demonstrated the association between FSH, LH, and inhibin production (22, 23). There is a direct endocrine role for inhibin A in the negative feedback on pituitary FSH production, whereas inhibin B does not contribute to the dynamic changes within a menstrual cycle (24-27). Decremental follicular phase FSH levels (effectively restricting the time where FSH levels remain above the threshold, referred to as the FSH window) appear to be crucial for selection of a single dominant follicle from the recruited cohort (28). As FSH levels fall, all but the dominant follicle (with its increased sensitivity to FSH) lose the stimulus to further development and become atretic (29).

The important concept of increased sensitivity of the dominant follicle for FSH has been confirmed by human studies showing developing follicles to exhibit variable tolerance to GnRH antagonist-induced gonadotropin withdrawal (30, 31). Recent evidence also points to a central role for LH in monofollicular selection and dominance in the normal ovulatory cycle. Although granulosa cells from early antral follicles respond only to FSH, those from mature follicles exhibit receptors to both gonadotropins. The maturing dominant follicle may become less dependent on FSH because of the ability to respond to LH (32, 33). Cyclic variation in the expressed isoforms of FSH (differing in oligosaccharide structure, the degree of terminal sialylation and sulfation) has been described (34). A greater proportion of less acidic circulating FSH isoforms are observed during the late follicular phase and midcycle (35, 36). The half-life of human FSH secreted 2–3 days before ovulation is considerably shorter than during the early follicular phase (37). It has been suggested that the preferential secretion of short-lived isoforms during the periovulatory period indicates the existence of regulatory mechanisms that control the intensity and duration of the FSH signal delivered to the ovary (38).

Evolution of ovulation induction

The idea of the existence of the endocrine pituitary-gonadal axis, and its control over ovulation, arose early in the 20th century when it was observed that lesions of the anterior pituitary resulted in atrophy of the genitals. Fevold et al. in 1931 (39) gave the first convincing evidence supporting the existence of two separate gonadotropins (initially referred to as Prolan A and Prolan B), and both LH and FSH were subsequently isolated and purified. Three years before, Ascheim and Zondek (40) described the capacity of urine from pregnant women to stimulate gonadal function. In 1940, Hamblen (41) reported the ability of purified pregnant mare serum to induce ovulation in humans by intravenous administration. However, these early attempts were unsuccessful due to species differences and resulting antibody formation impacting on efficacy and safety. Clinical experiments in the late 1950s demonstrated that extracts derived from the human pituitary could be used to stimulate gonadal function (42). Subsequently, experiments involving the extraction of both the gonadotropic hormones LH and FSH from urine of postmenopausal women led to the development of human menopausal gonadotropin (hMG) preparations. From the early 1960s, these were used for the stimulation of gonadal function in the human (43). The FSH to LH bioactivity ratio of registered hMG preparations is 1:1. As purity improved, it was necessary to add hCG to maintain this ratio of bioactivity (44). Improved protein purification technology allowed for the production of hMG with reduced amounts of contaminating nonactive proteins and eventually the development of purified urinary FSH (uFSH) preparations by using monoclonal antibodies since the early 1980s (45). Through recombinant DNA technology and the transfection of human genes encoding into Chinese hamster ovary cell lines, the large scale in vitro production of human recombinant FSH (recFSH) has been realized (46, 47). The first pregnancies using this preparation in ovulation induction (48) and in IVF (49, 50) were reported in 1992. The first report on the design of a long-acting FSH agonist was by Boime and co-workers (51), who used site-directed mutagenesis and gene transfer techniques to manufacture FSH-carboxy-terminal peptide (CTP). a recent dose-finding study showed that a single dose of FSH-CTP can indeed induce and maintain multifollicular growth for an entire week (52).

Clomiphene citrate (CC)

A second important development allowing for ovarian stimulation on a large scale arose when the first estrogen antagonist tested in cancer patients was found to induce ovulation. It is now more than 45 years since Greenblatt first reported a new compound, the anti-estrogen MRL-41, capable of inducing ovulation for anovulatory women (53). Otherwise, known as clomiphene citrate (CC), this preparation became the first-line treatment for women with absent or irregular ovulation due to hypothalamic–pituitary dysfunction associated with normal basal levels of endogenous estradiol (WHO group II). Many years later, a further suggested indication was its empirical use for ovarian stimulation in unexplained infertility. With the introduction of assisted reproductive technology, it became widely used as in conjunction with intrauterine insemination (IUI) and as adjuvant to gonadotrophin stimulation of the ovaries in IVF. The fact that CC is an orally administered, relatively cheap preparation has proved an enormous advantage over its competitors.

CC contains an unequal mixture of two isomers as their citrate salts, enclomiphene and zuclomiphene. Zuclomiphene is much the more potent of the two for induction of ovulation, accounts for 38% of the total drug content of one tablet and has a much longer half-life than enclomiphene, being detectable in plasma one month following its administration (54). CC, a non-steroidal compound closely resembling estrogen, is capable of blocking hypothalamic estrogen receptors, signaling a lack of circulating estrogen to the hypothalamus and inducing a change in the pattern of pulsatile release of GnRH and FSH from the anterior pituitary enough to lead to ovulation. In normal women, CC increases the frequency rather than the amplitude of gonadotrophin pulses (55), while in patients with PCOS an increase in the amplitude has been reported (56). When CC is given to normal women for 5 days, FSH concentrations increase during the period of administration and decline after the end of the treatment (57). This opens a window of FSH increase similar to the intercycle rise of FSH which occurs in normal women during the critical period of follicle recruitment and selection (58, 59).

Many trials defined the place of CC in the field of ovulation induction. A systematic review of four crossover RCTs that compared CC with placebo in patients with amenorrhoea/oligomenorrhoea, including PCOS found that all doses of CC were associated with increased pregnancy rates per treatment cycle (OR 3.41, 95% CI 4.23 to 9.48) and with increased ovulation (OR 4.6, 95% CI 2.84 to 7.45) (60). These RCTs involved women with a variety of ovulatory disorders, including some who had low oestrogens and would not be expected to benefit from anti-oestrogen treatment, so this may be an under-estimate of the effectiveness in women with PCOS. In view of the available data, there were no sufficient evidences about when to start CC and for how long should the treatment continue.

CC and tamoxifen have been shown to have similar effects on pregnancy rate and ovulation in anovulatory women with infertility (61). Similar results were found in three other studies, including a quasi-randomised study (62-64). One RCT showed that tamoxifen/clomifene citrate combination therapy did not improve pregnancy rate per cycle (65). About 70% of anovulatory women ovulate in response to CC treatment, (66, 67) and they do so at a dose of 50–100 mg, (68) the maximum dose being 250 mg. Although the British National Formulary recommends a maximum of six cycles of clomifene citrate (69) this relates to the number of cycles in one course of treatment. In clinical practice, many women will require more than one course of treatment and this will result in administration of more than six cycles of CC. There may be benefit in receiving CC in up to 12 cycles as cumulative pregnancy rates continue to rise after six treatment cycles before reaching a plateau, comparable to that of the normal fertile population, by cycle 12 (70, 71) However, use of CC for 12 or more cycles has been associated with an increased risk of ovarian cancer in one study (72). In women with unexplained infertility, CC treatment compared

with no treatment increased clinical pregnancy rates per woman and per treatment cycle (73). The RCTs identified by the review were generally of poor quality and underpowered and so this small treatment effect could be offset by one further medium-sized trial if one becomes available.

A compilation of published results regarding ovulation and pregnancy rates following treatment with CC reveals an ovulation rate of 73% and a pregnancy rate of 36% (74). The 27% of anovulatory women with normal FSH concentrations who do not respond at all are considered to be 'CC resistant'. Inability of CC to induce ovulation is more likely in patients who are obese, insulin resistant and hyperandrogenic compared with those who do respond (75-77). In anovulatory women, there is a significant association between clomifene citrate treatment failure and increased BMI (BMI greater than 27.2 kg/m2 or greater than 30.6 kg/m2) (78, 79). A weight loss program may improve ovulation and pregnancy outcomes in women who are obese and infertile for all forms of fertility treatment, including ovulation induction, IUI and IVF (80, 81). Advice on weight reduction may improve response to CC treatment; a modest weight reduction of 5% of initial body weight can result in improvement in endocrine and ovulatory function of obese women with PCOS (82).

It is frustrating that the restoration of ovulation by CC does not produce a much higher pregnancy rate. This discrepancy between ovulation and pregnancy rates (only 50% of those who ovulate will conceive) may be partly explained by the peripheral anti-estrogenic effects of CC at the level of the endometrium and cervical mucus or by hypersecretion of LH (83). While the depression of the cervical mucus may be overcome by performing IUI, suppression of endometrial proliferation, unrelated to dose or duration of treatment but apparently idiosyncratic, indicates a poor prognosis for conception if the endometrial thickness on ultrasound scanning does not reach 8mm at ovulation (55). CC not only increases the desired FSH but also produces an increase in LH concentrations. This increase in LH, whose basal level is often already high in women with PCOS, may compromise pregnancy rates in those receiving CC (83).

However, there is little or no compelling evidence to support these notions. The quality and quantity of cervical mucus production in CC treatment cycles may sometimes be reduced (84), but rarely to an extent that jeopardizes the effective capture, survival, or transport of sperm. Limited endometrial proliferation has been observed in some CC-treated patients (53), but the effect is minor or not at all evident in the large majority of women (85-87). Although some studies have suggested that fecundity may relate to endometrial thickness, others have failed to demonstrate any significant correlation. CC has indeed been shown to inhibit steroid hormone production by cultured avian (88), ovine (89), and human granulosa/luteal cells (90), but estrogen and P levels in CC-induced cycles are typically significantly higher, not lower, than in spontaneous cycles. Adverse effects of CC on mouse ovum fertilization and embryo development have been demonstrated in vitro (91), but circulating levels of CC never reach the concentrations required to produce these effects, even after several consecutive treatment cycles (92). Taken together, available evidence and accumulated clinical experience suggest that any adverse antiestrogenic effects of CC present no significant obstacle in the majority of treated women.

Adjuvant therapies in ovulation induction

In order to improve the outcome of treatment with CC, several adjuvant therapies have been suggested such as human chorionic gonadotropin (hCG), dexamethazone, metformin, bromocriptine and N-Acetyl cysteine (NAC). N-Acetyl cysteine (NAC) is the acetylated variant of the amino acid L-cysteine. It is an excellent source of sulfhydryl groups and is converted in vivo into metabolites that stimulate glutathione production, promote detoxification, and act directly as free-radical scavengers. It is primarily a powerful antioxidant (93); it has activity on insulin secretion in pancreatic cells and on insulin receptors on human erythrocytes (94-98). NAC

has antiapoptotic effects (99). Apoptosis is responsible for the process of follicular atresia. N-Acetyl cysteine was found to inhibit apoptosis in cultured ovarian primordial germ cells. It also preserves vascular integrity, lowers serum homocysteine levels, and is protective against ischemic injuries (100-103). N-Acetyl cysteine is immunologically active; it has an anticytokine effect and inflammatory-modulating capacity (104). Animal studies proved that the drug is neither teratogenic nor mutagenic (105). Overdose of the drug causes minimal allergy, especially after IV administration, and there are no contraindications for its use, apart from known hypersensitivity to the NAC (106).

Because it is an insulin sensitizer, NAC was proposed as an adjuvant to clomiphene citrate for ovulation induction in patients with polycystic ovary syndrome. Bedaiwy et al. (2004) used NAC for ovulation induction in unexplained infertility while Rizk et al. (2005) showed the efficacy of NAC plus CC in treatment of anovulation in polycystic ovary syndrome (107, 108).

Aromatase inhibitors (AIs)

Aromatase is a microsomal cytochrome P450 hemoprotein-containing enzyme (the product of the CYP19 gene). It catalyzes the rate-limiting step of the conversion of androstenedione and testosterone to estrone and estradiol, respectively (109). Aromatase activity is present in many tissues, e.g. ovaries, brain, adipose tissue, muscle, liver, breast tissue, and malignant breast tumors. A large number of aromatase inhibitors (AIs) have been developed over the last 30 years with the most recent, third-generation AIs used mainly for breast cancer treatment in postmenopausal women. The third-generation AIs have been developed over the last 10 years after the second- (Fadrozole and Formestane, developed around 15 years ago) and first-generation AIs (aminoglutethimide, developed around 30 years ago). The unsuccessful career of the first two generations was mainly due to unsatisfactory potency or specificity in inhibiting the aromatase enzyme and significant side effects associated with their use (110-112).

The third-generation AIs include two nonsteroidal preparations, anastrozole and letrozole, and a steroidal agent, exemestane. Letrozole and anastrozole are reversible, competitive AIs with considerably greater potency than aminoglutethimide (>1000 times) and, at doses of 1–5 mg/d, reduce serum estrogen levels by 97% to 99%. AIs are completely absorbed after oral administration with mean terminal half-life of 30–60 h with clearance mainly by the liver (113-115). Exemestane is a steroidal, irreversible inhibitor of aromatase enzyme with a circulating half-life of approximately 9 hours (116).

It was postulated that it would be possible to block estrogen-negative feedback, without depletion of estrogen receptors by administration of an AI in the early part of the menstrual cycle. Both circulating estrogen (produced mainly by the ovarian follicles and peripheral conversion of androgens in fat and other tissues) and locally produced estrogen in the brain exert negative feedback on gonadotropin release (117-120). Inhibition of aromatization will block estrogen production from all sources and release the hypothalamic/pituitary axis from estrogenic negative feedback. The resultant increase in gonadotropin secretion will stimulate growth of ovarian follicles. Withdrawal of estrogen centrally also increases activins, which are produced by a wide variety of tissues including the pituitary gland (121) and will stimulate synthesis of FSH (122). Because AIs do not deplete estrogen receptors normal central feedback mechanisms remain intact. As the dominant follicle grows and estrogen levels rise, normal negative feedback occurs centrally, resulting in suppression of FSH and atresia of the smaller growing follicles. A single dominant follicle, and mono-ovulation, should occur in most cases. This might be of advantage in cases of PCOS thereby avoiding the risk of ovarian hyperstimulation syndrome (OHSS).

A second hypothesis that may contribute to the mechanism of action of the AIs in ovarian stimulation involves an increased follicular sensitivity to FSH. This could result from temporary accumulation of intraovarian androgens because conversion of androgen substrate to estrogen is blocked by aromatase inhibition. Testosterone was found to augment follicular FSH receptor expression in primates (123-125). Also, androgen accumulation in the follicle stimulates IGF-I, which may synergize with FSH to promote folliculogenesis (126-129). It is possible that aromatase inhibition, with suppression of estrogen concentrations in the circulation and peripheral target tissues, results in up-regulation of estrogen receptors in the endometrium, leading to rapid endometrial growth once estrogen secretion is restored. As a result, normal endometrial development and thickness should occur by the time of follicular maturation.

As a result of the mechanisms of action of the AIs it could be used alone for induction of ovulation or as an adjuvant in conjunction with exogenous FSH or other medications to improve the outcome of ovulation induction. A major advantage of an AI used alone is the ability to achieve restoration of monofollicular ovulation in anovulatory infertility, e.g. PCOS. An AI could also be used in conjunction with FSH injections to increase the number of preovulatory follicles that develop and improve the outcome of treatment.

Mitwally and Casper (2000) performed the first trial of aromatase inhibitors for ovulation induction in a group of PCOS women who had failed to respond to CC (130, 131). Twelve women with PCOS received letrozole 2.5 mg daily from d 3 to 7. Ovulation occurred in nine patients (75%), and pregnancy was achieved in three cycles (25%), two of which were singleton clinical pregnancies and one was a chemical pregnancy. Patients in the second group were ovulatory women with unexplained or mild male factor. All women ovulated with endometrial thickness greater than 7 mm. A singleton clinical pregnancy (10%) resulted from timed intrauterine insemination (IUI) in one unexplained infertility couple who developed two follicles greater than 1.5 cm.

Another prospective, randomized, controlled trial of CC vs. an AI for augmentation of ovulation in women with unexplained infertility was carried out by Sammour and colleagues (132). Twenty-four patients were randomized to receive CC 100 mg daily from cycle d 3 to 7, and 26 patients received letrozole 2.5 mg daily from d 3 to 7. Using standard hormonal and ultrasound monitoring, the investigators showed that the CC group had a mean of two preovulatory follicles, whereas the letrozole group produced a mean of one. Estradiol levels on the day of hCG were significantly elevated in the CC group (2300 pmol/liter), compared with the letrozole group (600 pmol/liter). Endometrial thickness and blood flow were significantly less with CC (6.9 mm and pulsatility index of 3.6), compared with letrozole (8.6 mm and pulsatility index of 3.1) on the day of hCG. The pregnancy rate was 5.6% in the CC group vs. 16.7% in the letrozole group ($P>0.05$). Both groups of investigators concluded from these preliminary studies that AIs were an effective alternative to CC, particularly in cases with recurrent CC failure. Since this first study, some studies supporting the success of AIs in ovulation induction for infertility treatment has been accumulating (133-137).

Most studies of aromatase inhibitors have employed letrozole. However, anastrozole, another third-generation AI similar to letrozole, was used in other studies. It is currently not known whether there are any clinically significant pharmacological differences between letrozole and anastrozole, especially regarding efficacy of ovulation induction (138).

Many trials investigated the idea of combining AIs with FSH injection to reduce the dose of FSH required to achieve optimum controlled ovarian stimulation (COH), without adverse

antiestrogenic effects. All the trials found a significant reduction in the FSH dose required (from 45 to 55%). They concluded that friendly protocols of stimulation utilizing AIs will markedly reduce the cost of infertility treatment by decreasing the FSH dose required for optimum ovarian stimulation. In an observational cohort study by Mitwally and Casper (2002) (139), 12 patients with unexplained infertility and a poor response to ovarian stimulation with FSH in at least two cycles were studied. Letrozole was given at a dose of 2.5 mg from d 3 to 7 after onset of menses, and FSH injection was started on d 7 of the menstrual cycle. The mean number of mature follicles was significantly higher than in FSH-only cycles. The amount of FSH required was significantly lower in the letrozole plus FSH cycles than the FSH-only cycles (616 ± 454 vs. 1590 ± 708 IU, respectively). Three of the women conceived a clinical pregnancy with the combined letrozole and FSH treatment. In this clinical trial, there was a benefit of aromatase inhibition in improving ovarian response to FSH stimulation in poor responders.

The optimal dose of each AI is not yet clear. In most of the studies to date, the dose of letrozole (2.5 mg) or anastrozole (1.0 mg) typically used for breast cancer treatment in postmenopausal women has been chosen. Biljan et al. (140) in a randomized study comparing 2.5 and 5.0 mg of letrozole in women with unexplained infertility suggested that the higher dose might be associated with more follicles developing. However, the study was not large enough to demonstrate a significant advantage. Another study by Healey et al. (141) used a dose of 5.0 mg of letrozole together with FSH in women undergoing IUI. Similarly, a study using 7.5 mg letrozole from cycle d 3 to 7 showed, for the first time, a thinning of the endometrium similar to CC (142).

Aromatase inhibition is associated with significantly lower serum estrogen levels at midcycle and per mature follicle than found with CC (143, 144). The question whether low or very low intrafollicular estrogen is compatible with follicular development, ovulation, and corpus luteum formation has been reviewed before (145). Markedly reduced to absent intrafollicular concentrations of estrogen are known to be compatible with follicular expansion, retrieval of fertilizable oocytes, and apparently normal embryo development (146). The rapid clearance of the AIs, the reversible nature of enzyme inhibition, and elevated levels of FSH, which induces new expression of aromatase enzyme, are factors that limit accumulation of androgens and likely result in increasing estrogen production that should be relatively normal at the time of ovulation. This conclusion has now been confirmed by the use of AIs in in vitro fertilization (IVF) reviewed below.

There is no evidence that CC treatment increases the overall risk of birth defects or of any one anomaly in particular (147, 148). Early studies suggested that the incidence of spontaneous abortion in pregnancies resulting from CC treatment was increased over that observed in spontaneous pregnancies. However, a number of more recent studies have described abortion rates that are not different from those observed in spontaneous pregnancies (10% to 15%) (149). Recently, the safety of letrozole and may be other aromatase inhibitors for ovulation induction were seriously questioned. Health Canada and Novartis Pharmaceuticals issued a warning that letrozole should not be used for ovulation induction because of the potential for fetal toxicity and malformations. This warning was based on an abstract by Biljan et al. (2005) (150) in which six congenital abnormalities and one case of hepatocellular carcinoma were reported among 150 births resulting from the use of letrozole for ovulation induction. Another cohort study by Mitwally et al. (2005) compared the outcome of pregnancies achieved after letrozole and other ovarian stimulation treatments with a control group of pregnancies spontaneously conceived without ovarian stimulation. Pregnancies conceived after AI treatment was associated with comparable miscarriage and ectopic pregnancy rates, compared with all other groups including the spontaneous conceptions.

Despite recent developments in ovarian stimulation and assisted reproductive technologies, there has not been a corresponding increase in implantation rates. Supraphysiological levels of estrogen may explain some of the adverse effects of ovarian stimulation on infertility treatment, including deleterious effects on the endometrium (151, 152) and embryo (153, 154). Basir et al. (155) have shown that high response to COH for IVF results in endometrial glandular and stromal dyssynchrony as assessed by morphometric analysis. A step-down protocol has been proposed for IVF to lower E2 concentrations and improve successful implantation (156). An alternative approach is using an oral gent for ovarian stimulation such as CC and AI to significantly suppress E2 levels around d 3–7 of the menstrual cycle. In a randomized, controlled study, Goswami et al. (157) compared an AI plus FSH protocol with a standard GnRH agonist and FSH protocol in poor responders undergoing IVF. Although this was a small pilot study including only 38 patients, the results suggested that the addition of an AI to a small dose of FSH resulted in a similar number of oocytes retrieved, embryos transferred, and pregnancy rate as observed in the women on the standard protocol. The authors concluded that AIs could be a low-cost alternative to natural-cycle IVF in patients who are poor responders to FSH. Garcia-Velasco et al. (158) evaluated the use of an AI as an adjuvant to FSH treatment in IVF cycles of poor-responder patients. In this study, 147 low responders were enrolled. The study demonstrated that patients receiving an AI had higher numbers of oocytes retrieved and had a higher implantation rate despite receiving the same doses of FSH/human menopausal gonadotropin as the control group.

Women with breast cancer who are undergoing ovarian stimulation to have oocytes for cropreservation before starting chemotherapy have unique situation in which low estrogen level is required. Oktay et al. (159) studied 60 women with breast cancer. 29 patients underwent 33 ovarian stimulation cycles with either tamoxifen 60 mg/d alone or in combination with low-dose FSH or letrozole 5 mg in combination with FSH. Compared with women receiving tamoxifen alone, there was a significant increase in the mean number of mature oocytes retrieved in the group receiving letrozole-IVF (8.5) and TamFSH-IVF (5.1). The mean number of embryos cryopreserved was also significantly increased (5.3 and 3.8, respectively). Peak E2 levels were significantly lower with letrozole-IVF, compared with TamFSH-IVF. After almost 2 yr of follow-up, the cancer recurrence rate was similar between IVF and control patients. The authors concluded that both tamoxifen and letrozole added to FSH could increase the number of oocytes retrieved for IVF in breast cancer patients, but the letrozole protocol may be preferred because it resulted in lower peak E2 levels.

Aim of the thesis

The aim of this thesis is to address a number of inquiries regarding oral agents used for ovulation induction. From the brief introduction, we can observe that many basic concepts concerning the application of oral agents have been taken for granted without a real evidence base such as when to start CC, at what dose and for how many days therapy should continue. We can also observe that the widespread use of CC was associated with the existence of 10-40% of patients who were either resistant to CC or failed to achieve pregnancy. Second line therapies for these patients include the use of gonadotropins or laparoscopic ovarian drilling. These approaches have disadvantages related to costs and risk of ovarian hyperstimuation, multiple pregnancies or pelvic adhesions. For many women, particularly in developing countries, the cost of therapy is very critical which affects seriously the availability of the drug to the patients. Hence, before announcing the failure of cheap and safe drug like CC and moving forwards to another therapy, we tried to improve CC performance by manipulating its start or duration of use or by addition of other materials such as N-acetyl cysteine.

In chapter (2) a randomized controlled trial is presented to test the hypothesis that commencing CC in the late luteal phase of the preceding cycle would improve the rates of monofollicular development, increase the endometrial thickness, ovulation rate and pregnancy rate. In chapter (3) a randomized controlled trial is described to test the effect of extended CC therapy (beyond the standard 5 days) on the pregnancy outcome. Chapter (4) addresses the possible adjuvant effect of NAC when combined with CC for the treatment of polycystic ovary syndrome in a cross-over trial. In chapter (5), the role of NAC as an adjuvant to CC in unexplained infertility is investigated in a randomized, double-blind, controlled trial.

There has been a growing interest in the new group of aromatase inhibitors e.g letrozole and anastrozole as oral ovulation induction agents because of their theoretical advantages over CC. There was a scarcity of comparative RCTs to indicate the oral drug of first choice in induction of ovulation. In chapter (6) a prospective randomized controlled trial is presented to compare 5 mg of letrozole with 100 mg CC for inducing ovulation in infertile women with PCOS (438 patients and 1063 cycles). In chapter (7) a randomized controlled trial is presented to compare anastrozole (1 mg) with CC (100 mg) meant for inducing ovulation in women with infertility caused by PCOS. In chapter (8), a prospective, randomized trial is depicted to compare the effects of 2.5 mg of letrozole with 1 mg of anastrozole for ovulation induction in infertile women with CC resistant PCOS. Based on current data, the optimal dose of letrozole has not yet been determined. In chapter (9) the presented trial compare three different doses of letrozole (2.5 mg, 5 mg and 7.5 mg) in women undergoing ovulation induction and timed intercourse for treatment of unexplained infertility.

The safety issue of AIs is still unclear and continues to attract controversy. In a prospective randomized controlled trial (chapter 10), we present the pregnancy and neonatal outcomes after the use of aromatase inhibitors (letrozole and anastrozole) and clomiphene citrate for ovulation induction in comparison with the outcome after spontaneous (non-stimulated) pregnancy. By the end of this thesis we might be able to suggest a step-by-step scheme for ovarian stimulation and induction of ovulation in women with chronic anovulation.

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Chapter 2

Luteal phase clomiphene citrate for ovulation induction in women with polycystic ovary syndrome: A novel protocol

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Abstract

Objective: To test a novel protocol of luteal phase clomiphene citrate for ovulation induction in women with polycystic ovary syndrome.

Design: Prospective randomized controlled trial.

Setting: University teaching hospital and private practice settings.

Patients: The study comprised a total of 212 women (438 cycles) with PCOS.

Interventions: Patients in early CC group had 100 mg of CC daily starting next day of finishing MBA for 5 days (110 patients, 227 cycles) while patients in late CC group had 100 mg of CC daily for 5 days daily starting day 3 of the menses for 5 days (102 patients, 211 cycles).

Outcome measures: Number of growing and mature follicles, serum E2 (pg/ml), serum progesterone (ng/ml), endometrial thickness (mm), occurrence of pregnancy and miscarriage.

Results: The number of ovulating patients was not significantly different between the early and late CC groups (59.1% vs. 51.9%). The total numbers of follicles and the number of follicles ≥14 mm and ≥18 mm during stimulation were significantly greater ($P<0.05$) in the early CC group (Table 2). The endometrial thickness at the time of hCG administration was significantly greater ($P<0.05$) in the early CC group (9.1 ± 0.23 vs. 8.2 ± 0.60 mm). Serum E2 and progesterone were not significantly different between the two groups ($P>0.05$). Pregnancy occurred in 23/110 cycles in the early CC group (20.9%) and 16/102 cycles (15.7%) in the late CC group and the difference was not statistically significant ($P>0.05$). The miscarriage rate was similar in the two groups.

Conclusion: Early administration of CC in patients with PCOS will lead to more follicular growth and endometrial thickness which might result in higher pregnancy rate.

Introduction

Clomiphene citrate (CC) has had a remarkably sustained career as the first-line treatment for women with absent or irregular ovulation due to hypothalamic–pituitary dysfunction associated with normal basal levels of endogenous estradiol (WHO group II) (1). The vast majority of these patients probably some 80% are due to polycystic ovary syndrome (PCOS). Although CC is very successful in inducing ovulation, there is usually discrepancy between ovulation and pregnancy rates (only 50% of those who ovulate will conceive). This may be partly explained by the peripheral anti-estrogenic effects at the level of the endometrium and cervical mucus, by hypersecretion of LH or due to negative effects of CC on oocyte or granulosa cells. It can be argued that these negative effects are augmented by the relatively long half-life of CC (2). If treatment is started late in the cycle, those negative effects are more likely to extend into the sensitive peri-implantation period. Some studies reported better pregnancy rate when CC was started on day 1 rather than day 5 of menses (3, 4). So, the research question we wished to address was whether starting CC even before day 1 of menses improves the number of growing and mature follicles, endometrial thickness (mm) and occurrence of pregnancy. In this study, we tested a novel protocol of luteal phase clomiphene citrate for ovulation induction in women with polycystic ovary syndrome.

Materials and Methods

The study comprised 212 women (438 cycles) with PCOS among those attending the Gynecology outpatient clinic in Mansoura University Hospitals, Mansoura University, Egypt and a private practice setting in the period from November 2004 and March 2007. Diagnosis of PCOS based on the Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (5). All women had patent Fallopian tubes proved by hysterosalpingography and normal semen analysis for their partners.

Withdrawal bleeding was achieved using 10 mg medroxyprogesterone acetate (MBA) tablets for 10 days prior to stimulation. Patients were then randomly allocated using a computer-generated random table into 2 treatment groups: Early CC group (110 patients, 227 cycles) and late CC

group (102 patients, 211 cycles). The study was approved by the Research Ethics Committee and all participants gave informed consent before inclusion in the trial.

Patients in early CC group had 100 mg of CC (Clomid©, Hoechest Marion Russel, Cairo, Egypt) daily starting next day of finishing MBA (before withdrawal bleeding) for 5 days (110 patients, 227 cycles) while patients in late CC group had 100 mg of CC daily for 5 days daily starting day 3 of the menses for 5 days (102 patients, 211 cycles). All patients were monitored by trans-vaginal ultrasound for the mean follicular volume and thickness of the endometrium in the days 10, 12 and 14 of the cycle. Serum E2 (pg/ml) was measured at the time of human chorionic gonadotropine (hCG) injection by RIA using direct double antibody kits (Pantex, Santa Monica, CA) and serum progesterone (ng/ml) was measured in the day 21 to 23 of the cycle by RIA using antibody coated-tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA). Human chorionic gonadotropin (hCG) injection (5000-10000 IU IM) was given when at least one follicle measured at least 18 mm. Patients were advised for intercourse 24-36 hours after hCG injection. Serum hCG was determined two weeks after hCG injection in the absence of menstruation for diagnosis of pregnancy.

The primary outcome measures were number of growing and mature follicles, serum E2 (pg/ml), serum progesterone (ng/ml) and endometrial thickness (mm). Secondary outcome measure was the occurrence of pregnancy and miscarriage.

Statistical analysis

Data obtained were statistically analyzed using SPSS computer package (SPSS Inc., Chicago, USA) by Student's *t* test. Proportions were analyzed using the χ^2 test. Results were expressed as mean and standard error of the mean. The differences were considered to be statistically significant if $P<0.05$ at CI 95%.

Results

The study comprised 212 patients (438 cycles) in total. There were no statistical significant differences between the two groups as regards the age, duration of infertility, body weight, height and body mass index (BMI) or the presenting symptoms and signs (Table 1). Basal FSH and LH were significantly higher in the late CC group ($P<0.05$). The number of ovulating patients was more in the early CC group (59.1% vs. 51.9%) without significant differences. The total numbers of follicles and the number of follicles ≥14 mm and ≥18 mm during stimulation were significantly more ($P<0.05$) in the early CC group (Table 2). There was no significant difference in the pretreatment endometrial thickness between the two groups but endometrial thickness at the time of hCG administration was significantly more ($P<0.05$) in the early CC group (9.1 ± 0.23 vs. 8.2 ± 0.60 mm). Serum E2 and progesterone were not significantly different between the two groups ($P>0.05$).

Pregnancy occurred in 23/110 cycles in the early CC group (20.9%) and 16/102 cycles (15.7%) in the late CC group and the difference was not statistically significant ($P>0.05$). Miscarriage rate was similar in the two groups.

Discussion

The question we tested in this study was: does starting CC before day 1 of menses, in the late luteal phase, improve the ovarian response to ovulation induction. CC, as a non-steroidal compound closely resembling estrogen, blocks hypothalamic estrogen receptors, signaling a lack of circulating estrogen to the hypothalamus and induces a change in the pattern of pulsatile release of GnRH. CC is capable of inducing a discharge of FSH from the anterior pituitary and

this is often enough to reset the cycle of events leading to ovulation into motion. It is always noticed that the success rate of treatment with CC is excellent, with ovulation rates of 80% to 85%; the conception rate, however, is 40% (6). This marked discrepancy may be due to negative effects of CC on oocytes or granulosa cells, or because of prolonged antiestrogenic effects of CC on endometrial receptivity and cervical mucus. Approximately 15% of women who take CC have poor post-coital test results, and intrauterine insemination is recommended for these women (7). In the past, estrogen was administered from day 10 to day 16 of the menstrual cycle to improve mucus production, but there are now reasons to believe that estrogen administration is ineffective (7). It can be argued that these negative effects are augmented by the relatively long half-life of CC. Therefore, if treatment is started late in the cycle, these negative effects are more likely to be extended into the sensitive peri-implantation period (2).

In this study, the total numbers of follicles and the numbers of follicles ≥14 mm and ≥18 mm during stimulation were significantly more when CC was started early. In some studies, as in the study by Chung and colleagues (1989), there were no significant differences in ovarian response and pregnancy rates between groups starting CC on the second, third, fourth, or fifth day of the cycle (3). However, Bilijan and colleagues found a more rapid follicular growth and a higher pregnancy rate when CC treatment began on day 1 rather than day 5 of the cycle (2). Marrs et al. (1984) found that the number of follicles produced was markedly increased, oocytes were retrieved and fertilized in cycles in which CC was initiated on day 5 (8). Dhebashi et al. (2006), as well, found that, during therapy, gonadotropin levels increased for 10 to 14 days after initiation of CC in both groups, a result similar to results in previous studies (5). The number of significant follicles (14 mm or more) and maximum size of follicles were markedly higher in day 5 women than day 1. They concluded that in women who are candidates for in vitro fertilization or embryo transfer, it can be recommended to initiate CC on day 5 of the cycle.

Initiation of growth of primordial follicles, referred to as "primary recruitment", occurs continuously and in a random fashion. Follicle development from the primordial to the preovulatory stage usually takes several months (3, 4). The great majority of primordial follicles that enter this development phase undergo atresia before reaching the antral follicle stage through a process of apoptosis. The degree to which early stages of follicle developments are influenced by FSH remains unclear. It can be proposed that early start of CC in the later part of the luteal phase of the preceding cycle will be responsible for more follicular recruitment. Although the pregnancy rate was more in the early group, however, the difference was not statistically significant. We believe that when larger numbers of patients are going to be included in a similar study, a significant difference might appear in favor of the early protocol.

In conclusion, this study suggests that early administration of CC in patients with PCOS might lead to more follicular growth and endometrial thickness which might reflect on higher pregnancy rate.

Table 1 Patients' Characteristics

	Early CC group (n=110)	Late CC group (n=102))	t	P value
Number of cycles	227	211		
Age (years)	25.1 ± 2.11	24.3 ± 3.0	0.26	0.71
Parity	0.4 ± 0.23	0.3 ± 0.15	0.39	0.32
Height (cm)	155.3 ± 5.21	159.1 ± 5.34	1.06	0.12
Weight (kg)	75.3 ± 5.41	79.1 ± 4.22	2.51	0.08
Clinical presentation Oligo/anovulation Hyperandrogenism Polycystic ovaries	 90 (81.8%) 56 (50.9%) 88 (80.0%)	 85 (83.3%) 48 (47.1%) 81 (79.4%)	X2 0.08 0.31 0.01	 0.77 0.57 0.91
BMI (kg/m2)	30.1± 3.25	31.6 ± 2.61	0.22	0.83
FSH (IU/ml)	5.1 ± 2.71	7.1 ± 2.33	5.8	0.04*
LH (IU/ml)	11.1 ± 1.86	13.3 ± 2.11	6.1	0.04*

*Significant difference as P< 0.05

Table 2 Outcome in CC and gonadotropin groups

	Early CC group (n=110)	Late CC group (n=102)	t	P value
Number of ovulating patients	65 (59.1%)	53 (51.9%)	1.09	0.29
Total number of follicles	5.1 ± 0.41	2.8 ± 0.31	11.2	0.001*
Number of follicles >14 mm	3.0± 0.28	1.5 ± 0.18	12.4	0.001*
Number of follicles >18 mm	2.1 ± 0.15	1.3± 0.32	8.9	0.01*
Pretreatment endometrial thickness (mm)	3.7 ± 0.43	3.9 ± 0.51	1.6	0.09
Endometrial thickness at hCG (mm)	9.1 ± 0.23	8.2 ± 0.6	3.8	0.036*
Serum E2 (pg/ml)	311.1 ± 64.21	288 ± 91.30	0.71	0.56
Serum progesterone (ng/ml)	10.2 ± 0.81	10.3 ± 1.12	0.31	0.84
Pregnancy/cycle	23/110(20.9%)	16/102 (15.7%)	X^2=0.96	0.32
Miscarriage/patient	4 (17.4%)	3(18.8%)	X^2=0.08	0.77

*Significant difference as P< 0.05

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Chapter 3

Extended clomiphene citrate for ovulation induction in clomiphene-resistant women with polycystic ovary syndrome: A randomized controlled trial

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Abstract

The purpose of this study was testing the effect of extended CC in comparison to the gonadotropin therapy in the management of clomiphene-resistant women with polycystic ovary syndrome (PCOS). The study comprised 318 women (802 cycles) with clomiphene-resistant PCOS. Patients in CC group had 100 mg of CC daily starting day 2 of the menses for 9 days (160 patients, 405 cycles) while patients in gonadotropin group had hMG 75 IU intramuscularly daily for 5 days daily starting day 3 of the menses (158 patients, 397 cycles). The number of ovulating patients was significantly more ($P< 0.05$) in the gonadotropin group (57.6% vs. 28.1%). The total numbers of follicles during stimulation were significantly more ($P<0.05$) in the gonadotropin group (6.7 ± 0.3 vs. 4.1 ± 0.4). The endometrial thickness at the time of hCG administration was significantly more in the gonadotropin group (10.2 ± 0.6 vs. 8.2 ± 0.3 mm) as well. Serum progesterone was significantly higher in the gonadotropin group. Pregnancy occurred in 47/405 cycles in the CC group (11.3%) and 80/397cycles (20.1%) in the gonadotropin group and the difference was statistically significant ($P<0.05$). The extended CC regimens resulted in a modest ovulation and pregnancy rates and no side effects were reported. This therapy seems to offers economic, efficacy, and safety advantages and it worth trying before moving to more expensive or sophisticated alternatives.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in young women which manifests itself in a variety of clinical ways. Many of PCOS patients (55-75%) are infertile due to chronic anovulation (1, 2). Clomiphene citrate (CC) is still the typical therapy used for inducing ovulation in this condition. Traditionally, Clomiphene is given for 5 days following the onset of a spontaneous or a progestagen-induced period (3). It is not, however, equally effective in all situations due to different reasons. Clomiphene-resistance which refers to persistence of anovulation after standard CC therapy occurs in 15-20% of patients (4). Alternatives for PCOS women with CC-resistant anovulation include aromatase inhibitors, gonadotropins and laparoscopic ovarian drilling. Aromatase inhibitors might be useful in clomiphen-resistant women but still much more expensive than CC (5). Gonadotropin therapy is expensive and associated with a risk of high order multifetal gestation and ovarian hyperstimulation syndrome. Laparoscopic ovarian drilling in patients with PCOD can restore temporarily normal menses and might allow successful CC restimulation in many who remain anovulatory after resection. However, it remains expensive and the ovary is at risk for postoperative adhesion formation or premature failure (6). In clomiphene resistance, some advocate a treatment period of >5 days with this drug (7, 8) The purpose of this study was testing the effect of extended CC in comparison to the gonadotropin therapy in the management of clomiphen-resistant women with polycystic ovary syndrome.

Materials and Methods

The study comprised 318 women (802 cycles) with clomiphene-resistant PCOS among those attending the Gynecology outpatient clinic in Mansoura University Hospitals, Mansoura University, Egypt and a private practice setting in the period from May 2004 till May 2007. Diagnosis of PCOS based on the Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (9). By clomiphene- resistance in this study we meant failure of ovulation after 150 mg CC for 5 days. All women had patent Fallopian tubes proved by hysterosalpingography and normal semen analysis for their partners according to the modified criteria of WHO. All patients had normal serum prolactin, TSH and 17-OH progesterone.

Withdrawal bleeding was achieved using 10 mg levo-norgesterel tablets for 10 days prior to stimulation. Patients were then randomly allocated using a computer-generated random table into

2 treatment groups: CC group (160 patients, 405 cycles) and gonadotropin group (158 patients, 397 cycles). The study was approved by the hospital Research Ethics Committee and all participants gave informed consent before inclusion in the trial.

Patients in CC group had 100 mg of CC (Clomid©, Hoechest Marion Russel, Cairo, Egypt) daily starting day 2 of the menses for 9 days (160 patients, 405 cycles) while patients in gonadotropin group had hMG 75 IU (Menogon©, Ferring Pharmaceuticals, Malmo, Sweden) intramuscularly daily for 5 days daily starting day 3 of the menses (158 patients, 397 cycles). All patients were monitored by trans-vaginal ultrasound for the mean follicular volume and thickness of the endometrium in the days 10, 12 and 14 of the cycle. Serum E2 (pg/ml) was measured at the time of human chorionic gonadotropin (hCG) injection by radioimmunoassay (RIA) using direct double antibody kits (Pantex, Santa Monica, CA) and serum progesterone (ng/ml) was measured in the day 21 to 23 of the cycle by RIA using antibody coated-tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA). Human chorionic gonadotropin (hCG) injection (5000 IU IM) was given when at least one follicle measured at least 18 mm. Patients were advised for intercourse 24-36 hours after hCG injection. Serum hCG was determined two weeks after hCG injection in the absence of menstruation for diagnosis of pregnancy.

The primary outcome measures were number of growing and mature follicles, serum E2 (pg/ml), serum progesterone (ng/ml) and endometrial thickness (mm). Secondary outcome measures were the occurrence of pregnancy and miscarriage.

Statistical analysis

Data obtained were statistically analyzed using SPSS computer package (SPSS Inc., Chicago, USA) by Student's *t* test. Proportions were analyzed using the χ^2 test. Results were expressed as mean and standard error of the mean. The differences were considered to be statistically significant if $P<0.05$ at CI 95%. Considering the pregnancy rate as the outcome with expected difference of >15% between the two groups, the sample size was calculated using Statcalc program (EpiInfo version 6) and found that 286 patients (143 patients in each group) are needed to have a power of 80% at CI 95%.

Results

The study comprised 318 patients (802 cycles) in total. There were no statistical significant differences between the two groups as regards the age, duration of infertility, body weight, height and body mass index (BMI) or the presenting symptoms and signs (Table 1). Basal FSH was significantly higher in the gonadotropin group ($P<0.05$). The number of ovulating patients was significantly more in the gonadotropin group (57.6% vs. 28.1%). The total numbers of follicles during stimulation were significantly more ($P<0.05$) in the gonadotropin group (6.7 ± 0.3 vs. 4.1 ± 0.4). The number of follicles ≥14 mm and ≥18 mm were significantly higher ($P<0.05$) in the gonadotropin group (Table 2). There was no significant difference in the pretreatment endometrial thickness between the two groups but endometrial thickness at the time of hCG administration was significantly more ($P<0.05$) in the gonadotropin group (10.2 ± 0.6 vs. 8.2 ± 0.3 mm). Serum progesterone was significantly higher in the gonadotropin group ($P<0.05$).

Pregnancy occurred in 47/405 cycles in the CC group (11.3%) and 80/397cycles (20.1%) in the gonadotropin group and the difference was statistically significant ($P<0.05$). One twin pregnancy occurred in the CC group and 4 in the gonadotropin group. Two moderate ovarian hyperstimulation syndromes occurred in gonadotropin group and recovered.

Discussion

Inability of CC to induce ovulation, CC resistance, is unpredictable and foremost unexplainable event. Some studies showed that CC resistance is more likely in patients who are obese, insulin resistant and hyperandrogenic (10, 11). Nevertheless, it is virtually impossible to predict who will respond to which dose of CC, if at all. So far, there is no general agreement on a standard regimen for management of CC-resistant PCOS patients. Traditional alternatives to CC include aromatase inhibitors, gonadotropins and laparoscopic ovarian drilling. However, these alternatives are costly and sometimes risks are inherent to their use. Addition of adjuvants to CC such as N-acetyl cysteine (12), metformin (13, 14) and glucocorticoides (15) may give a hope for better ovarian response.

Our experience with a regimen of extended CC (from day 2-10 of menses) resulted in a modest rate of ovulation and pregnancy (28.1% and 11.2% respectively) in a group of patients who were previously resistant to CC. However, ovulation and pregnancy rates, as expected, were significantly more with the use of gonadotropins. The rationale of using this extended regimen based on understanding the physiology of follicular growth. Decremented follicular phase FSH levels (referred to as the FSH window) appear to be crucial for selection of a single dominant follicle from the recruited cohort (16). As FSH levels fall, all but the dominant follicle (with its increased sensitivity to FSH) lose the stimulus to further development and become atretic (17). The concept of extending the FSH window by administering exogenous FSH or extending the duration of CC therapy in the midfollicular phase will maintain FSH levels above the threshold allowing multifollicular development to occur.

It is always frustrating that the restoration of ovulation by CC does not produce a much higher pregnancy rate. This discrepancy between ovulation and pregnancy rates (only 50% of those who ovulate will conceive) may be partly explained by the peripheral anti-estrogenic effects of CC at the level of the endometrium and cervical mucus or by hypersecretion of LH. However, all these unwanted effects of CC are not related to the duration of CC therapy and it can not be claimed that extended CC therapy will compromise more the pregnancy rate. The depression of the cervical mucus, occurring in 15% of patients, may be overcome by addition of estrogen in the midfollicular period or performing IUI. Suppression of endometrial proliferation is unrelated to dose or duration of treatment but apparently idiosyncratic (18). It indicates a poor prognosis for conception if the endometrial thickness on ultrasound scanning does not reach 8mm at ovulation and if noted in the first cycle of treatment with CC, it will almost certainly be seen in repeated cycles in the same woman. There is little point in persisting after even one cycle, and a step-up to other forms of ovulation induction is recommended. The main action of CC, indirectly stimulating GnRH secretion, not only increases the desired FSH release but also produces an undesirable increase in LH concentrations. This increase in LH, whose basal level is often already high in women with PCOS, may compromise pregnancy rates in those receiving CC (19,20).

We were not expecting that our extended therapy will be more effective than gonadotropins in this situation. However, it resulted in a modest ovulation and pregnancy rates. No side effects were reported on extending the treatment. This therapy seems to offers economic, efficacy, and safety advantages and it worth trying before moving to more expensive or sophisticated alternatives.

Table 1 Patients' Characteristics

	CC group (n=160)	**Gonadotropin group (n=158)**	**t**	**P value**
Number of cycles	405	397		
Age (years)	24.1 ± 3.1	26.3 ± 3.0	0.35	0.41
Parity	0.3 ± 0.2	0.3 ± 0.3	0.06	0.88
Height (cm)	160.3 ± 6.2	158.1 ± 5.8	0.02	0.91
Weight (kg)	78.3 ± 6.4	81.1 ± 4.2	0.13	0.67
Clinical presentation			X^2	
Oligo/anovulation	136 (85.0%)	140 (88.6%)	0.9	0.34
Hyperandrogenism	76 (47.5%)	70 (44.3%)	0.33	0.56
Polycystic ovaries	111 (69.4%)	103 (65.2%)	0.63	0.42
BMI (kg/m2)	30.5 ± 3.1	32.5 ± 2.9	0.24	0.78
FSH (IU/ml)	4.1 ± 2.7	5.1 ± 2.1	0.87	0.10
LH (IU/ml)	10.9 ± 1.8	13.1 ± 2.2	3.8	0.04*

*Significant difference as P< 0.05

Table 2 Outcome in CC and gonadotropin groups

	CC group (n=160)	Gonadotropin group (n=158)	t	P value
Number of ovulating patients	45 (28.1%)	91 (57.6%)	28.2	0.001*
Total number of follicles	4.1 ± 0.4	6.7 ± 0.3	8.6	0.01*
Number of follicles >14 mm	2.0± 0.3	3.5 ± 0.1	9.6	0.02*
Number of follicles >18 mm	2.1 ± 0.1	3.3± 0.3	6.5	0.03*
Pretreatment endometrial thickness (mm)	4.7 ± 0.4	4.9 ± 0.5	0.84	0.12
Endometrial thickness at hCG (mm)	8.2 ± 0.3	10.2 ± 0.6	7.88	0.01*
Serum E2 (pg/ml)	215.1 ± 64.2	328 ± 91.3	2.06	0.09
Serum progesterone (ng/ml)	8.2 ± 0.8	10.3 ± 1.1	5.5	0.04*
Pregnancy/cycle	46/405(11.3%)	80/397 (20.1%)	X^2=4.27	0.03*
Miscarriage/patient	5 (20.8%)	4(13.8%)	X^2=0.10	0.74

*Significant difference as P< 0.05

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Chapter 4

N-Acetyl cysteine and clomiphene citrate for induction of ovulation in polycystic ovary syndrome: a cross-over trial

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Abstract

Objective: To compare clomiphene citrate plus N-acetyl cysteine versus clomiphene citrate for inducing ovulation in patients with polycystic ovary syndrome.

Design: Prospective cross-over trial.

Setting: University teaching hospital and a private practice setting.

Patients: Five hundred and seventy-three patients were treated with clomiphene citrate for one menstrual cycle among which 470 patients were treated with clomiphene citrate plus N-acetyl cysteine for another cycle. All women suffered from polycystic ovary syndrome.

Interventions: Patients had clomiphene citrate 50-mg tablets twice daily alone or with N-acetyl cysteine 1,200 mg/day orally for 5 days starting on day 3 of the menstrual cycle.

Outcome measures: Primary outcomes were number of mature follicles, serum E2, serum progesterone, and endometrial thickness. Secondary outcome was the occurrence of pregnancy.

Results: Ovulation rate improved significantly after the addition of N-acetyl cysteine (17.9% versus 52.1%). Although the number of mature follicles was more in the N-acetyl cysteine group (2.19/0.88 versus 3.29/0.93), the difference was not statistically significant. The mean E2 levels (pg/ml) at the time of human chorionic gonadotropine injection, serum progesterone levels (ng/ml) on days 21_23 of the cycle, and the endometrial thickness were significantly improved in the N-acetyl cysteine group. The overall pregnancy rate was 11.5% in the N-acetyl cysteine group. Insulin resistance occurred in 260 patients (55.4%). There was no significant difference between the insulin resistance group (n=260) and non-insulin resistance group (n=210) as regards ovulation rate, number of follicles, serum E2 (pg/ml), serum progesterone (ng/ml), endometrial thickness (mm), or pregnancy rate.

Conclusion: N-Acetyl cysteine is proved effective in inducing or augmenting ovulation in polycystic ovary patients.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in young women. It is a heterogeneous disorder that manifests itself in a variety of clinical ways extending from oligomenorrhea to amenorrhea; some patients are obese and others are not and some experience no hirsutism while others show mild to severe virilization (1). Nevertheless, 55-75% of PCOS patients are infertile due to chronic anovulation (2, 3). Clomiphene citrate (CC) is the standard drug for inducing or augmenting ovulation. However, it is not equally successful in all situations. Clomiphene resistance refers to persistence of anovulation after standard clomiphene therapy, which occurs in 15-20% of patients (4). Adjuvants to the effect of CC such as glucocorticoids (5, 6), dopaminergic drugs (7, 8), oral contraceptive pills (9), and insulin-sensitizing drugs (10, 11) have attracted attention for a long time. Recently, N-acetyl cysteine (NAC) was suggested as one of these adjuvants (12, 13). NAC is the acetylated variant of the amino acid L-cysteine. It is a potent antioxidant, which is converted in vivo into metabolites stimulating glutathione production (14-18). NAC is a mucolytic in a variety of respiratory illnesses; however, it has been proved effective in other conditions such as HIV infection, cancers, heart diseases, smoking, heavy metal poisoning, and prevention of influenza, epilepsy, and acetaminophene poisoning (19-26). Recently, some reports discussed the possible beneficial effects of NAC on ovulation (12, 13). It has an activity on insulin secretion in pancreatic cells and on insulin receptors on human erythrocytes (27-30). Being an insulin sensitizer, NAC was suggested as an adjuvant to CC for ovulation induction in patients with PCOS. NAC also has an antiapoptotic effect (31), preserves vascular integrity (32), and has an immunologic effect (33). The multiplicity of actions of NAC and the encouraging preliminary reports stimulated us in this study to investigate the effects of NAC in promoting the effects of CC on ovulation in PCOS patients.

Materials and Methods

The study comprised 573 women diagnosed to have PCOS among those attending the Fertility Outpatient Clinic in Mansoura University Hospitals and a private practice setting during the period from December 2003 till June 2005. Diagnosis of PCOS was based on clinical, laboratory, and ultrasound criteria. Clinical criteria included amenorrhea/ oligomenorrhea (cycle length >35 days or </6 cycles/year). Biochemical criteria included elevated menstrual LH/FSH ratio (>2.5) or elevated serum testosterone >2.8 nmol/l. Ultrasound criteria used for the diagnosis were enlarged ovary with >10 peripherally arranged small follicular cysts and hyperechogenic central stroma (34, 35). All women had patent Fallopian tubes proved by hysterosalpingography and normal semen analysis for their partners according to the modified criteria of WHO. All patients had normal serum prolactin, TSH, and 17-OH progesterone. The study protocol was approved by the ethical committee at Mansoura University, Egypt and all patients gave informed consent. Withdrawal bleeding was achieved using 10 mg levonorgestrel tablets for 10 days. Patients were on standard carbohydrate diet and no other medications were given before recruitment in the study. All patients were treated with CC 50-mg tablets (2 tablets daily for 5 days) starting on day 3 of the menstrual cycle. They were monitored by transvaginal ultrasound for the mean follicular number and volume and thickness of the endometrium on days 10, 12, and 14 of the cycle, serum E2 (pg/ml) at the time of human chorionic gonadotropine (hCG) injection, and serum progesterone (ng/ml) on days 21-23 of the cycle. Ovulation was documented when serum progesterone was ≥5 ng/ml. hCG injection (5,000 IU) was given when at least one follicle measured at least 18 mm. Patients were advised to have intercourse 24-36 h after the hCG injection. Serum hCG was determined two weeks after hCG injection in the absence of menstruation for diagnosis of pregnancy. After one cycle of treatment, 470 patients (82.1%) failed to achieve ovulation. Two months later, patients were treated using CC 50-mg tablets twice daily for 5 days starting on the second day of the menstrual cycle and NAC 1,200 mg/day orally (sachets, 200 mg each, as two sachets thrice daily) starting on the second day of the cycle for 5 days.

Cycles were monitored, hCG injection was given, and patients were advised to have intercourse. Again serum hCG was determined two weeks after hCG injection in the absence of menstruation for diagnosis of pregnancy. Plasma glucose and fasting insulin were measured after an overnight fast on the third day of menstruation. Insulin resistance (IR) was determined by static fasting glucose and serum insulin level, which are highly related to the dynamic measurements after glucose loading (36, 37). IR was defined as abnormal fasting insulin (>/20 mIU/l) and glucose/insulin ratio (< 0.25).

Treatment had continued to one cycle and the primary outcome measures were number of mature follicles (>18 mm) in the ovary, serum E2 levels (pg/ml), serum progesterone levels (ng/ml), and endometrial thickness. Secondary outcome measure was the occurrence of pregnancy.

Obtained data were statistically analyzed using the SPSS computerized package by Fisher's exact test to compare differences in rates and Student's t- test for differences in parametric data. A p value <0.05 was considered significant. Considering the pregnancy rate as the outcome with expected difference of 12% between the two groups, the sample size was calculated using Statcalc program (EpiInfo version 6) and found that 486 patients (243 patients in each group) are needed to have a power of 80% at CI 95%.

Results

The study contained 470 patients, with their characteristics presented in Table I. Ovulation rate improved significantly after the addition of NAC (17.9% versus 52.1%). Although the number of mature follicles (> 18 mm) was higher in the NAC group (2.19 ±/0.88 versus 3.29/±/0.93), the

difference was not statistically significant. The mean E2 levels (pg/ml) at the time of hCG injection, serum progesterone levels (ng/ml) on days 21-23 of the cycle, and the endometrial thickness were significantly improved in the NAC group. The overall pregnancy rate was 11.5% in the NAC group (Table II). There were no cases of drug allergy or ovarian hyperstimulation syndrome. There were 7 cases of twin pregnancy in the study group (12.9%). Miscarriage in the first trimester occurred in 6 cases (11.1%). IR occurred in 260 patients (55.4%). There was no significant difference between the IR group (n=260) and the non-IR group (n=210) as regards ovulation rate, number of follicles, serum E2 (pg/ ml), serum progesterone (ng/ml), endometrial thickness (mm), or pregnancy rate (Table III).

Discussion

NAC is a potent antioxidant. Most of the beneficial effects of orally administered NAC are due to its ability to either reduce extracellular cystine to cysteine or to be a source of SH metabolites. NAC, which can stimulate GSH synthesis, promotes detoxification and acts directly on reactive oxidant radicals (38, 39). NAC is a safe drug and its LD50 is very high. Animal studies proved that the drug is neither teratogenic nor mutagenic (40, 41). None of the patients in this study reported any side effects from the use of the drug. There were no ovarian hyperstimulations and only 7 multiple pregnancies (12.9%) in the study and control groups. Miscarriage rate (11.1%) was not higher than expected in spontaneous pregnancy. We recruited the same cohort of patients who failed to achieve ovulation (470 patients) as study and control groups. The second part of the study commenced 2 months after the first part to eliminate the possibility of a cumulative effect of the CC because of its significantly greater half-time for clearance (2 weeks) in the body. In this study, there was a significant improvement in ovulation rate after introduction of NAC in addition to CC (17.9% versus 52.1%). Although the number of mature follicles per patient was more in the NAC group, the difference was not statistically significant. The mean serum estrogen and mid-luteal progesterone were higher in the NAC group, which may explain the significant improvement in the endometrial thickness in the same group. Consequently, the pregnancy rate was 11.5% in the NAC group. These results are in agreement with those recently reported by Rizk et al. (12) and Bedaiwy et al. (13). Many mechanisms are proposed to explain the unique action of NAC on ovulation. Borgstro¨m et al. demonstrated the insulin-sensitizing activity of NAC (27). Other authors have shown the value of NAC in patients with PCOS through its action on hyperinsulinemia and IR (28, 29). Fulghesu et al. found that NAC caused significant reduction in circulating insulin levels, peripheral IR, serum testosterone levels, and free androgen index in PCOS (30). Normoinsulinemic patients did not report any change in C-peptide and insulin levels or in peripheral insulin sensitivity after NAC treatment. The decrease in circulating insulin level was followed by a significant reduction in testosterone levels and free androgen index in patients responding to ovulation treatment, while no changes were observed when insulin levels were not modified (30). They stressed the value of NAC in the presence of IR. This was not the situation in this study group, as IR was reported in 55.4% of patients. There was no significant difference between the IR and non-IR groups as regards the ovulation rate, number of follicles, serum estrogen, serum progesterone, or pregnancy rates. There should be other mechanisms that explain the beneficial effects of NAC which are as important as its insulin-sensitizing effects. Odetti et al. reported that NAC has antiapoptotic effects on the ovary. Apoptosis is definitely responsible for the process of follicular artesia. NAC also preserves the vascular integrity, lowers serum homocysteine levels, and is protective against ischemic injuries (18, 32). NAC has an immunological activity. It is shown to have an anticytokine effect and inflammatory-modulating ability (33). All these actions, other than insulin sensitizing activity, might explain the results in this study. We observed another beneficial effect for NAC in combination with CC which may adversely affect the cervical mucous. Being a mucolytic, NAC usually improves the character of

the cervical mucous without additional estrogen supply. Further studies are required to prove these effects and to compare the efficacy of NAC versus other insulin sensitizing drugs.
In view of the results of this study, NAC is proved effective in inducing or augmenting ovulation in polycystic ovary patients. It is equally effective in both IR and non-IR patients, which suggest the presence of other mechanisms of action. NAC can confidently be recommended as an adjuvant to CC in such a situation.

Table 1 Patients' characteristics

	(n=470)
Age (years)	27.1 ± 3.2
Infertility (years)	4.1 ± 2.9
Height (m)	163.3 ± 6.1
Weight (kg)	98.3 ± 6.4
BMI (kg/m2)	28.1 ± 3.2
FSH (IU/ml)	4.1 ± 3.1
LH (IU/ml)	11.2 ± 1.8
FSH/LH	2.4
Insulin resistance	210 (44.6%)

Table 2 Outcome in CC group and CC + NAC group

	Group I (CC group)	Group II (CC + NAC group)	p
Ovulation	103/573 (17.9%)	245/470 (52.1%)	S
Number of follicles	2.1 ± 0.88	3.2 ± 0.93	NS
Serum E2 (pg/ml)	110.0 ± 30.4	452.1 ± 290.3	S
Serum progesterone (ng/ml)	2.3 ± 1.2	6.4 ± 2.3	S
Endometrial thickness (mm)	4.3 ± 1.2	7.8 ± 3.1	S
Pregnancy	0	54 (11.5%)	S
OHSS	0	0	

Table 3 Outcome in IR group and non-IR groups

	IR group	Non-IR group	p
Number	260 (55.4%)	210 (44.6%)	
Ovulation	129 (49.6%)	116 (47.3%)	NS
Number of mature follicles	3.1 ± 0.89	3.2 ± 0.93	NS
Serum E2 (pg/ml)	468.0 ± 280.4	452.1 ± 290.3	NS
Serum progesterone (ng/ml)	7.3 ± 1.2	6.4 ± 2.3	NS
Endometrial thickness (mm)	8.3 ± 1.2	7.8 ± 3.1	NS
Pregnancy	30 (11.5%)	24 (11.4%)	NS

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Chapter 5

Clomiphene citrate plus *N*-acetyl cysteine versus clomiphene citrate for augmenting ovulation in the management of unexplained infertility: a randomized double-blind controlled trial

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Abstract

Objective: To compare clomiphene citrate with *N*-acetyl cysteine vs. clomiphene citrate alone for augmenting ovulation in management of unexplained infertility.

Design: Prospective randomized double-blind controlled trial.

Setting: Department of obstetrics and gynecology in a university medical faculty in Egypt.

Patient(s): Four hundred four patients as a study group (clomiphene citrate plus *N*-acetyl cysteine group) and 400 patients as a control group (clomiphene citrate–alone group). All women had unexplained infertility.

Intervention(s): Patients in the study group were treated with clomiphene citrate (50-mg tablets) twice per day and with *N*-acetyl cysteine (1,200 mg/d orally) for 5 days starting on day 2 of the cycle. Patients in the control group were treated with clomiphene citrate with sugar powder.

Main Outcome Measure(s): The primary outcomes were number and size of growing follicles, serum E2, serum P, and endometrial thickness. The secondary outcome was the occurrence of pregnancy.

Result(s): There were no statistically significant differences between the two groups in the number of follicles sized >18 mm, mean E2 levels, serum P, or endometrial thickness. Pregnancy rate was comparable in both groups (22.2% vs. 27%). Miscarriage rate was comparable in both groups (6.7% in the study group vs. 7.4% in the control group).

Conclusion(s): *N*-Acetyl cysteine is ineffective in inducing or augmenting ovulation in patients with unexplained infertility and cannot be recommended as an adjuvant to clomiphene citrate in such patients.

Introduction

Clomiphene citrate remains the standard drug for inducing or augmenting ovulation. However, as a singular drug, it is not equally effective in all situations, and therefore it may require additional expensive drugs such as gonadotropins to achieve ovulation with their potential side effects; mainly, ovarian hyperstimulation syndrome. The introduction of antioxidant therapy to ovarian stimulation was proven to have many benefits regarding cell mitosis and apoptosis (1, 2). *N*-acetyl cysteine, as an antioxidant, has been suggested as an adjuvant in clomiphene-resistant cases (3, 4). *N*-Acetyl cysteine (NAC) is the acetylated variant of the amino acid L-cysteine. It is an excellent source of sulfhydryl groups and is converted in vivo into metabolites that stimulate glutathione production, promote detoxification, and act directly as free-radical scavengers. Historically, NAC is a mucolytic in a variety of respiratory illnesses; however, it appears to be beneficial in other conditions, such as HIV infection, cancers, heart diseases, smoking, heavy-metal poisoning, prevention of influenza, epilepsy, and acetaminophen poisoning (5–12). *N*-Acetyl cysteine has different actions inside the body. It is primarily a powerful antioxidant (13–17); it has activity on insulin secretion in pancreatic cells and on insulin receptors on human erythrocytes (18–22). *N*-Acetyl cysteine has antiapoptotic effects (23); it can preserve vascular integrity (24) and has immunological functions (25).

Recently, some reports discussed the possible beneficial effects of NAC on ovulation (3, 4). Because it is an insulin sensitizer, NAC was proposed as an adjuvant to clomiphene citrate for ovulation induction in patients with polycystic ovary syndrome. Encouraging results in those patients stimulated us to investigate the effects of NAC in promoting the augmenting effects of clomiphene citrate on ovulation in nonpolycystic patients in this randomized, double-blind, controlled study.

Materials and Methods

The study included 804 patients among those who were attending the fertility outpatient clinic in the department of obstetrics and gynecology of Mansoura University (Mansoura, Egypt) for management of primary subfertility problems during the period from October 2003 to April 2005.

All women had at least 1 year of continuous marriage without conception. They all had had the preliminary investigations for infertility performed and had proved normal. They had patent fallopian tubes as proven by hysterosalpingography; had normal ovulating cycles as proven by mid-luteal serum P levels; and had normal laparoscopic findings, in addition to normal semen analysis for their partners according to the modified criteria of the World Health Organization. Therefore, by definition, all women had unexplained infertility.

All patients underwent superovulation with normal timed intercourse as a line of management of their problem. All women were included in the study after we had obtained written informed consent, and the study was approved by the ethical committee of Mansoura University. Patients were allocated randomly to either the study group (404 women) or the control group (400 women) using sealed envelopes. Patients in the study group were treated with clomiphene citrate (50 mg tablets twice daily; (Hoechst Marion Russel, Arab Republic of Egypt) for 5 days, starting on the 2nd day of the menstrual cycle, and with *N*-acetyl cysteine (1,200 mg/d orally; Sedico-Cairo, ARE), for 5 days, starting on the 2nd day of the cycle. Patients in the control group were treated with clomiphene citrate in the same dose, in combination with sugar powder, which was used at the same volume as that of NAC, starting on day 2, for 5 days. Patients in the study and control groups were blinded to the real drug and placebo.

Cycles were monitored by transvaginal ultrasound for the mean follicular volume and thickness of the endometrium on days 10, 12, and 14 of the cycle. Serum E2 assay by RIA with direct double-antibody kits (Pantex, Santa Monica, CA) was performed at the time of hCG injection; serum P was measured between day 21 and day 23 of the cycle by RIA by using the antibody coated-tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA). Both radiologist and laboratory personnel also were blinded to the study and placebo group. Injection of hCG was given when at least 1 follicle measured >18 mm. Patients were advised to have intercourse 24–36 hours after the hCG injection. Serum hCG was determined 2 weeks after the hCG injection, in the absence of menstruation, for diagnosis of pregnancy. Treatment was continued to one cycle. The primary outcome measures were the following: number of growing follicles sized >18 mm in the ovary, serum E2 levels, serum P levels, and endometrial thickness. The secondary outcome measure was the occurrence of pregnancy.

Data obtained were statistically analyzed using the SPSS computer package (SPSS, Inc., Chicago, IL) and Fisher's exact test to compare differences in rates and Student's *t* test to assess differences in parametric data. A value of $P<.05$ was considered significant.

Results

The study included 804 patients in total. There were no statistically significant differences between the two groups regarding age, duration of infertility, body weight, height, and body mass index (Table 1). There were no statistically significant differences between the two groups in the number of follicles sized >18 mm in both ovaries, mean E2 levels at the time of hCG injection, serum P levels in days 21 to 23 of the cycle, or the endometrial thickness. Pregnancy rate was comparable in both groups (Table 2). There were no cases of ovarian hyperstimulation syndrome. There were 8 cases of twin pregnancy in the study group (8.9%), and there were 12 cases in the control group (11.1%) with no higher order pregnancies. Miscarriage rate was comparable in both groups (6.7% in the study group vs. 7.4% in the control group).

Discussion

N-Acetyl cysteine recently has been proposed as an adjuvant to the clomiphene citrate in inducing or augmenting ovulation in different situations (3, 4). *N*-Acetyl cysteine is a potent antioxidant.

Most of the beneficial effects of orally administered NAC are theorized to be due to its ability to either reduce extracellular cystine to cysteine or to be a source of sulfhydryl metabolites. As a source of sulfhydryl groups, NAC can stimulate glutathione synthesis, enhance glutathione-*S*-transferase activity, promote detoxification, and act directly on reactive oxidant radicals (26). *N*-Acetyl cysteine is used primarily as a mucolytic drug, but it has been proven effective for many other uses, such to promote detoxification, to enhance the effects of nitroglycerin on the heart, to serve as a hepatoprotectant, to lower lipoprotein levels, and to lower homocysteine levels. *N*-Acetyl cysteine is a safe drug, and its LD50 is very high.

Animal studies proved that the drug is neither teratogenic nor mutagenic (27, 28). Overdose of the drug causes minimal allergy, especially after IV administration, and there are no contraindications for its use, apart from known hypersensitivity to the NAC (29). None of the patients in this study reported any side effects from the use of the drug. We had no ovarian hyperstimulations and only 20 multiple pregnancies in the study and control groups. Miscarriage rate was not higher than expected in spontaneous pregnancy. *N*-Acetyl cysteine is a very promising drug. Studies have established many possible mechanisms of action to it that might be beneficial in augmenting ovulation. Borgstrom et al. (18) demonstrated the insulin-sensitizing activity of NAC. Other investigators have shown the value of NAC in patients with polycystic ovarian syndrome through its action on hyperinsulinemia and insulin resistance (19–21). Many other mechanisms have been proposed, such as antiapoptotic effects (1, 23). Apoptosis is responsible for the process of follicular atresia. *N*-Acetyl cysteine was found to inhibit apoptosis in cultured ovarian primordial germ cells. It also preserves vascular integrity, lowers serum homocysteine levels, and is protective against ischemic injuries (17, 24, 27, 30). *N*-Acetyl cysteine is immunologically active; it has an anticytokine effect and inflammatory-modulating capacity (25).

In the present study, however, NAC was proven to be ineffective in improving the ovulation or pregnancy rates in the study cohort. *N*-Acetyl cysteine did not add to the ovulatory effect of the clomiphene citrate, and moreover, the results even were better in its absence. These results are different from those that others were reporting (3). Kleinveld et al. (30) reported that in healthy individuals, NAC may act as a pro-oxidant and may lower the glutathione and increase the amount of oxidized glutathione. Although NAC is reported to be a novel beneficial adjuvant to the clomiphene citrate in inducing and augmenting ovulation in polycystic ovarian disease, this was not the case in women with unexplained infertility. In women with polycystic ovary syndrome, insulin resistance is a frequent finding (31, 32), and serum homocysteine is significantly higher than in nonpolycystic patients, which might indicate a role for the use of NAC.

We reported another beneficial effect of NAC when combined with clomiphene citrate; the latter adversely affects the cervical mucus. *N*-Acetyl cysteine is mucolytic and therefore usually improves the character of the cervical mucus without the need for an estrogen supply. Further studies are required on a larger cohort of women to prove these effects, to compare the efficacy of NAC vs. other insulin-sensitizing drugs, and to assess the effect of NAC on short- and longterm complications of polycystic ovary disease. In view of the results of the present study, NAC has proved ineffective in inducing or augmenting ovulation in nonpolycystic ovarian disease and cannot be recommended as an adjuvant to clomiphene citrate in such a situation.

Table 1 Patients' characteristics in study and control groups.

Characteristic	Group 1 (n =404)	Group 2 (n =400)
Age (y)	27.9 ± 4.2	28.1 ± 3.7
Infertility (y)	5.1 ± 2.9	4.3 ± 2.7
Height (m)	168.0 ± 4.9	165.3 ± 5.1
Weight (kg)	78.9 ± 6.2	80.3 ± 5.4
BMI (kg/m2)	27.9 ± 3.4	28.5 ± 3.1
Serum FSH (IU/mL)	4.3 ± 2.1	4.6 ± 2.2

Note: P<.05 for all characteristics, group 1 vs. group 2. All data are mean ± SD. BMI=body mass index.

Table 2 Outcome measures in study and control groups.

Outcome	Group 1 (n =404)	Group 2 (n =400)
No. of follicles	3.1 ± 0.88	3.2 ± 0.97
Serum E2 (pg/mL)	398.0 ± 230.4	452.1 ± 290.3
Serum P (ng/mL)	8.3 ± 1.2	8.4 ± 2.3
Endometrial thickness (mm)	8.3 ± 2.2	8.8 ± 3.1
Pregnancy, n (%)	90 (22.2)	108 (27)

Note: P<.05. for all outcome measures, group 1 vs. group 2. All data are mean ± SD.

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Chapter 6

Clomiphene citrate or letrozole for ovulation induction in women with polycystic ovarian syndrome: a prospective randomized controlled trial

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Abstract

Objective: To compare the effects of letrozole (5 mg) and clomiphene citrate (100 mg) for ovulation induction in women with polycystic ovary syndrome (PCOS).
Design: Prospective randomized controlled trial.
Setting: University teaching hospital and private practice setting.
Patient(s): The study comprised a total of 438 infertile women (1063 cycles) with PCOS.
Intervention(s): Patients were randomized to treatment with 5 mg of letrozole daily (218 patients, 545 cycles) or 100 mg of clomiphene citrate daily (220 patients, 518 cycles) for 5 days starting on day 3 of menses. Timed intercourse was advised 24 to 36 hours after hCG injection.
Main Outcome Measure(s): Number of follicles, serum estradiol, serum progesterone, endometrial thickness, and pregnancy and miscarriage rates.
Result(s): The mean age, parity, and duration of infertility in both groups were similar. The total number of follicles was statistically significantly greater in the clomiphene citrate group (6.8±0.3 versus 4.4±0.4). Endometrial thickness at the time of hCG administration was statistically significantly greater in the CC group (9.2±0.7 mm versus 8.1±0.2 mm). The duration to reach a dominant follicle was statistically significantly longer in the letrozole group (12.1±1.3 versus 8.8±2.9 days). Ovulation occurred in 365 out of 540 cycles (67.5%) in letrozole group and 371 out of 523 cycles (70.9%) without a statistically significant difference. Levels of serum estradiol and progesterone were statistically significantly higher in the clomiphene citrate group. The pregnancy rate per cycle was 15.1% in the letrozole group and 17.9% in the clomiphene citrate group without statistically difference between the groups.
Conclusion(s): The results of this study did not show any advantage to the use of letrozole over clomiphene citrate as a first-line treatment for induction of ovulation in women with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in young women. It manifests itself in a variety of clinical ways and 55% to 75% of PCOS patients are infertile due to chronic anovulation (1–3). Clomiphene citrate (CC) is still the standard drug for inducing or augmenting ovulation. It is not, however, equally successful in all situations. Clomiphene resistance, which refers to persistence of anovulation after standard CC therapy, occurs in 15% to 20% of patients (4). Also, CC may have a negative effect on the cervical mucus and endometrium. Treatment with CC is associated with a discrepancy between ovulation and conception rates, and the incidence of miscarriage has been claimed to be higher than in the general population (4, 5).

Alternative treatments to CC with insulin-sensitizing drugs such as metformin have attracted attention. Letrozole is an aromatase inhibitor that has been widely used in women with breast cancer (6). It works by suppressing estrogen production, and has been used to induce ovulation. But does letrozole add anything to the art of ovulation induction and represent a real alternative to CC? This prospective randomized trial compared the effects of 5 mg of letrozole with 100 mg CC for inducing ovulation in infertile women with PCOS.

Materials and Methods

The study comprised 438 women (1063 cycles) with PCOS among those attending the Fertility Outpatient Clinic in Mansoura University Hospitals, Mansoura University, Egypt, and private practices in the period from January 2004 and September 2006. The diagnosis of PCOS was based on the Revised 2003 consensus diagnostic criteria for PCOS (7). All women had patent fallopian tubes proved by hysterosalpingography, and their partners had normal semen analysis parameters according to the modified criteria of the World Health Organization.

All patients had normal serum prolactin, thyroidstimulating hormone (TSH) and 17-OH progesterone. Withdrawal bleeding was achieved using 10-mg levonorgestrel tablets for 10 days before stimulation. Patients then were randomly allocated using a computer-generated random table into two treatment groups: letrozole group (218 patients, 575 cycles) and CC group (220 patients, 588 cycles). The study was approved by the hospital research ethics committee, and all participants gave informed consent before inclusion in the trial. Patients in the letrozole group had 5 mg of letrozole (Novartis Pharma Services, Basel, Switzerland) daily for 5 days starting on day 3 of menses (218 patients, 575 cycles); patients in the CC group had 100 mg of CC (Hoechst Marion Roussel, ARE) daily starting day 3 of the menses for 5 days (520 patients, 588 cycles). All patients were monitored by transvaginal ultrasound for the mean follicular volume and thickness of the endometrium on the days 10, 12, and 14 of the cycle. The serum estradiol (E2, pg/mL) concentration was measured at the time of human chorionic gonadotropin (hCG) injection by radioimmunoassay (RIA) using direct double-antibody kits (Pantex, Santa Monica, CA), and serum progesterone (ng/mL) concentration was measured on days 21 to 23 of the cycle by RIA using the antibody coated tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA). The hCG injection (5000–10,000 IU intramuscular) was given when at least one follicle measured R18 mm. Patients were advised to have intercourse 24 to 36 hours after the hCG injection. The serum hCG concentration was determined 2 weeks after the hCG injection in the absence of menstruation for diagnosis of pregnancy. The primary outcome measures were the number of growing and mature follicles, the concentrations of serum E2 (pg/mL) and progesterone (ng/mL), and the endometrial thickness (mm). The secondary outcome measure was the occurrence of pregnancy and miscarriage.

Statistical Analysis

Data were statistically analyzed using SPSS computer package (SPSS Inc., Zonguldak Karaelmas University, Zonguldak, Turkey) by Student's t test. Proportions were analyzed
using the chi-square test. Results were expressed as mean and standard error of the mean. $P<.05$ was considered a statistically significant difference. Considering the pregnancy rate as the outcome with expected difference of 15% between the two groups, the sample size was calculated using Statcalc program (EpiInfo version 6) and found that 412 patients (206 patients in each group) are needed to have a power of 80% at CI 95%.

Results

The study comprised 438 patients (1063 cycles). There were no statistically significant differences between the two groups in age, duration of infertility, body weight, height, body mass index (BMI), or presenting symptoms and signs (Table 1). The total number of follicles during stimulation was statistically significantly greater in the CC group (6.8 0.3 vs. 4.4 ± 0.4). The number of follicles R14 mm and R18 mm was statistically significantly higher in the CC group (Table 2). There was no statistically significant difference in pretreatment endometrial thickness between the two groups, but endometrial thickness at the time of hCG administration was statistically significantly greater in the CC group (9.2 ± 0.7 vs. 8.1 ± 0.2 mm). The amount of time needed to achieve a dominant follicle was statistically significantly longer in the letrozole group (12.1 ± 1.3 days vs. 8.8 ± 1.9 days). Ovulation occurred in 365 out of 540 cycles (67.5%) in letrozole group and 371 out of 523 cycles (70.9%) in the CC group, without a statistically significant difference between the two groups. Serum E2 and progesterone concentrations were statistically significantly higher in the CC group. Pregnancy occurred in 94 out of 523 cycles in the CC group (17.9%) and 81 out of 540 cycles (15.1%) in the letrozole group; the difference was not statistically significant. Three twin pregnancies occurred in the CC group and none in the letrozole group. No higher order pregnancies or cases of ovarian hyperstimulation syndrome occurred in either group.

Discussion

Clomiphene citrate is not equally effective in all situations for induction of ovulation or superovulation. Clomiphene resistance occurs in 15% to 20% of patients. The use of CC may be associated with poor cervical mucous and endometrial thinning in 15% to 50% of patients due to prolonged estrogen-receptor depletion in the endometrium and possibly in the cervix (8–12). Alternatives to clomiphene citrate such as letrozole have been used for this indication, but it has not been clear whether these alternatives are as effective as CC for inducing ovulation, especially in PCOS, the most common ovulation disorder in women. Letrozole (4,40-[1H-1,2,4-triazol-1-ylmethylene]-bisbenzonitrile) is a type IIa third-generation aromatase inhibitor. It was postulated that blocking estrogen production by inhibiting aromatization, the conversion of androstenedione and testosterone to estrogen, in the ovary would release the hypothalamic/pituitary axis from estrogenic negative feedback. As a result, follicle-stimulating hormone (FSH) secretion increases, stimulating the development of ovarian follicles. Preliminary studies reported that aromatase inhibitors were useful for inducing ovulation and in superovulation.

Mitwally and Casper (13) described the use of 2.5 mg of letrozole on days 3 to 7 of menses in 12 patients with PCOS. Ovulation occurred in nine patients (75%), and pregnancy was achieved in three (25%). The proper dosage of letrozole for ovulation induction has yet to be determined, but the most commonly used dosage in previous studies has been between 2.5 and 7.5 mg; therefore, our study used a dosage of 5 mg daily for letrozole. Compared with letrozole, the use of CC led to a statistically significant increase in the number of developing and mature follicles (14 mm and 18 mm follicles). Although the pregnancy rate was slightly greater in the CC group, the difference was not statistically significant. The miscarriage rate was similar in both groups. Bayar et al. (14) reported similar results in women with ovulatory infertility. In contrary, Al-Fouzan et al. (15) reported better results in the letrozole group for the number of developing and mature follicles than in the CC group. In another study, Mitwally and Casper (16) reported improved response to exogenous FSH stimulation with letrozole cotreatment in poor ovarian responders. Healey et al. (17) reported similar findings; although endometrial thickness was decreased, no negative effect on pregnancy rates was noted in this study. However, when letrozole was compared with CC combined with gonadotropin, Jee et al. (18) reported that the number of mature follicles and serum E2 level on the day of hCG administration were statistically significantly lower in the letrozole group than the CC group. Clomiphene citrate results in central estrogen receptor depletion for a lengthy time because of its greater half-time for clearance (2 weeks) (5). As a result, supraphysiologic levels of estrogen can occur without central suppression of FSH because the normal estrogen receptor–mediated feedback mechanisms are blocked. This results in multiple follicle growth and higher multiple pregnancy rates with CC than are found in letrozole cycles. When the development of multiple mature follicles is needed, letrozole alone is probably not the optimal choice for ovulation induction.

In our study, the greater number of mature follicles in the CC group did not result in a statistically significantly higher pregnancy rate, which leads to the discussion about the effect of both drugs on the endometrium. The endometrium was, astoundingly, statistically significantly thicker in the CC group. This may be due to more growing follicles and the higher levels of estrogen and progesterone in the CC group. Mitwally and Casper (14) showed that letrozole has minimal effect on the endometrium; compared with CC, letrozole is associated with a thicker endometrium. Cortinez et al. (19) found normal morphologic features of endometrium and full expression of pinopodes during the implantation window when letrozole was used. On the other hand, no significant difference has been noted by other studies as regards the effect of either drug on the endometrium (20, 21). Information on the teratogenic capacity of letrozole in humans is lacking, but animal studies have shown that low doses of letrozole are effective in inducing noxious effects on the developing conceptus (22). In the rat, teratogenic effects, including fetal

domed head and cervical/centrum vertebral fusion, resulted from exposure to 0.03 mg/kg. In the rabbit, embryotoxic effects were induced by exposure to 0.002 mg/kg (approximately 1/100,000 the daily maximum recommended human dose on a mg/m2 basis). Although extrapolation of animal data to humans is a complex process, these findings suggest that letrozole might have the capacity to elicit teratogenesis. Mitwally et al. (23) reported favorable pregnancy outcomes and low multiple gestation rates with aromatase inhibitors for ovarian stimulation. Clomiphene citrate has been reported to elicit various ocular side effects in 1.5% to 10.0% of patients taking CC. The cost of letrozole per cycle is much higher than CC, especially when higher doses of letrozole are required (500 versus 5 Egyptian pounds, respectively). Bedaiwy et al. (14) have suggested that a letrozole-FSH combination could be more cost-effective than FSH alone for ovarian stimulation in intrauterine insemination cycles. Our study found no advantages to using letrozole rather than CC as a first-line treatment for inducing ovulation in women with PCOS. Multiple follicular growths occur in CC-treated patients without any corresponding statistically significant increase in multiple pregnancies or miscarriage rates, which represents an advantage to using CC in many situations. As the ideal dose of letrozole has yet to be determined, a higher dose than the 5 mg used in our study may achieve better results. However, raising the dosage will further increase the cost, which is an important consideration in choosing the appropriate therapy.

Table 1 Characteristics of the patients.

	Letrozole group (n=218)	CC group (n=220)	t	P value
Number of cycles	540	523		
Age (years)	27.1 ± 3.2	29.3 ± 2.9	2.05	0.68
Parity	0.3 ± 0.1	0.4 ± 0.2	3.42	0.11
Height (cm)	163.3 ± 6.1	158.1 ± 5.2	2.63	0.44
Weight (kg)	98.3 ± 6.4	91.1 ± 4.2	2.24	0.071
Clinical presentation				
Oligo/anovulation	196 (89.9%)	190 (86.3%)	-	0.32
Hyperandrogenism	117 (53.6%)	129 (58.6%)	-	0.28
Polycystic ovaries	156 (71.5%)	143 (65%)	-	0.34
Body mass index (kg/m2)	28.1 ± 3.2	27.1 ± 3.1	1.98	0.72
FSH (IU/mL)	4.1 ± 3.1	5.1 ± 2.2	1.08	0.81
LH (IU/mL)	11.2 ± 1.8	14.1 ± 2.2	2.88	0.07

Note: None of the differences were statistically significant (P>.05)

Table 2 Outcome in letrozole and clomiphene citrate (CC) groups.

	Letrozole group (n=218)	CC group (n=220)	t	P value
Total number of follicles	4.4 ± 0.4	6.8 ± 0.3	4.3	0.042[a]
Number of follicles >14mm	2.1 ± 0.3	3.7 ± 0.5	6.13	0.008 [a]
Number of follicles >18mm	2.3 ± 0.1	3.1 ± 0.8	5.03	0.0.3 [a]
Prettreatment endometrial thickness	4.5 ± 0.4	4.3 ± 0.5	1.41	0.52
Endometrial thickness at hCG (mm)	8.1 ± 0.2	9.2 ± 0.7	5.44	0.021 [a]
Serum E2 (pg/ml)	255.1 64.2	384 ± 91.3	4.12	0.022 [a]
Serum progesterone (ng/ml)	7.1 0.9	11.1 ± 1.2	6.33	0.024 [a]
Duration of stimulation (days)	12.1 ± 1.38	8 ± 2.9	4.91	0.036 [a]
Pregnancy/cycle	82/540 (15.1%)	94/523 (17.9%)	1.33	0.72
Miscarriage/patient	4 (12.1%)	4 (9.7%)	1.73	0.43

[a] Statistically significant difference: P<.05

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Chapter 7

Clomiphene citrate or anastrozole for ovulation induction in women with polycystic ovary syndrome? A prospective controlled trial

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Abstract

Objective: To compare the effects of anastrozole (1 mg) and clomiphene citrate (CC; 100 mg) used for ovulation induction in women with polycystic ovary syndrome.
Design: Prospective controlled trial.
Setting: University teaching hospital and private-practice setting.
Patient(s): The study comprised a total of 216 infertile women (469 cycles) with polycystic ovary syndrome.
Intervention(s): Patients received anastrozole (1 mg/d; 115 patients, 243 cycles) for 5 days, starting on day 3 of menses. A matched historical group of patients with polycystic ovary syndrome who were treated with CC (100 mg/d; 101 patients, 226 cycles) was used as a control group. Timed intercourse was advised 24–36 hours after hCG injection.
Main Outcome Measure(s): Number of follicles, serum E2, serum P, endometrial thickness, and pregnancy and miscarriage rates.
Result(s): The mean age, parity, and duration of infertility in both groups were similar, but statistically significantly more polycystic ovaries were found in the anastrozole group (odds ratio =2.44; 95% confidence interval =1.19–5.02). The total numbers of follicles were significantly higher in the CC group (3.8 ± 0.6 vs. 3.4 ± 0.5). Endometrial thickness at the time of hCG administration was significantly greater in the anastrozole group (10.1 ± 0.22 mm vs. 8.2 ± 0.69 mm). The duration of stimulation was similar in the two groups. Ovulation occurred in 165 (67.9%) of 243 cycles in the anastrozole group and in 150 (68.6%) of 226 cycles in the CC group without significant difference. Serum P was significantly higher in the CC group (7.1 ± 1.11 vs. 8.1 ± 0.88 ng/mL). The pregnancy and miscarriage rates were similar in the two groups.
Conclusion(s): Anastrozole was associated with significantly fewer mature and growing follicles, thicker endometrium, and slightly higher pregnancy rate. Anastrozole may be helpful in situations in which multiple pregnancy is not desirable or the risk of ovarian hyperstimulation syndrome is high.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in young women that manifests itself in a variety of clinical ways. Many patients with PCOS (55%–75%) are infertile because of chronic anovulation (1–3). Clomiphene citrate (CC) still is the typical therapy that is used for inducing or augmenting ovulation in this condition. It is not, however, equally triumphant in all situations, for different reasons. Clomiphene resistance, which refers to persistence of anovulation after standard CC therapy, occurs in 15%– 20% of patients (4). Because of its anti-estrogenic actions, CC may have a negative consequence on the cervical mucus and endometrium. The miscarriage rate after CC treatment is claimed to be higher than that in the general population (4).

Adjuvants to CC that are used to improve its performance, such as N-acetyl cysteine, have been used successfully in patients with PCOS (5) but not in the setting of unexplained infertility (6). Alternatives to CC, such as aromatase inhibitors, for example, anastrozole (2.2'[5-(1H-1,2,4-triazol-1-ylmethyl]-1,3-phenlene]bis(2ethylpropiononitrile), have been used for ovulation induction in case of CC failure. Anastrozole is an aromatase inhibitor that has been used widely in women with breast cancer (7). It works by suppressing estrogen production, which gives a concession in inducing ovulation. The aim of this prospective randomized trial was to compare the effects of anastrozole (1 mg) with CC (100 mg) meant for inducing ovulation in women with infertility caused by PCOS.

Materials and Methods

The study comprised 216 women with PCOS, from among those attending the Fertility Outpatient Clinic at Mansoura University Hospitals, Mansoura University, Egypt, and a private-practice setting, in the period from September 2005 to January 2007. The diagnosis of PCOS was made on the basis of the revised 2003 consensus on diagnostic criteria and longterm health risks related to polycystic ovary syndrome by the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (8). All women who were included in the study had patent fallopian tubes, as proved by hysterosalpingography, and their partners had normal semen analysis (sperm count of ≥20 millions, ≥25% forward progressive motility, and ≥30% normal morphology). All patients had normal serum PRL, TSH, and 17a-hydroxyprogesterone. The study was approved by the hospital's research ethics committee, and all participants gave informed consent before inclusion in the trial.

Withdrawal bleeding was achieved by using medroxyprogesterone acetate (10-mg tablets) for 10 days before stimulation when menses were irregular. One hundred fifteen successive patients received anastrozole (Arimidex, 1 mg; Zeneka Pharma International, United Kingdom) daily, starting on day 3 of the menses, for 5 days (115 patients, 243 cycles). All patients were monitored by transvaginal ultrasound for mean follicular volume and thickness of the endometrium on days 10, 12, and 14 of the cycle. At the time of hCG injection, serum E2 was measured by RIA, using direct doubleantibody kits (Pantex, Santa Monica, CA). Serum P was measured in days 21 to 23 of the cycle by using RIA, using the antibody coated-tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA). Injection of hCG (5,000–10,000 IU, IM) was given when at least one follicle measured R18 mm. Patients were advised to have intercourse 24–36 hours after hCG injection. For diagnosis of pregnancy, serum hCG was determined 2 weeks after hCG injection when menstruation was absent. The primary outcome measures were the following: number of growing and mature follicles, serum E2 (pg/mL), serum P (ng/mL), and endometrial thickness (mm). The secondary outcome measure was the occurrence of pregnancy and miscarriage.

Control Group

A historically matched group of 101 patients with PCOS who were treated during the same period of the trial with CC (100 mg/d; Hoechst Marion Roussel, Cairo, Egypt) for 5 days, starting on day 3 of menses (101 patients, 226 cycles), was used as a control group. All women had the same inclusion criteria as those in the study group.

Statistical Analysis

The data obtained were analyzed statistically by using Student's t-test in SPSS (version 13; SPSS Inc., Chicago, IL). Proportions were analyzed by using the X2 test. Results were expressed as mean and SEM. The differences were considered to be statistically significant if P was <.05.

Results

The study comprised 216 patients (463 cycles) in total. There were no statistically significant differences between the two groups as regards the age, duration of infertility, body weight, height, and body mass index. More polycystic ovaries were reported by ultrasonography in the anastrozole group (Table 1). The total numbers of follicles during stimulation were significantly higher in the CC group (3.8 ± 0.6 vs. 3.4 ± 0.5). The number of follicles sized R14 mm and R18 mm were significantly higher in the CC group (Table 2). There was no significant difference in the pretreatment endometrial thickness between the two groups, but the mean endometrial thickness at the time of hCG administration was significantly greater in the anastrozole group (10.1 ± 0.22 mm vs. 8.2 ± 0.69 mm). The duration to reach a dominant follicle was similar in the two groups. Ovulation occurred in 165 (67.9%) of 243 cycles in the anastrozole group and in 150

(68.6%) of 226 cycles in the CC group, which was not a statistically significant difference. Serum P was significantly higher in the CC group (7.1 ± 1.11 vs. 8.1 ± 0.88). Pregnancy occurred in 18 (7.9%) of 226 cycles in the CC group and in 25 (10.2%) of 243 cycles in the anastrozole group, and this difference was not statistically significant. There was one twin pregnancy in the CC group, but no ovarian hyperstimulation syndrome occurred in either group.

Discussion

Clomiphene citrate (CC) is not equally effective in all situations of induction of ovulation or superovulation. Clomiphene resistance occurs in 15%–20% of patients. The use of CC may be associated with poor cervical mucus and endometrial thinning in 15%–50% of patients, as a result of prolonged estrogen receptor depletion in the endometrium and possibly in the cervix (9–12). Alternatives to CC, such as aromatase inhibitors, were used for the same indication. To our knowledge, this is the first trial comparing anastrozole with CC used alone for ovulation induction in women with PCOS.

Anastrozole is a third-generation aromatase inhibitor. Blocking estrogen production by inhibiting aromatization will stop the conversion of androstenedione and T to estrogen in the ovary. This hypoestrogenic state would release the hypothalamic– pituitary axis from estrogenic negative feedback, which in turn would increase FSH secretion and the development of ovarian follicles. In their original work, Mitwally and Casper (13) described the use of letrozole (2.5 mg) on days 3–7 of menses in 12 patients with PCOS. Ovulation occurred in nine patients (75%), and pregnancy was achieved in three (25%) (13). So far, there has been no general agreement on the recommended dose of aromatase inhibitors for inducing ovulation. In this study, a 1 mg dose of anastrozole was used because this dose has been found to be roughly equivalent to 2.5 mg of letrozole in terms of inhibiting E2 production over a 6-week time period (14).

Compared with anastrozole, the use of CC led to significantly more developing and mature follicles (14- and 18-mm follicles). Although the pregnancy rate was slightly higher in the anastrozole group, that difference was not statistically significant. The miscarriage rate was similar in both groups. There have been other trials that compared letrozole and CC, with diverse results. Bayar et al. (15) reported better outcomes in the CC group, but on the contrary, Al-Fozan et al. (16) reported better results in the letrozole group than in the CC group, regarding the number of developing and mature follicles. In a study performed by our group and published elsewhere, we found that letrozole did not give any advantages over CC in the context of ovulation induction (17). Sipe et al. (18) compared the ovarian and endometrial effects of anastrozole and CC when used with gonadotropins in a combination protocol. Women were randomized to receive either anastrozole (1 mg) or CC (100 mg) for 5 days, followed by FSH injections (days 7–11) for ovulation induction. Anastrozole cycles were associated with fewer total follicles, fewer mature follicles, lower serum E2, and the same endometrial stripe thickness, compared with CC cycles. Serum P was higher in the anastrozole group. It is not clear whether CC causes more abnormal LH secretion than anastrozole or whether the difference is caused by another mechanism. Pregnancy rates were similar between CC (20%) and anastrozole (12%) cycles. Sipe et al. (18) concluded that a combination protocol of anastrozole and gonadotropins may be safer for patients who are at higher risk of hyperstimulation and multiple births.

Clomiphene citrate results in central estrogen receptor depletion for a long duration because of its significantly greater half-time for clearance (2 wk) (9, 10). As a result, supraphysiological levels of estrogen can occur without central suppression of FSH, because the normal estrogen receptor–mediated feedback mechanisms are blocked. This results in multiple follicle growth and in higher multiple pregnancy rates with CC than occurs in anastrozole cycles. In many situations in which multiple mature follicles are needed to develop, anastrozole alone probably is not the optimal

choice for ovulation induction. In this study, the greater number of mature follicles in the CC group did not result in a significantly higher pregnancy rate, which leads to the discussion about the effect of both drugs on the endometrium. As expected, the endometrial thickness was significantly more in the anastrozole group. Mitwally and Casper (13) showed that aromatase inhibitors have minimal effects on the endometrium, compared with CC (19). Cortinez et al. (20) found normal morphology of endometrium and full expression of pinopodes during the implantation window when aromatase inhibitors were used.

Information on teratogenic capacity in human beings is lacking, and extrapolation of animal data to human beings is a complex process. Mitwally et al. (21) reported favorable pregnancy outcomes and a low multiple-gestation rate for the use of aromatase inhibitors for ovarian stimulation. Clomiphene citrate has been reported to elicit various ocular side effects in 1.5%–10% of patients taking it (22). Toxicology studies have shown that anastrozole is well tolerated at 1 and 6 months. Oral administration of anastrozole to pregnant rats and rabbits caused no teratogenic effects (23). The cost of anastrozole per cycle is much higher than that of CC, especially because higher doses of anastrozole are required (respective costs, 500 vs. 5 Egyptian pounds per cycle).

We are aware of the limitations of the study, which would have been strengthened if prospective randomization had been performed. However, the results of this study showed that anastrozole was associated with significantly fewer mature and growing follicles, thicker endometrium, and a slightly higher pregnancy rate. Anastrozole may be helpful in situations in which multiple pregnancy is not desirable or in which the risk of ovarian hyperstimulation syndrome is high.

Table 1 Patients' Characteristics

	Anastrozole group (n=115)	CC group (n=101)	**t**	P value	CI
Number of cycles	243	226			
Age (years)	23.8± 3.1	25.3 ± 2.9	1.04	0.67	-0.12-0.15
Parity	0.3 ± 0.12	0.3 ± 0.16	0.98	0.71	0.30-0.06
Height (cm)	158.3 ± 5.12	155.1 ± 4.20	1.65	0.08	0.002-0.15
Weight (kg)	80.3 ± 5.42	79.1 ± 4.22	0.22	O.95	0.26-0.45
Clinical presentation Oligo/anovulation Hyperandrogenism Polycystic ovaries	 110 (95.6%) 51 (44.3%) 98 (85.2%)	 92 (91.0%) 42 (41.5%) 71 (70.2%)	X2= 1.84 0.17 7	 1.75 0.68 0.008**	 0.36-1.31 0.63-1.99 1.05-1.41
BMI (kg/m2)	31.1 ± 2.91	29.1 ± 3.12	1.4	0.31	-0.02-5.4
FSH (IU/ml)	6.1 ± 2.92	6.3 ± 2.22	2.43	0.06	0.07-2.1
LH (IU/ml)	13.2 ± 1.82	12.1 ± 3.11	2.55	0.052	0.06-3.2

** Statistical significant differences as P <0.05

Table 2 Outcome in Letrozole and clomiphene citrate (CC) groups

	Anastrozole group (n=115)	CC group (n=101)	t	P value	CI
Total number of follicles	3.4 ± 0.5	3.8 ± 0.6	2.45	0.044*	0.0004-0.27
Number of follicles >14 mm	2.1± 0.41	2.7 ± 0.38	3.32	0.02*	0.081-1.53
Number of follicles >18 mm	1.9 ± 0.11	2.1 ± 0.69	3.16	0.02*	0.08-1.6
Prettt endometrial thickness (mm)	5.5 ± 0.33	5.3 ± 0.45	1.32	0.65	-0.25-1.6
Endometrial thickness at hCG (mm)	10.1 ± 0.22	8.2 ± 0.69	4.21	0.01**	0.46-3.55
Serum E2 (pg/ml)	391.5 ± 89.33	448.1 ± 54.23	2.24	0.08	-0.62-0.44
Serum progesterone (ng/ml)	8.1 ± 0.88	7.1 ± 1.11	3.05	0.03*	0.1-1.6
Duration of stimulation (days)	10.1 ± 1.32	10.3 ± 2.88	0.95	0.21	-1.6-5.4
Pregnancy/cycle	25/243 (10.2%)	18/226 (7.9%)	X2= 0.76	0.38	0.72-2.3
Miscarriage/patient	4 (16%)	3(16.6%)	X2=0.04	0.83	0.27-5.11

- Statistical significant differences as $P < 0.05$

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Chapter 8

Anastrozole or letrozole for ovulation induction in clomiphene-resistant women with polycystic ovarian syndrome: A prospective randomized controlled trial

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Abstract

Objective: To compare the effects of letrozole (2.5 mg) and anastrozole (1 mg) meant for ovulation induction in clomiphene (CC)-resistant women with PCOS.

Design: Prospective randomized controlled trial.

Setting: University teaching hospital and private practice setting.

Patient(s): The study comprised a total of 220 infertile women (574 cycles) with CC-resistant PCOS.

Intervention(s): Patients were randomized to treatment with 2.5 mg of letrozole daily (111 patients, 295 cycles) or 1 mg of anastrozole daily (109 patients, 279 cycles) for 5 days from day 3 of menses.

Main Outcome Measure(s): Number of follicles, serum E2, serum P, endometrial thickness, pregnancy rate (PR), and miscarriage rate.

Result(s): The total number of follicles was significantly more in the anastrozole group (5.4 ± 0.4 vs. 5.8 ± 0.4). The number of follicles >14mm (3.1 ± 0.3 vs. 2.7 ± 0.2) and >18mm (2.3 ± 0.1 vs. 3.1 ± 0.2) were significantly higher in the anastrozole group. The endometrial thickness at the time of hCG administration was significantly more in the anastrozole group (9.1 ± 0.2 vs. 10.2 ± 0.7 mm). The duration to reach a dominant follicle was longer in the letrozole group (12.1 ± 1.3 days vs. 8.8 ± 1.9 days) but without statistical significant difference. Ovulation occurred in 183/295 cycles (62%) in the letrozole group and 177/279 cycles (63.4%) in the anastrozole group, whereas pregnancy occurred in 36/295 cycles (12.2%) in the letrozole group and 42/279 cycles (15.1%) in the anastrozole group and the differences were not statistically significant.

Conclusion(s): The results of this study did not show a significant difference in PR or miscarriage rate between anastrozole and letrozole when used for ovulation induction in women with CC-resistant PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in young women. It manifests itself in a variety of clinical ways and 55%–75% of patients with PCOS are infertile due to chronic anovulation (1, 2). Clomiphene citrate (CC) is still the standard drug for inducing or augmenting ovulation. It is not, however, equally successful in all situations. Clomiphene resistance refers to persistence of anovulation after standard CC therapy, which occurs in 15%–20% of patients (3). Clomiphene citrate may have a negative effect on the cervical mucus and endometrium and is associated with a discrepancy between ovulation and conception rates (3, 4). Adjuvants to CC such as N-acetyl cysteine (NAC) were used successfully in patients with PCOS (5), but not in the setting of unexplained infertility (6). Alternatives to CC such as gonadotropins are used but they are expensive and increase the risk of multiple pregnancy and ovarian hyperstimulation syndrome (OHS). Aromatase inhibitors drugs (e.g., letrozole and anastrozole) have attracted the attention for a long time to induce ovulation. Letrozole and anastrozole have been widely used in women with breast cancer (7). They are highly selective, orally administered, and easy to monitor (8). The aim of this prospective, randomized trial is to compare the effects of 2.5 mg of letrozole with 1 mg of anastrozole meant for inducing ovulation in infertile women with CC resistant PCOS.

Materials and Methods

The study comprised of 220 women (574 cycles) with CCresistant PCOS among those attending the Fertility Outpatient Clinic in Mansoura University Hospitals, Mansoura University, Egypt, and a private practice setting in the period from May 2005 to January 2007. Diagnosis of PCOS based on the revised 2003 consensus on diagnostic criteria and long-term health risks related to PCOS (9). All women were previously treated with 100 mg of CC daily for 5 days per cycle, for two to three cycles with persistent anovulation or ovulate with very thin endometrium <5 mm at

the time of hCG administration. They had patent Fallopian tubes proved by hysterosalpingography and normal semen analysis for their partners according to the modified criteria of World Health Organization. The study was approved by the local Research Ethics Committee and all participants gave informed consent before inclusion in the trial. Patients were randomly allocated using a computer-generated random table into two treatment groups: letrozole group (111 patients, 295 cycles) and anastrozole group (109 patients, 279 cycles). Withdrawal bleeding was achieved using 10 mg of dydrogesterone tablets for 10 days before stimulation. Patients in letrozole group took 2.5 mg of letrozole oral tablets (Femara; Novartis Pharma Services, Switzerland) daily from day 3 of the menses for 5 days (111 patients, 295 cycles), whereas patients in the anastrozole group took 1 mg of anastrozole oral tablets (Arimidex; Zeneka Pharma International, United Kingdom) daily from day 3 of the menses for 5 days (109 patients, 279 cycles).

All patients were monitored by transvaginal ultrasound for the mean follicular diameter and endometrial thickness in the days 10, 12, and 14 of the cycle. Serum E2 (pg/mL) was measured at the time of hCG injection by RIA using direct double antibody kits (Pantex, Santa Monica, CA) and serum P (ng/ mL) was measured on days 21–23 of the cycle by RIA using antibody coated-tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA). Human chorionic gonadotropin injection (5,000–10,000 IU IM, Pregneyl; Organon, Holland) was given when one follicle measured at least 18 mm was found. Patients were advised to have intercourse 24–36 hours after hCG injection. Serum hCG was determined 2 weeks after hCG injection in the absence of menstruation for diagnosis of pregnancy. The primary outcome measures were the number of growing and mature follicles, serum E2 (pg/ml), serum P (ng/mL), and endometrial thickness (mm). Secondary outcome measures were the occurrence of pregnancy and miscarriage.

Statistical Analysis

Data obtained were statistically analyzed using SPSS computer package (SPSS Inc., Zonguldak Karaelmas University, Zonguldak, Turkey) by Student's t-test. Proportions were analyzed using the c2 test. Results were expressed as mean and standard error of the mean. The differences were considered to be statistically significant if $P<.05$.

Results

The study comprised 220 patients (574 cycles) in total. There were no statistical significant differences between the two groups with regard to age, duration of infertility, body weight, height, body mass index (BMI), or the presenting symptoms and signs (Table 1). The total number of follicles during stimulation was significantly more in the anastrozole group (5.4 ± 0.4 vs. 5.8 ± 0.4). The number of follicles >14 mm and >18 mm were significantly higher in the anastrozole group (Table 2). The endometrial thickness at the time of hCG administration was significantly more in the anastrozole group (9.1 ± 0.2 mm vs. 10.2 ± 0.7 mm). The duration to reach a dominant follicle was longer in the letrozole group (12.1 ± 1.3 days vs. 8.8 ± 1.9 days) but without statistical significant difference.

Ovulation occurred in 183/295 cycles (62%) in the letrozole group and 177/279 cycles (63.4%) in the anastrozole group without significant difference between two groups. Serum E2 and P were higher in the anastrozole group but without a statistical significant difference (Table 2). Pregnancy occurred in 36/295 cycles in the letrozole group (12.2%) and 42/279 cycles (15.1%) in the letrozole group and the difference was not statistically significant. Two twin pregnancies occurred in the letrozole group and none in the anastrozole group. No higher order pregnancies or OHS occurred in both groups.

Discussion

Letrozole (4, 40-[1H-1, 2, 4-triazol-1-ylmethylene]-bis-benzonitrile) and anastrozole (2.20[5-(1H-1,2,4-triazol-1- ylmethyl]- 1,3-phenlene]bis (2-methylpropiononitrile) are third generation aromatase inhibitors. Administering aromatase inhibitors early in the follicular phase can induce ovulation by releasing the hypothalamus or pituitary from estrogen (E) negative feedback on GnRH and gonadotropin secretion, which would stimulate ovarian follicular development. An alternative hypothesis is that aromatase inhibitors may act locally in the ovary to increase follicular sensitivity to FSH by accumulation of intraovarian androgens (10). In addition, androgen accumulation in the follicle may stimulate insulinlike growth factor I (IGF-I), along with other endocrine and paracrine factors, which may synergize with FSH to promote folliculogenesis (11). Mitwally and Casper (12) described the use of 2.5 mg letrozole on days 3–7 of menses in women with PCOS. Ovulation occurred in nine patients (75%) and pregnancy occurred in three (25%). Thereafter, many studies proved the efficacy of aromatase inhibitors in inducing ovulation in different clinical situations (13–17). So far, there is no general agreement on the recommended dose of aromatase inhibitors for inducing ovulation. In this study, 1 mg of anastrozole was used because this dose has been found to be roughly equivalent to 2.5 mg of letrozole in terms of inhibiting E2 production. In a pharmacologic study of menopausal women, 1 mg of anastrozole suppressed E2 levels to the same extent as 2.5 mg of letrozole. Letrozole (2.5 mg) was slightly more effective in inhibiting total body aromatization during 6 weeks (18). The investigators concluded that there may be differences in the aromatase inhibition by the two drugs, but the differences are likely to be small. In a clinical randomized trial comparing different doses of letrozole to induce ovulation in patients with unexplained infertility, higher doses of letrozole (5 and 7.5 mg), although having the potential advantage of requiring a shorter period of stimulation, have been found to offer no significant advantage in comparison to the use of 2.5 mg in terms of PR (19).

In this study, anastrozole resulted in significantly more mature and medium-sized follicles and more endometrial thickness than letrozole. Although, the serum level of E2 and P in the anastrozole group were higher, the differences were not statistically significant. This E2 level allowed the growth of the endometrium to an adequate thickness on the day of hCG administration, showing the absence of the antiestrogenic effects seen with CC. Mitwally and Casper (12) showed that letrozole, as an aromatase inhibitor, has minimal effect on the endometrium and compared with CC, letrozole is associated with a thicker endometrium. Cortinez et al. (20) found normal morphology of the endometrium and full expression of pinopodes during the implantation window when aromatase inhibitors were used. These differences in number of follicles or endometrial thickness in this study, however, did not show significant improvement in the ovulation or pregnancy rate (PR) in the anastrozole group in comparison to the letrozole group. To our knowledge, only one small trial compared anastrozole (1 mg) and letrozole (2.5 mg) for induction of ovulation in 40 CC-resistant PCOS patients (21). In contrary to our results, letrozole was associated with significantly more endometrial thickness, size of follicles, E2 levels, ovulation rate, and PR. Those investigators reported no difference between the two groups with regard to the number of follicles, duration of stimulation, or the serum P level. This was a preliminary small trial of limited power to endow with reliable conclusions.

Recently, the safety of letrozole was seriously questioned. An abstract presented at the American Society for Reproductive Medicine (ASRM) meeting in 2005, examining a relatively small number of letrozole pregnancies (130 pregnancies) compared to a large control group of spontaneous conceptions, suggested that the use of letrozole for infertility treatment might be associated with a higher risk of congenital cardiac and bone malformations in the newborns (22). As a result of this small study, on November 17, 2005, Novartis Pharmaceutical, the

manufacturer, issued a statement to physicians in Canada and worldwide advising that letrozole use in premenopausal women, specifically its use for ovulation induction, is contraindicated (23). A more recent multicenter retrospective study in Canada by Tulandi et al. (24) on the pregnancy outcome after letrozole induction concluded that the concern about letrozole use for ovulation induction was unproved. Toxicology studies showed that anastrozole is well tolerated at 1 and 6 months. Oral administration of anastrozole to pregnant rats and rabbits caused no teratogenic effects (25).

The cost of letrozole and anastrozole/cycle are comparable, but both are much more expensive than CC, especially when higher doses of letrozole/anastrozole are required (200 Egyptian pounds vs. 5 Egyptian pounds). The results of this study showed more growing and mature follicles and greater endometrial thickness in the anastrozole group. However, it did not show significant advantage for anastrozole over letrozole with regard to PR or miscarriage rates when used for ovulation induction in women with CC-resistant PCOS.

Table 1 Patients' characteristics

	Letrozole group (n=111)	Anastrozole group (n=119)	t	X^2	P value
Number of cycles	295	279			
Age (years)	28.2 ± 2.8	26.3 ± 2.5	2.66		0.1
Parity	0.3 ± 0.1	0.3 ± 0.2	1.05		0.38
Clinical presentation					
Oligo/anovulation	80 (72.0%)	87 (73.1%)		0.03	0.86
Hyperandrogenism	45 (40.5%)	56 (47.1%)		0.99	0.32
Polycystic ovaries	79 (71.2%)	81 (68.1%)		0.26	0.60
Height (cm)	166.3 ± 5.2	162.1 ± 5.1	2.95		0.08
Weight (kg)	85.3 ± 6.4	88.1 ± 4.2	2.88		0.09
BMI (kg/m2)	29.1 ± 3.1	30.1 ± 2.1	2.05		0.12
FSH (IU/ml)	4.6 ± 2.1	5.1 ± 2.2	3.88		0.06
LH (IU/ml)	11.4 ± 2.8	11.8 ± 2.6	3.90		0.061

No statistical significant differences as P >0.05

Table 2 Outcome in letrozole and anastrozole groups

	Letrozole group (n=111)	Anastrozole group (n=119)	t	P value
Total number of follicles	5.4 ± 0.4	5.8 ± 0.4	5.21	0.01*
Number of follicles >14 mm	3.1± 0.3	2.7 ± 0.2	5.33	0.004*
Number of follicles >18 mm	2.3 ± 0.1	3.1 ± 0.2	8.62	0.001*
Prettt endometrial thickness (mm)	5.5 ± 0.5	5.3 ± 0.6	1.31	0.22
Endometrial thickness at hCG (mm)	9.1 ± 0.2	10.2 ± 0.7	4.45	0.04*
Serum E2 (pg/ml)	455.1 ± 64.2	484 ± 91.3	2.39	0.08
Serum progesterone (ng/ml)	9.2 ± 0.9	10.1 ± 1.2	2.81	0.06
Duration of stimulation (days)	11.9 ± 1.3	10.8 ± 2.2	2.30	0.21
Pregnancy/cycle	36/295 (12.2%)	42/279 (15.1%)	0.99	0.31
Miscarriage/patient	4 (11.1%)	4(9.5%)	0.01	0.92

* Statistical significant differences as P <0.05

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Chapter 9

Randomized controlled trial of three doses of letrozole for ovulation induction in patients with unexplained infertility

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Abstract
The aromatase inhibitor letrozole is a novel agent that can be used as an alternative to clomiphene citrate for ovulation induction in patients with unexplained infertility. The dose of letrozole used has varied between studies, and this study aimed to compare the three most commonly used doses: 2.5, 5 and 7.5 mg. A total of 179 patients were randomly recruited in this prospective study with 58, 61 and 60 patients in each dosage group respectively. This study reports a significantly higher ($P< 0.05$) number of follicles (total, > 14 mm and ≥ 18 mm) on the day of administration of human chorionic gonadotrophin in the 7.5 mg group, associated with significantly fewer ($P < 0.05$) days of stimulation. However the pregnancy and miscarriage rates were similar in the three groups. In conclusion, it seems that the use of higher doses of letrozole offers no advantage in terms of pregnancy rates over the lower (2.5 mg) dose.

Introduction

Among couples experiencing difficulty conceiving, unexplained infertility is a fairly common diagnosis, occurring in approximately 10–20% of cases (1). One of the options available to women with unexplained infertility is ovulation induction, which is most commonly achieved using clomiphene citrate. However, clomiphene citrate can produce side effects, such as hot flushes and adverse effects on the cervical mucus and endometrium, due mostly to its long half-life and anti-oestrogenic effects (2). More recently, the aromatase inhibitor letrozole has been investigated as a possible alternative, either as a first treatment or in patients in whom clomiphene has been ineffective (2-5). Different doses of letrozole have been used in previous studies. The dose has varied from 2.5 to 7.5 mg, and in one study the use of a single 20 mg dose has been described (6). The aim of this study was to compare three different doses of letrozole (2.5 mg, 5 mg and 7.5 mg) in women undergoing ovulation induction and timed intercourse for treatment of unexplained infertility.

Materials and Methods

Study population

This was an open-label, parallel-group randomized controlled trial, including 179 women with unexplained infertility attending the infertility clinic of a tertiary referral centre (Mansoura University Hospitals, Egypt) and a private assisted conception unit, both in Mansoura, Egypt, between January 2005 and September 2006The study was approved by the local research ethics committee and all participants gave informed consent before inclusion in the trial.

Inclusion criteria

Patients were included in the trial if they fulfilled all of the following inclusion criteria: (i) they were less than 40 years old; (ii) they had had at least one year of infertility; (iii) patent Fallopian tubes had been proven by hysterosalpingography or laparoscopy; (iv) they had ovulatory mid-luteal progesterone concentrations; (v) the husband's semen analysis was normal.

Randomization

Patients were randomly allocated using a computer-generated random table into three letrozole treatment groups: 2.5 mg (n = 58), 5 mg (n = 61) and 7.5 mg (n = 60).

Cycle monitoring

After at least one cycle without ovulatory therapy, patients were given 2.5 mg, 5 mg or 7.5 mg of letrozole (Novartis Pharma Services, Switzerland) daily, starting on day 3 of the menses, for 5 days. Therapy continued for one to three menstrual cycles. All patients were monitored throughout the study period, as described below. A transvaginal ultrasound scan was carried out on day 2 of the cycle toensure the absence of any ovarian cysts. Scans were then performed on

days 10, 12 and 14 of the cycle to determine mean follicular diameter and endometrial thickness. The mean follicular diameter was measured by taking the mean of three internal follicular diameters. The endometrial thickness was measured in the saggital plane and the maximum anteroposterior endometrial diameter was measured. Serum oestradiol (pg/ml) was measured at the time of human chorionic gonadotrophin (HCG) injection by radioimmunoassay using direct double-antibody kits (Pantex, Santa Monica, CA, USA). Serum progesterone (ng/ml) was measured on days 21 to 23 of the cycle by radioimmunoassay using an antibody-coated tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). HCG (Pregnyl, Organon, Holland; 5000– 10000 IU i.m.) was given when at least one follicle measured at least 18 mm. Timed intercourse was advised daily from the night of HCG administration for 1 week. Serum βHCG was measured two weeks after HCG administration in the absence of menstruation for diagnosis of pregnancy. Pregnancy was diagnosed on the basis of a βHCG concentration greater than 2 IU/l, and a positive gestational sac viewed by transvaginal ultrasound when the βHCG concentration was more than 1500 IU/l.

Statistical analysis

Data analysis was performed using SPSS version 13. Results were expressed in the form of mean ± SD and percentages. The chi-squared test was used to compare proportions. Student's ttest and one-way analysis of variance (ANOVA) were used to compare means, as appropriate. Statistical significance was set at $P < 0.05$.

Results

Demographic characteristics of the study groups

A total of 179 patients were recruited and none were lost to follow-up. Patients receiving the 7.5 mg dose had a significantly longer duration of prior infertility compared with the 2.5 mg group ($P = 0.02$), otherwise the three groups were similar regarding all other demographic characteristics (Table 1).

Ovarian stimulation

The total number of follicles and the number of intermediate (14 mm) and mature (18 mm) follicles were all significantly higher in the 7.5 mg group compared with the other two groups. The number of days needed to achieve a mature follicle was also significantly less and the mid-luteal serum progesterone concentration was significantly higher in the 7.5 mg group compared with the other two groups. There was no evidence for any significant difference between the three groups regarding the pre-treatment endometrial thickness, pregnancy or miscarriage rates. Characteristics of the stimulated cycles are presented in Table 2 and details of significant relationships between the three groups (post hoc test) are presented in Table 3.

Discussion

Letrozole is a third-generation aromatase inhibitor that blocks oestrogen production by inhibiting the conversion of androstendione and testosterone to oestrogen in the ovary, thus releasing the hypothalamic/pituitary axis from oestrogenic negative feedback. FSH secretion thus increases, stimulating the development of ovarian follicles.

Despite several studies that have shown the effectiveness of letrozole for ovarian stimulation, there have been very few studies to determine the optimal dose. Most studies have used a 2.5 mg dose (4, 6, 7); others have used a range from 2.5–5 mg (5), while at least one study has used a 7 mg dose (2). One study (8) has compared two of these doses, 2.5 versus 5 mg, and found that 5 mg of letrozole was associated with more follicles and a higher pregnancy rate. However, there are no studies to date that have compared all of the three commonly-used doses. This study

reports on the different responses elicited by administration of 2.5 mg, 5 mg and 7.5 mg of letrozole in patients with unexplained infertility.

Similar to the findings of Al-Fadhli et al. (2006), this study reports a significant ($P < 0.05$) dose-dependent increase in the total number of follicles, the number of intermediate (>14 mm) and mature (≥18 mm) follicles on the day of HCG administration, with the most follicles yielded following the 7.5 mg dose. The number of days needed to achieve a mature follicle also showed a significant ($P < 0.05$) dose-dependent decrease with increasing doses of letrozole (8). This is in contrast with the findings of Al-Fadhli et al. (2006) (8), who found no significant difference in the days needed for stimulation for patients receiving 2.5 or 5 mg of letrozole. This discrepancy in the results may be due to recruitment of different populations.

The exponential increase in the number of mature follicles with increasing doses of letrozole obviously raises the fear of a parallel increase in oestradiol concentrations that may ultimately result in an increased risk of ovarian hyperstimulation syndrome. However, these results are reassuring in that although the number of follicles significantly ($P < 0.05$) increased with the 5 mg and 7.5 mg doses, there was no significant difference in the peak oestradiol concentrations between the three groups. As letrozole is an aromatase inhibitor an increased dose should theoretically be associated with decreased oestradiol levels, which may affect the cervix as well as the endometrium. This was not found to be the case in this study and up to 7.5 mg of the drug did not show any detrimental effect. The increase in the number of mature follicles is not, however, paralleled by a similar increase in pregnancy rate between the three groups. This, again, is in contrast with the findings of Al-Fadhli et al. (2006) (8) who found that, compared with the 2.5 mg dose, the 5 mg dose was associated with a higher pregnancy rate. The higher concentrations of mid-luteal progesterone and thicker endometria seen in the 5 mg and 7.5 mg groups would lead to an initial expectation of a better luteal phase and lower miscarriage rate with the use of higher doses of letrozole. However this was not the case and, although not statistically significant, we observed a slightly higher miscarriage rate associated with the use of the 5 mg and 7.5 mg doses compared with the 2.5 mg dose (Table 2).

These findings may therefore suggest that higher doses of letrozole may be associated with unfavourable effects on the endometrium. Letrozole, through inhibition of aromatase activity, prevents the conversion of androgens to oestrogen, thus increasing testosterone concentrations (7). Although there are data to suggest that testosterone may augment follicular FSH-receptor expression in primates and promote follicular growth by amplifying FSH effects (7, 9,10), it may be that higher concentrations of testosterone are produced with higher doses of letrozole, causing an adverse effect on the endometrium. However, testosterone concentrations were not measured in this study and this hypothesis therefore warrants further investigation in future studies. In conclusion, use of higher doses of letrozole (5 and 7.5 mg), although having the potential advantage of requiring a shorter period of stimulation, has been found to offer no significant advantage over the use of 2.5 mg in terms of pregnancy rates. Further studies examining endometrial receptivity and androgen profiles with letrozole treatment are needed.

Table 1 Demographic characteristics of the study groups

	Letrozole 2.5 mg (n=58)	Letrozole 5 mg (n=61)	Letrozole 7.5 mg (n=60)	p
Age (y)	28.8 ± 0.3	31.8 ± 0.6	29.7 ± 0.5	NS
Parity	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.3	NS
Duration of infertility (y)	2.8 ± 0.3	3.1 ± 0.3	4.1 ± 0.4	0.02*
BMI	24.1 ± 3.2	23.1 ± 3.3	25.1 ± 3.1	NS
FSH (IU/L)	4.6 ± 0.7	4.9 ± 0.5	3.9 ± 0.5	NS
LH (IU/L)	4.8 ± 0.5	5.2 ± 0.4	4.7 ± 0.4	NS

There was evidence for a significant difference in the duration of infertility between the 2.5 mg and the 7.5 mg groups (one way ANOVA)

Table 2 Cycle characteristics during stimulation in the three groups

	Letrozole 2.5 mg (n=61) Group 1	Letrozole 5 mg (n=58) Group 2	Letrozole 7.5 mg (n=60) Group 3	p[a]
Total number of follicles	4.2 ± 0.4	5.8 ± 0.3	6.9 ± 0.3	< 0.05
No. of follicles >14 mm	1.6 ± 0.1	2.1 ± 0.1	4.1 ± 0.2	<0.05
No. of follicles ≥18 mm	1.0 ± 0.0	1.4 ± 0.1	3.4 ± 0.3	<0.05
No. of days of stimulation[b]	12.4 ± 0.3	11.6 ± 0.5	9.6 ± 0.4	<0.05
Pre-treatment endometrial thickness (mm)	4.4± 0.5	4.1 ± 0.5	4.3 ± 0.4	NS
Endometrial thickness (mm)	8.5 ± 0.3	8.2 ± 0.3	10.2 ± 0.4	<0.05
Serum E2 (pg/ml)	257.1 ± 68.2	284 ± 91.3	302 ± 71.3	NS
Serum progesterone (ng/ml)	7.1 ± 0.8	9.1 ± 1.2	11.3 ± 1.3	<0.05
Pregnancy rate/cycle (%)	8 (4.8%)	7 (4.3%)	10 (6.5%)	NS
Miscarriage/patient (%)	7 (11.6%)	9 (15%)	9 (15%)	NS

NS: non significant

Values are mean ± SD unless otherwise stated; NS = Not statistically significant.

[a]Statistical significance was tested pairwise for all three doses of letrozole.
[b]Patients were treated for 5 days per cycle over 1–3 cycles; the value given represents the mean duration of treatment.

Table 3 Post Hoc test for p values between the three groups where a significant relationship exists

Parameter	Group 1 Vs group 2	Group 2 Vs group 3	Group 1 Vs Group 3
Total number of follicles	0.002	0.004	0.001
No. of follicles >14 mm	0.04	0.03	0.02
No. of follicles ≥18 mm	0.03	0.002	0.002
No. of days of stimulation	NS	0.03	0.03
Endometrial thickness	NS	0.03	0.04
Serum progesterone	NS	0.07	0.001

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Chapter 10

Pregnancy outcome after ovulation induction with aromatase inhibitors or clomiphene citrate

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Abstract

The aim of this prospective trial was to evaluate the pregnancy outcome after ovulation induction with aromatase inhibitors or clomiphene citrate (CC). The study comprised a total of 796 infertile women (1100 cycles) and 200 spontaneously pregnant women (298 cycles) as a control group. Patients were randomly allocated to treatment either with 100 mg of CC daily (420 patients, 634 cycles), 5 mg of letrozole daily (269 patients, 323 cycles) or 1 mg of anastrozole daily (107 patients, 143 cycles) for 5 days starting on day 3 of menses. Timed intercourse was advised 24-36 hours after hCG injection. The outcome measures were the occurrence of pregnancy, miscarriage and neonatal outcome. The mean age, parity, BMI and duration of infertility in all groups were similar. Pregnancy occurred in 167/1398 cycles (11.9%) in total without statistical significant differences between all groups. The total miscarriage rate was 16.1% (varied between 14.2% in CC group to 19.9% in anastrozole group) without difference between spontaneous and stimulated pregnancies. There were 129 deliveries in all groups. There were no statistical significant differences between the stimulated and spontaneous pregnancies as regards mean gestational age, premature deliveries, birth weight, SGA <10 percentile or 5 minutes Apgar score. There was one case of complete cleft palate and one case of major congenital heart problem in the letrozole group. There were 2 cases of talipus equinovarus in CC and spontaneous pregnancy group. In conclusion, aromatase inhibitors and CC are equally effective in inducing and augmenting ovulation in many situations. They resulted in favorable pregnancy outcomes and average miscarriage rates. Safety of the drugs to both the mother and fetuses were documented.

Introduction

Letrozole (4, 4′-[1H-1, 2, 4-triazol-1-ylmethylene]-bis-benzonitrile) and anastrozole (2.2'[5-(1H-1,2,4-triazol-1- ylmethyl]- 1,3-phenlene]bis (2-methylpropiononitrile) are third-generation aromatase inhibitors. Administering aromatase inhibitors early in the follicular phase can induce ovulation by releasing the hypothalamus or pituitary from estrogen negative feedback on GnRH and gonadotropin secretion, which would stimulate ovarian follicular development. Mitwally and Casper (2001) described the use of 2.5 mg letrozole on days 3–7 of menses in polycystic ovary syndrome (PCOS) (1). Ovulation occurred in nine patients (75%) and pregnancy occurred in three (25%). Thereafter, many studies discussed the efficacy of aromatase inhibitors in inducing ovulation in different clinical situations (2-6).

Recently, the safety of letrozole and may be other aromatase inhibitors for ovulation induction were seriously questioned. Biljan et al. (2005) suggested that the use of letrozole for infertility treatment might be associated with a higher risk of congenital cardiac and bone malformations in the newborns. In this prospective controlled trial, we presented the pregnancy and neonatal outcomes after the use of aromatase inhibitors and clomiphene citrate for ovulation induction in comparison with the outcome after spontaneous (non-stimulated) pregnancy (7).

Materials and Methods

The study comprised of 796 women with unexplained infertility attending the Fertility Outpatient Clinic in Mansoura University Hospitals, Mansoura University, Egypt and private practice settings in the period from October 2003 to March 2007. All women had at least 1 year of continuous marriage without conception. All patients had patent fallopian tubes as proven by hysterosalpingography; had normal ovulating cycles as proven by midluteal serum P levels; in addition to normal semen analysis for their partners according to the modified criteria of the WHO (sperm count ≥20 x 10^5/ml, ≥25% forward progressive motility and ≥30% normal morphology). Therefore, by definition, all women had unexplained infertility to nullify the effect of co-morbidities such as polycystic ovary syndrome on the pregnancy outcome. The study was approved by the hospital Research Ethics Committee and all participants gave informed consent

before inclusion in the trial. Patients were randomly allocated to treatment with different ovulation induction protocols using Clomiphene citrate (CC), letrozole and anastrozole using computer-generated random table. Clomiphene citrate (CC) group included 420 patients who completed 634 cycles of treatment with 100 mg of CC (Hoechest Marion Russel, ARE) daily starting day 3 of the menses for 5 days. Anastrozole group included 107 patients (143 cycles) who received 1 mg anastrozole (Arimidex@, Zeneka Pharma International, UK) daily starting day 3 of the menses for 5 days. Letrozole group included 269 patients (323 cycles) who were treated with 5 mg of letrozole (Novartis Pharma Services, Switzerland) daily starting day 3 of the menses for 5 days. All patients were monitored for side effects of treatment. Human chorionic gonadotropine (hCG) injection (5000-10000 IU IM) was given when at least one follicle measured at least 18 mm. Patients were advised for intercourse 24-36 hours after hCG injection. Serum hCG was determined two weeks after hCG injection in the absence of menstruation for diagnosis of pregnancy. The outcome measures were the occurrence of pregnancy, miscarriage and neonatal condition. Babies were examined by a neonatologist for the gestational age, body weight, Apgar score at 1, 5 and 10 minutes and any congenital anomalies or neonatal complications.

Control group
An age-matched group of 200 women (298 cycles) who naturally conceived during the same period of the trial were used as a control group.

Statistical analysis
Data analysis was performed using SPSS version 13 (SPSS Inc., Chicago, IL). Results were expressed in the form of mean ± SD and percentages. The chi-squared test was used to compare proportions. Student's *t* test and one-way analysis of variance (ANOVA) were used to compare means, as appropriate. Statistical significance was set $P < 0.05$.

Results

The study comprised of 996 patients (1398 cycles) in total. There were no statistical significant differences between all groups as regards the age, duration of infertility, body weight, height and body mass index (BMI) (Table 1).

Pregnancy occurred in 36/323 cycles in the letrozole group (11.1%), in 15/143 cycles in the anastrozole group (10.5%), in 77/634 cycles in CC group (12.1%) and in 21/298 cycles in the spontaneous pregnancy group (7.0%) without statistical significant differences between all groups. The total miscarriage rate was 16.1% (varies between 14.2% in CC group to 19.9% in the anastrozole group) without difference between spontaneous and stimulated pregnancies. There were 3 twin pregnancies in the letrozole group, one in anastrozole group which ended in early miscarriage, 7 in the CC group and one in the spontaneous pregnancy group. There were no higher order pregnancies or ovarian hyperstimulation syndrome in any of the groups. There was one ectopic pregnancy in the CC group may be due to undetected tubal factor and one molar pregnancy in the letrozole group.

There were 129 deliveries in all groups. There were no statistical significant differences between the stimulated and spontaneous pregnancies as regards mean gestational age, premature deliveries, birth weight, SGA <10 percentile or 5 minutes Apgar score. Two cases of congenital anomalies occurred in the letrozole group; one case of complete cleft palate and one case of major congenital heart problem in a mother with gestational diabetes which ended in early neonatal death. There were 2 cases of talipus equinovarus in CC and spontaneous pregnancy group.

Discussion

Clomiphene citrate (CC) is the standard drug for ovulation induction but is not equally effective in all situations. Clomiphene-resistance occurs in 15-20% of patients. The use of CC may be associated with poor cervical mucous and endometrial thinning in 15%–50% of patients due to prolonged estrogen receptor depletion in the endometrium and possibly in the cervix (8-10). Alternatives to clomiphene citrate such as aromatase inhibitors were employed for the same indication. Many studies discussed the efficacy of different aromatase inhibitors in comparison with CC but, not much was written regarding the outcome and safety of these drugs.

In this study, all stimulation protocols were equally effective in achieving pregnancy which came in agreement with many previous reports (11, 12). The rates of pregnancy loss after ovarian stimulation, with different protocols, were not higher than that after spontaneous pregnancy. Many reports referred to increased overall rate of miscarriage in infertile patients (13, 14). The trend towards preclinical pregnancy loss was, usually, due to very early diagnosis of pregnancy by sensitive serum hCG assays rather than actual increase in early pregnancy loss. The claim of higher miscarriage rates after CC due to its effect on the endometrium was not proved in this study or many studies before (11, 12).

Although the CC group witnessed the highest multiple pregnancy rate, the differences from the other groups were not significant. CC results in central estrogen receptor depletion for long duration (2 weeks) compared to the aromatase inhibitors (8-10). This results in multiple follicle growth and may be higher multiple pregnancy rates with CC than occurs in aromatase inhibitors cycles. The multiple follicular growths in CC treated patients without corresponding significant increase in the multiple pregnancies or miscarriage rates represent an advantage to CC in many situations. Aromatase inhibitors are the ideal choice when limited number of follicles is required or there is a risk of hyperstimulation syndrome (12).

Recently, the safety of letrozole was seriously questioned after an abstract presented in the American Society for Reproductive Medicine (ASRM) meeting 2005, examining letrozole pregnancies (130 pregnancies) compared to a large control group of spontaneous conceptions. It suggested that the use of letrozole for infertility treatment might be associated with a higher risk of congenital cardiac and bone malformations in the newborns (7). As a result of this study, on November 17th, 2005, Novartis Pharmaceutical, the manufacturer, issued a statement to physicians in Canada and worldwide advising that letrozole use in premenopausal women, specifically its use for ovulation induction, is contraindicated.

There is no evidence that the exposure of oocytes to letrozole can increase birth defects. A recent study on aromatase-overexpressing mice showed that when these animals were treated with high doses of letrozole for 6 weeks and allowed to conceive 2 weeks later, there was no difference between treated and control animals in terms of litter size, birth weight, and anomalies (15). Mitwally et al. (2005) reported favorable pregnancy outcome and low multiple gestation rate of aromatase inhibitors for ovarian stimulation (16). A more recent multicenter retrospective study in Canada by Tulandi et al. (2006) on the pregnancy outcome after letrozole induction of ovulation came out. They concluded that the concern about letrozole use for ovulation induction was unproved (17). We had one case of complete cleft palate and one case of major congenital heart problem which ended in early neonatal death in the letrozole group. The mother in this last pregnancy was diabetic which might explain the occurrence of this serious congenital heart disease. CC has been reported to elicit various ocular side effects in 1.5-10% of patients taking clomiphene (18). In this study, there were 2 cases of talipus equinovarus in CC and spontaneous pregnancy groups. No congenital anomalies were reported in the anastrozole group. Toxicology

studies showed that anastrozole is well tolerated in 1 and 6 month. Oral administration of anastrozole to pregnant rats and rabbits caused no teratogenic effects (19). It was reported that in vitro exposure of mouse follicles to anastrozole, did not increase meiotic spindle defects in oocytes and birth anomalies (20).

This prospective controlled trial confirmed what others previously reported that both aromatase inhibitors and CC are equally effective in inducing and augmenting ovulation in many situations. Both drugs resulted in favorable pregnancy outcomes and average miscarriage rates. Safety of the drugs to both the mother and fetuses were documented. The use of least effective doses will result in lower incidence of multiple pregnancies and usually avoids ovarian hyperstimulation syndrome.

Table 1 Patients' Characteristics

	letrozole group (n=269)	Anastrozole group (n=107)	CC group (n=420)	Control group (n=200)	F	P
Number of cycles	323	143	634	298		
Age (years)	25.8± 3.2	24.3 ± 2.9	23.5±2.6	25.0±1.9	1.09	0.67
Parity	0.4 ± 0.12	0.3 ± 0.18	0.6 ± 0.20	0.3 ± 0.11	1.10	0.62
Duration of infertility	3.2 ± 1.22	4.1 ±1.61	3.7 ± 1.21	3.3 ± 0.11	1.2	0.08
Height (cm)	158.3 ± 5.12	155.1 ± 4.20	157.2 ± 5.90	160.1 ± 3.51	2.6	0.55
Weight (kg)	80.3 ± 5.42	79.1 ± 4.22	75.1± 4.13	74.1 ± 5.11	6.4	0.07
BMI (kg/m2)	30.1 ± 2.91	29.1 ± 3.12	27.2 ± 2.33	26.2 ± 3.44	7.3	0.08

No statistical significant differences as P >0.05

Table 2 Pregnancy outcome in study and control groups

	letrozole group (n=269)	Anastrozole group (n=107)	CC group (n=420)	Control group (n=200)	Total (n=996)
Number of cycles	323	143	634	298	1398
Chemical pregnancy	42/323 (13.0%)	19/143 (13.3%)	83/634 (13.0%)	23/298 (7.7%)	167/1398 (11.9%)
Clinical pregnancy	36/323 (11.1%)	15/143 (10.5%)	77/634 (12.1%)	21/298 (7.0%)	149/1398 (10.7%)
Multiple pregnancy	3/36 (8.3%)	1 (6.6%)	7/77(9.1%)	1/21 (4.7%)	12/149 (8.1%)
Early miscarriage	4/36 (11.1%)	2/15 (13.3%)	8/77(10.3%)	3/21(14.3%)	17/149 (11.4%)
Late miscarriage	2/36 (5.5%)	1 (6.6%)	3/77 (3.9%)	1/21 (4.7%)	7/149 (4.7%)
Ectopic pregnancy	0	0	1 (1.3%)	0	1/149 (0.67%)
Molar pregnancy	1/36 (2.7%)	0	0	0	1/149 (0.67%)

	X	P
Chemical pregnancy	6.4	0.09
Clinical pregnancy	5.4	0.13
Multiple pregnancy	0.46	0.92
Early miscarriage	0.31	0.95
Late miscarriage	0.30	0.96

Table 3 Neonatal outcome in study and control groups

	letrozole group (n=269)	Anastrozole group (n=107)	CC group (n=420)	Control group (n=200)	Total
Number of deliveries	30	11	65	23	129
Gestational age (weeks)	37.1 ± 2.3	37.3 ± 2.7	38.0 ± 1.8	38.1 ± 1.8	
Birth weight (gms)	2915 ± 354	3011 ± 401	2975 ± 410	3005 ± 398	
SGA <10 percentile*	2 (6.6%)	1 (9.1%)	3 (4.6%)	1 (4.3%)	7 (5.4%)
Premature birth	4 (13.3%)	1 (9.1%)	2 (3.1%)	0	7 (5.4%)
5-min Apgar <7	1 (3.3%)	1 (9.1%)	0	0	2 (1.5%)
Congenital anomalies	2 (6.6%)	0	1 (1.5%)	1 (4.3%)	4 (3.0%)
Early neonatal death	1 (3.3%)	0	0	0	1 (0.7%)

- Small for gestational age

	F	P
Gestational age (weeks)	2.40	0.32
Birth weight (gms)	3.10	0.11
SGA <10 percentile*	0.51	0.91
Premature birth	6.20	0.55
5-min Apgar <7	6.10	0.10
Congenital anomalies	2.27	0.51

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Chapter 11

Summary

The aim of this thesis was to address a number of potentially important unanswered questions regarding oral agents for inducing ovulation such as clomiphene citrate and aromatase inhibitors.

Chapter 1
In chapter 1 a brief introduction about the physiology of ovulation, evolution of ovulation induction and oral agents used for inducing ovulation is presented. At the end of this chapter, the aim of the thesis is discussed.

Chapter 2
For more than 40 years, clomiphene citrate has held its place as a first line drug used for induction or stimulation of ovulation. Despite this, there is little data available on which to base clinical practice regarding when to start CC and for how long treatment should continue. We tested the hypothesis that starting CC therapy in the late luteal phase of the preceding cycle would increase number of mature follicles, endometrial thickness, ovulation rate and pregnancy rate compared with and current standard regimen by carrying out a randomized controlled trial chapter (2). The study comprised a total of 212 women (438 cycles) with PCOS. Patients in the early CC group commerce 100 mg of CC daily on the last 5 days of treatment with medroxy progesterone acetate (MBA) (110 patients, 227 cycles) while patients in the late CC group had 100 mg of CC daily for 5 days starting day 3 of the menses for 5 days (102 patients, 211 cycles). No significant difference in ovulate rate between the early and standard cc protocol was demonstrated. The total numbers of follicles and the number of follicles ≥14 mm and ≥18 mm during stimulation were significantly greater ($P<0.05$) in the early CC group. The endometrium at the time of hCG administration was significantly thicker in the early CC group (9.1 ± 0.23 vs. 8.2 ± 0.60 mm). Serum E2 and progesterone were not significantly different between the two groups ($P>0.05$). Pregnancy occurred in 23/110 cycles in the early CC group (20.9%) and 16/102 cycles (15.7%) in the late CC group and the difference was not statistically significant ($P>0.05$). Miscarriage rate was similar in the two groups. We concluded that early administration of CC in patients with PCOS will lead to more follicular growth and endometrial thickness which might result in higher pregnancy rate.

Chapter 3
In chapter 3 the effect of extended CC therapy (beyond the standard 5 days) on pregnancy rate was compared with gonadotropin therapy in a randomized controlled trial. The study comprised of 318 women (802 cycles) with clomiphene-resistant PCOS. Patients in the CC group were treated with 100 mg of CC daily starting on cycle day 2 for 9 days (160 patients, 405 cycles) while patients in the gonadotropin group had hMG 75 IU intramuscularly daily for 5 days starting on cycle day 3. The proportion of ovulating patients was significantly more in the gonadotropin group (57.6% vs. 28.1%). The total numbers of follicles during stimulation were also significantly increased in the gonadotropin group (6.7 ± 0.3 vs. 4.1 ± 0.4). The endometrial thickness at the time of hCG administration was significantly more in the gonadotropin group (10.2 ± 0.6 vs. 8.2 ± 0.3 mm). Finally, serum progesterone was significantly higher in the gonadotropin group. Pregnancy occurred in 47/405 cycles in the CC group (11.3%) and 80/397cycles (20.1%) in the gonadotropin group ($P<0.05$). The extended CC regimens resulted in a modest ovulation and pregnancy rates and no side effects were reported. From this study it can be concluded, that this therapy offers economic, efficacy, and safety advantages and it can be considered before moving to more expensive or sophisticated alternatives.

Chapter 4

A number of preliminary reports about the role of *N*-acetyl cysteine (NAC) with CC in ovulation induction have been published, but the role of this adjuvant therapy has not been widely investigated. In chapter 4 we present a cross-over trial to compare clomiphene citrate plus *N*-acetyl cysteine versus clomiphene citrate for inducing ovulation in patients with polycystic ovary syndrome. Five hundred and seventy-three polycystic ovary patients were treated with clomiphene citrate alone for one menstrual cycle among which 470 patients were treated with clomiphene citrate plus N-acetyl cysteine for another cycle. Patients had clomiphene citrate 50-mg tablets twice daily alone and then CC plus with N-acetyl cysteine 1,200 mg/day orally for 5 days starting on day 3 of the menstrual cycle. Primary outcomes were number of mature follicles, serum E2, serum progesterone, and endometrial thickness. The secondary outcome was the occurrence of pregnancy. The ovulation rate improved significantly after the addition of N-acetyl cysteine (52.1% versus 17.9%). Although the number of mature follicles was more in the N-acetyl cysteine group (2.19 ± 0.88 versus 3.29 ± 0.93), the difference was not statistically significant. The mean E2 levels (pg/ml) at the time of human chorionic gonadotropine injection, serum progesterone levels (ng/ml) on days 21-23 of the cycle, and the endometrial thickness were significantly improved in the N-acetyl cysteine group. The overall pregnancy rate was 11.5% in the N-acetyl cysteine group. Insulin resistance was found in 260 patients (55.4%). There was no significant difference between the insulin resistance group (n=260) and non-insulin resistance group (n=210) as regards ovulation rate, number of follicles, serum E2 (pg/ml), serum progesterone (ng/ml), endometrial thickness (mm), or pregnancy rate. In conclusion, N-Acetyl cysteine was proved effective as an adjuvant to CC for inducing ovulation in polycystic ovary patients.

Chapter 5

In chapter 5 we present a randomized, double-blind, controlled study to compare clomiphene citrate plus NAC versus clomiphene citrate alone for augmenting ovulation in management of unexplained infertility. We recruited 404 patients as a study group (clomiphene citrate plus *N*-acetyl cysteine group) and 400 patients as a control group (clomiphene citrate alone group). Patients in the study group were treated with clomiphene citrate (50mg tablets) twice per day and with *N*-acetyl cysteine (1200 mg/day orally) for 5 days starting on day 2 of the cycle. Patients in the control group were treated with clomiphene citrate with sugar powder. The primary outcomes were number and size of growing follicles, serum E2, serum P, and endometrial thickness. The secondary outcome was the occurrence of pregnancy. There were no statistically significant differences between the two groups in the number of follicles sized >18 mm, mean E2 levels, serum P, or endometrial thickness. Pregnancy rate was comparable in both groups (22.2% vs. 27%). Miscarriage rate was comparable in both groups (6.7% in the study group vs. 7.4% in the control group) as well. In conclusion, *N*-Acetyl cysteine was found ineffective as an adjuvant to clomiphene citrate in augmenting ovulation in patients with unexplained infertility and cannot be recommended in such situation.

Chapter 6

A number of randomized controlled trials have compared AIs and CC for ovulation induction. The number of patients in all trials, however, was small which affected seriously the validity of their conclusions. Chapter 6 presents a prospective randomized controlled trial to compare the effects of letrozole (5 mg) and clomiphene citrate (100 mg) for ovulation induction in women with PCOS. The study comprised a total of 438 infertile women (1063 cycles) with PCOS. Patients were randomized to treatment with 5 mg of letrozole daily (218 patients, 545 cycles) or 100 mg of clomiphene citrate daily (220 patients, 518 cycles) for 5 days starting on day 3 of menses. Timed intercourse was advised 24 to 36 hours after hCG injection. Number of follicles, serum estradiol, serum progesterone, endometrial thickness, and pregnancy and miscarriage rates

represented the outcome measures. The total number of follicles was statistically significantly greater in the clomiphene citrate group (6.8±0.3 versus 4.4±0.4). Endometrial thickness at the time of hCG administration was statistically significantly greater in the CC group (9.2±0.7 mm versus 8.1±0.2 mm). The number of days required to reach follicles (18 mm or more) was statistically significantly longer in the letrozole group (12.1±1.3 versus 8.8±2.9 days). Ovulation occurred in 365 out of 540 cycles (67.5%) in letrozole group and 371 out of 523 cycles (70.9%) without a statistically significant difference. Levels of serum estradiol and progesterone were statistically significantly higher in the clomiphene citrate group. The pregnancy rate per cycle was 15.1% in the letrozole group and 17.9% in the clomiphene citrate group without statistically difference between the groups. In conclusion, this study did not show any advantage to the use of letrozole over clomiphene citrate as a first-line treatment for induction of ovulation in women with PCOS.

Chapter 7

Chapter 7 presents a prospective controlled trial to compare the effects of anastrozole (1 mg) and clomiphene citrate (100 mg) used for ovulation induction in women with polycystic ovary syndrome. The study comprised a total of 216 infertile women (469 cycles) with polycystic ovary syndrome. Patients received anastrozole (1 mg/day; 115 patients, 243 cycles) for 5 days, starting on day 3 of menses. A matched historical group of patients with polycystic ovary syndrome who were treated with CC (100 mg/day; 101 patients, 226 cycles) was used as a control group. Number of follicles, serum E2, serum P, endometrial thickness, and pregnancy and miscarriage rates were the outcome measures. The total numbers of follicles were significantly higher in the CC group (3.8 ± 0.6 vs. 3.4 ± 0.5). Endometrial thickness at the time of hCG administration was significantly greater in the anastrozole group (10.1 ± 0.22 mm vs. 8.2 ± 0.69 mm). The duration of stimulation was similar in the two groups. Ovulation occurred in 165 (67.9%) of 243 cycles in the anastrozole group and in 150 (68.6%) of 226 cycles in the CC group without significant difference. Serum P was significantly higher in the CC group (7.1 ± 1.11 vs. 8.1 ± 0.88 ng/mL). The pregnancy and miscarriage rates were similar in the two groups. In conclusion, anastrozole was associated with significantly fewer mature and growing follicles, thicker endometrium, and slightly higher pregnancy rate. Anastrozole may be helpful in situations in which multiple pregnancies are not desirable or the risk of ovarian hyperstimulation syndrome is high.

Chapter 8

Chapter 8 presents a prospective randomized controlled trial comparing letrozole (2.5 mg) and anastrozole (1 mg) for ovulation induction in clomiphene-resistant women with PCOS. The study comprised a total of 220 infertile women (574 cycles) with CC-resistant PCOS. Patients were randomized to treatment with 2.5 mg of letrozole daily (111 patients, 295 cycles) or 1 mg of anastrozole daily (109 patients, 279 cycles) for 5 days from day 3 of menses. The outcome measures were number of follicles, serum E2, serum P, endometrial thickness, pregnancy rate (PR), and miscarriage rate. The total number of follicles was significantly more in the anastrozole group (5.4 ± 0.4 vs. 5.8 ± 0.4). The number of follicles >14mm (3.1 ± 0.3 vs. 2.7 ± 0.2) and >18mm (2.3 ± 0.1 vs. 3.1 ± 0.2) were significantly higher in the anastrozole group. The endometrial thickness at the time of hCG administration was significantly more in the anastrozole group (9.1 ± 0.2 vs. 10.2 ± 0.7 mm). The duration to reach a 18 mm or more follicle was longer in the letrozole group (12.1 ± 1.3 days vs. 8.8 ± 1.9 days) but without statistical significant difference. Ovulation occurred in 183/295 cycles (62%) in the letrozole group and 177/279 cycles (63.4%) in the anastrozole group, whereas pregnancy occurred in 36/295 cycles (12.2%) in the letrozole group and 42/279 cycles (15.1%) in the anastrozole group and the differences were not statistically significant. In conclusion, the results of this study did not show a significant difference in PR or miscarriage rate between anastrozole and letrozole when used for ovulation induction in women with CC-resistant PCOS.

Chapter 9

This chapter addresses the optimal dose of letrozole for ovarian stimulation in patients with unexplained infertility. A total of 179 patients were randomly recruited in this prospective study and randomized to receive 2.5, 5 or 7.5 mg letrozole for 5 days. 58, 61 and 60 patients were recruited to each dosage group respectively. This study reported a significantly higher number of follicles (total, > 14 mm and ≥ 18 mm) on the day of administration of human chorionic gonadotrophin in the 7.5 mg group, associated with significantly fewer days of stimulation. However, pregnancy and miscarriage rates were similar in the three groups. In conclusion, it seemed that the use of higher doses of letrozole offers no advantage in terms of pregnancy rates over the lower (2.5 mg) dose.

Recently, the safety of letrozole and other aromatase inhibitors used for ovulation induction has been questioned. Chapter (10) presents a prospective randomized controlled trial to evaluate the pregnancy outcome after ovulation induction with aromatase inhibitors or clomiphene citrate (CC). The study comprised a total of 796 infertile women (1100 cycles) and 200 spontaneously pregnant women (298 cycles) as a control group. Patients were allocated to treatment with 100 mg of CC daily (420 patients, 634 cycles), 5 mg of letrozole daily (269 patients, 323 cycles) or 1 mg of anastrozole daily (107 patients, 143 cycles) for 5 days starting on day 3 of menses. Timed intercourse was advised 24-36 hours after hCG injection. The outcome measures were the occurrence of pregnancy, miscarriage and neonatal outcome. Pregnancy occurred in 167/1398 cycles (11.9%) in total without statistical significant differences between all groups. The total miscarriage rate was 16.1% (varied between 14.2% in CC group to 19.9% in anastrozole group) without difference between spontaneous and stimulated pregnancies. There were 129 deliveries in all groups. There were no statistical significant differences between the stimulated and spontaneous pregnancies as regards mean gestational age, premature deliveries, birth weight, SGA <10 percentile or 5 minutes Apgar score. There was one case of complete cleft palate and one case of major congenital heart problem in the letrozole group. There were 2 cases of talipus equinovarus in CC and spontaneous pregnancy group. In conclusion, aromatase inhibitors and CC were equally effective in inducing and augmenting ovulation in many situations. They resulted in favorable pregnancy outcomes and average miscarriage rates.

Chapter 12

Discussion

The aim of this thesis was to address a number of questions regarding oral agents used for ovulation induction. We were motivated to run the presented trials because of many reasons. Firstly, although oral agents, namely CC, have been in the market for decades, many basic aspects regarding the application of oral agents have been taken for granted without a real evidence base. These include when to start CC, at what dose and for how many days therapy should continue. Some of the early studies about CC date back to up to 35 years or more. Although these were pioneering studies, most of them were underpowered observational trials without trustworthy conclusions. Secondly, the widespread use of CC showed the existence of 10-40% of patients who were either resistant to CC or failed to achieve pregnancy. Second line therapies for these patients include gonadotropin ovulation induction and laparoscopic ovarian drilling. These approaches have disadvantages related to costs and risk of ovarian hyperstimuation, multiple pregnancies or pelvic adhesions. For many women, particularly in developing countries, the cost of therapy is very decisive. Hence, before announcing the failure of cheap and safe drug like CC and moving forwards to another therapy, we tried to improve its performance by manipulating its start or duration of use and by addition of N-acetyl cysteine. Lastly, there has been a growing interest in the new group of aromatase inhibitors e.g letrozole and anastrozole as ovulation induction agents because of their theoretical advantages over CC. 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, there was paucity of comparative RCTs to indicate the drug of first choice in this condition.

In chapter 2, the presented trial addressed the question whether starting CC before day 1 of menses, in the late luteal phase, could improve the ovarian response, compared with the current standard regimens starting on day 2-3 of the cycle. The rational of this novel protocol based upon our understanding of the principles of natural follicle recruitment and the possible side effects of CC on the cervix, endometrium, oocytes and embryos. In this study, the ovulation rate was 59.1% while the conception rate was 20.9% in the new protocol compared with 51.9% and 15.7% respectively in the control group. Again, the discernible discrepancy between ovulation and pregnancy rates may be, as reported before, due to negative effects of CC on oocytes or granulosa cells, or because of prolonged antiestrogenic effects of CC on endometrial receptivity and cervical mucus. Therefore, if treatment starts late in the cycle (day 2-5), these negative effects are more likely to extend into the sensitive peri-implantation period. Nevertheless, despite regarding many well designed studies, this approach remains a topic of lively debate.

In the presented study, the total numbers of follicles and the numbers of follicles ≥14 mm and ≥18 mm were significantly more when CC was started early. Although, the pregnancy rate was more in the early group although the difference was not statistically significant. There was no significant increase in the number of multiple pregnancies in either both groups. It can be argued that the observed increase in the number of follicles in the early start group will increase the number of multiple pregnancies and the attendant risks to the mother and fetus. However, this was not observed in this study. This study showed some rewards for this protocol such as more ovulating patients, more mature follicles and improved pregnancy rate. Further studies powered for pregnancy as an outcome are required to further eliminate the clinical value of the novel protocol.

In order to investigate other ways of improving the outcome of CC ovulation induction, the study (in chapter 3) was carried out. This study addresses the value of increasing the number of days of CC administration for ovulation induction in CC-resistant PCOS women by comparing outcomes with gonadotrophin ovulation induction. CC resistance, is usually unexplainable incident and it is virtually impractical to forecast who will respond to which dose of CC, if at all. So far, there is no general agreement on a standard regimen for management of CC-resistant PCOS patients. The

rationale of using this extended CC regimen was based on our understanding of other aspects of the physiology of follicular growth. Decremental follicular phase FSH levels (referred to as the FSH window) appear to be crucial for selection of a single dominant follicle from the recruited cohort. As FSH levels fall, all but the dominant follicle (with its increased sensitivity to FSH) lose the stimulus to further development and become atretic. The practice of extending the FSH window by administering exogenous FSH or extending the duration of CC therapy in the midfollicular phase will maintain FSH levels above the threshold allowing multifollicular development to occur (Introduction; ref. 6).

In this study, the proportion of ovulating patients was significantly greater in the gonadotropin group. The total numbers of follicles during stimulation, serum progesterone and endometrial thickness at the time of hCG administration were also significantly more in the gonadotropin group. Pregnancy occurred in 47/405 cycles in the CC group (11.3%) and 80/397cycles (20.1%) in the gonadotropin group and the difference was statistically significant. One twin pregnancy occurred in the CC group and 4 in the gonadotropin group. Two cases of moderate ovarian hyperstimulation syndrome occurred in the gonadotropin group and recovered. It was not anticipated that the extended therapy would be more effective than gonadotropins in this situation but sought to investigate whether it may obviate the need for more expensive drugs in some women. The extended CC protocol results in a modest ovulation and pregnancy rates and no side effects. This therapy appears to offer economic, efficacy, and safety advantages and may be worth considering before moving to more expensive or sophisticated alternatives. A larger randomized study is needed to compare this extended protocols with other modalities used for treatment of CC resistance.

In the same theme of the thesis, in chapter 4, the presented trial compared clomiphene citrate plus N-acetyl cysteine versus clomiphene citrate alone for inducing ovulation in patients with polycystic ovary syndrome to test the value NAC as an adjuvant to CC. The study contained 470 patients which was a sufficient number to give the study a good power. The study is a cross-over trial which may not be the best way to test a hypothesis but this design seemed suitable for studying a treatment modality for CC resistance. In this study, there was a significant improvement in ovulation rate after adding NAC to CC (17.9% versus 52.1%).

Many authors have demonstrated the insulin-sensitizing activity of NAC and have reported the value of NAC in the presence of IR. This was not the situation in this study group, as IR was reported in 55.4% of patients. There was no significant difference between the IR and non-IR groups as regards the ovulation rate, number of follicles, serum estrogen, serum progesterone, or pregnancy rates. There should be other mechanisms that explain the beneficial effects of NAC such as antiapoptotic effects, preservation of vascular integrity and useful immunological effects. We observed another favorable effect for NAC in combination with CC on the cervical mucous. Being a mucolytic, NAC generally improves the character of the cervical mucous without need fot supplementary estrogen supply. Further studies are required to attest these effects and to compare the efficacy of NAC versus other insulin sensitizing drugs. In this study, NAC was proved effective as an adjuvant to CC for inducing ovulation in polycystic ovary patients even in the absence of IR.

In chapter 5, we tested the adjuvant effect of NAC combined with CC in stimulating ovulation in unexplained infertility. The study comprised of 404 patients as a study group (clomiphene citrate plus N-acetyl cysteine group) and 400 patients as a control group (clomiphene citrate–alone group). The number of patients was enough to offer the study a sufficient power of 90% at CI 95% when 20% difference was expected in the outcome of drugs. In this study, NAC was proved

to be unsuccessful in improving the ovulation or pregnancy rates in the study cohort. N-Acetyl cysteine did not add to the ovulatory effect of clomiphene citrate, and moreover, the results were better in its absence. These results were not consistent with most previously published studies in this field. Although NAC may have a beneficial adjuvant effect to the clomiphene citrate in inducing ovulation in PCOS, this was not the case in unexplained infertility and it is not recommended in non-PCOS settings. Further studies are needed to determine the precise mechanism(s) of action of NAC to get the utmost benefit from this promising drug.

Letrozole and anastrozole are third-generation aromatase inhibitors which were extensively investigated as ovulatory drugs. But, are aromatase inhibitors real additions to the armamentarium of oral ovulatory drugs? In chapter 6, the presented prospective randomized controlled trial addresses a comparison between letrozole (5mg) and clomiphene citrate (100 mg) meant for ovulation induction in women with polycystic ovary syndrome (PCOS). The study comprised of 438 patients (1063 cycles) which gave the results of the trial a sufficient supremacy. Compared with letrozole, the use of CC led to increase in the number of 14 mm and 18 mm follicles. Although the pregnancy rate was slightly greater in the CC group, the difference was not statistically significant. The cost of letrozole per cycle was much higher than CC, especially when higher doses of letrozole are required (500 versus 5 Egyptian pounds, respectively). Multiple follicular growths occurred in CC-treated patients without any corresponding statistically significant increase in multiple pregnancies or miscarriage rates, which represents an advantage to using CC in many situations. As the ideal dose of letrozole has yet to be determined, a higher dose, than the 5 mg used in this study, may achieve better results. However, raising the dosage will further increase the cost, which is an important decisive issue in choosing the appropriate therapy. In contrary to others, this study found no advantages to use letrozole rather than CC for inducing ovulation in women with PCOS and it could not be recommended as a first-line treatment in this situation.

Chapter 7 presented a prospective randomized controlled trial to compare the effects of another aromatase inhibitor, anastrozole (1 mg) with clomiphene citrate (CC; 100 mg) used for ovulation induction in women with polycystic ovary syndrome. The study comprised of 216 infertile women (469 cycles) with polycystic ovary syndrome in total. To our knowledge, this was the first trial comparing anastrozole with CC used alone for ovulation induction in women with PCOS. In this study, a 1 mg dose of anastrozole was used because this dose has been found to be approximately equivalent to 2.5 mg of letrozole in terms of inhibiting E2 production over a 6-week time period. Compared with anastrozole, the use of CC led to significantly more developing and mature follicles (≥14mm and ≥18mm follicles). Although the pregnancy rate was slightly higher in the anastrozole group, this difference was not statistically significant. The miscarriage rate was similar in both groups. In many situations in which the development of multiple mature follicles is required, anastrozole alone probably is not the optimal choice for ovulation induction. As in letrozole, the cost of anastrozole per cycle is much higher than that of CC, especially when higher doses of anastrozole are required (respective costs, 500 vs. 5 Egyptian pounds per cycle). We are aware of the limitations of the study, which would have been strengthened if prospective randomization had been performed. The use of historical controls has some advantages such as saving the number and time of the trial. However, it has the possibility of limited protection against bias. To limit this risk, the controls were selected from patients receiving treatment in the same organization at the same time. The results of this study showed that anastrozole was associated with significantly fewer mature and growing follicles, thicker endometrium, and a slightly higher pregnancy rate. Anastrozole may be helpful in situations in which multiple pregnancy is not desirable or in which the risk of ovarian hyperstimulation syndrome is high.

In the previous study, anastrozole was shown to offer further advantages encouraging us to investigate whether it may be superior to letrozole in the context of ovulation induction. In chapter 8, a prospective randomized controlled trial compared letrozole (2.5 mg) and anastrozole (1 mg) meant for ovulation induction in clomiphene-resistant women with PCOS. The study comprised a total of 220 infertile women (574 cycles) with CC-resistant PCOS. In this study, anastrozole resulted in significantly more mature and medium-sized follicles and greater endometrial thickness than letrozole. Although the serum level of E2 and P in the anastrozole group was higher, the difference was not statistically significant. To our knowledge, only one small trial has previously compared anastrozole (1 mg) and letrozole (2.5 mg) for induction of ovulation in 40 CC-resistant PCOS patients. In contrary to our results, letrozole was associated with significantly greater endometrial thickness, higher size of follicles, increased E2 levels, higher ovulation rate, and higher pregnancy rate (PR). The results of our study showed more growing and mature follicles and greater endometrial thickness in the anastrozole group. However, it did not show significant advantage for anastrozole over letrozole as regards pregnancy or miscarriage rates when used for ovulation induction in women with CC-resistant PCOS. At present, there is insufficient data available to allow a conclusion to be drawn as to whether letrozole or anastrozole offer better outcomes.

Although letrozole is entering clinical practices for ovarian stimulation, the optimal data is still unknown. Chapter 9 presented a randomized controlled trial of three doses (2.5, 5 and 7.5 mg) of letrozole for ovulation induction in patients with unexplained infertility. A total of 179 patients were randomly recruited in this prospective study with 58, 61 and 60 patients in each dosage group respectively. This study reported a significantly larger number of follicles (total, $\geq$ 14 mm and $\geq$ 18 mm) on the day of administration of human chorionic gonadotrophin in the 7.5 mg group, associated with significantly fewer days of stimulation. However, the pregnancy and miscarriage rates were similar in the three groups. Although the number of patients in the study was small, it raised questions regarding the possible impact of aromatase inhibitors on endometrial receptivity. Although theoretically aromatase inhibitors have a favorable effect on the endometrium, sure they reduce supraphysiological levels of estradiol higher concentrations of testosterone produced with higher doses of letrozole may cause an adverse effect on the endometrium. However, testosterone concentrations were not measured in this study and this hypothesis therefore warrants further investigation in future studies. In conclusion, use of higher doses of letrozole (5 and 7.5 mg), although having the potential advantage of shorter period of stimulation, has been found to offer no significant benefit over the use of 2.5 mg in terms of pregnancy rates. Further studies examining endometrial receptivity and androgen profiles with letrozole treatment are still needed. According to the present state of knowledge, 2.5 mg is still the recommended dose for ovarian stimulation in patients with unexplained infertility. Further dose-finding studies in patients with PCOS, in particular, are still required.

Pregnancy outcome after the use of ovulation agents remains an important issue, because the safety of these agents was seriously questioned. Clomiphene citrate has been used for over four decades and several studies have shown that its use is not associated with an increased risk of congenital malformation even among women who took CC inadvertently during pregnancy. However, Federal Drug Administration (FDA) listed CC as category X medication. On the other hand, an American Society for Reproductive Medicine (ASRM) abstract, examining a relatively small number of letrozole pregnancies compared to a large control group of spontaneous conceptions, suggested that the use of letrozole for infertility treatment might be associated with a higher risk of congenital cardiac and bone malformations in the newborns. As a result of this small study, on November 17th, 2005, Novartis Pharmaceutical issued a statement to physicians in Canada and worldwide advising that letrozole use in premenopausal women, specifically its use for ovulation induction, is contraindicated. Chapter 10 presents a prospective randomized

controlled trial to evaluate the pregnancy outcome after ovulation induction with aromatase inhibitors or clomiphene citrate (CC). The study comprised a total of 796 infertile women (1100 cycles) and 200 spontaneously pregnant women (298 cycles) as a control group. Patients were allocated to treatment either with 100 mg of CC daily, 5 mg of letrozole daily or 1 mg of anastrozole daily. The average miscarriage rate was 16.1% without difference between spontaneous and stimulated pregnancies. There were no statistical significant differences between the stimulated and spontaneous pregnancies as regards mean gestational age, premature deliveries, birth weight, SGA <10 percentile or 5 minutes Apgar score. There was one case of complete cleft palate and one case of major congenital heart problem in the letrozole group. There were 2 cases of talipus equinovarus in CC and spontaneous pregnancy group. In conclusion, aromatase inhibitors and CC were observed to be equally effective in inducing and augmenting ovulation. They resulted in similarly favorable pregnancy outcomes and miscarriage rates.

The trials compiled in this thesis have many points of strength and weakness. All the studies were randomized controlled trials except 2 trials. Randomized controlled trials (RCTs) are the best way to test a hypothesis as the bias in the assignment of patients is eliminated. Treatment groups tend to be balanced in covariants whether or not these variants are known. Randomization guarantee the validity of the statistical tests of significance used to compare the treatments. One of the trials has a historical control which is definitely less valid than the RCT (chapter 7). When information is available about the efficacy of a standard treatment such as CC, through historical data, then it can be argued that it is logic to use these historical controls. It has the advantages that all patients in the trials receive the new treatment which lead to substantial saving in the number of patients required and therefore the length of the study. The other non-RCT was a cross over trial (Chapter 4). A crossover design has the advantage of eliminating individual subject differences from the overall treatment effect, thus enhancing statistical power. This design seemed suitable as 2 months passed between the two treatments to allow the effects of the first treatment to disappear before the next was applied and the infertility problem was not expected to change in 2 months. Although the sample size was not properly determined before the start of the studies, the number of patients in each trial was definitely more than the other similar trials. Calculation of the power of most of the trials exceeded 80% at a confidence interval (CI) of 95%. RCTs should follow the consolidated standards of reporting trials (CONSORT guidelines and power calculation). Overall, conclusions can be obtained from most of the trials compiled in the trials.

Implications for practice

From the trials included in this thesis, we can advocate the following for future practice:

1. Early administration of CC, in the late luteal phase of the preceding cycle, in patients with PCOS will lead to greater follicular growth and increased endometrial thickness which might result in higher pregnancy rate. This novel protocol may improve the performance of CC as ovulation induction agent.
2. Extended CC protocol (> 5 days therapy) starting day 2 of menses seems to offer economic, efficacy, and safety advantages and should be considered in some settings before moving to more expensive or sophisticated alternatives in cases of CC-resistant PCOS.
3. N-Acetyl cysteine may be an effective adjuvant treatment to CC for inducing ovulation in polycystic ovary patients but not in cases of unexplained infertility.
4. Letrozole does not show any advantage over clomiphene citrate as a first-line therapy for induction of ovulation in women with PCOS. Considering the cost of treatment, there is a remarkable difference in favor of CC. The same is true for anastrozole because CC is also cheaper in use- and more cost-effective. Anastrozole may be helpful in situations in which multiple pregnancies are not desirable or the risk of ovarian hyperstimulation syndrome is high such as PCOS.
5. When aromatase inhibitors are to be used, there are no significant difference expected in PR or miscarriage rate between anastrozole and letrozole employed for ovulation induction in women with CC-resistant PCOS.
6. When letrozole is used for ovulation induction, it seems that the use of higher doses of offers no advantage in terms of pregnancy rates over the lower (2.5 mg) dose.
7. Aromatase inhibitors and CC result in similar pregnancy outcomes and miscarriage rates. Our trial, in agreement with others, did not show any remarkable increase in the rate of congenital malformations.
8. The following scheme represents a suggestion for step-by step induction of ovulation in cases of chronic anovulation.

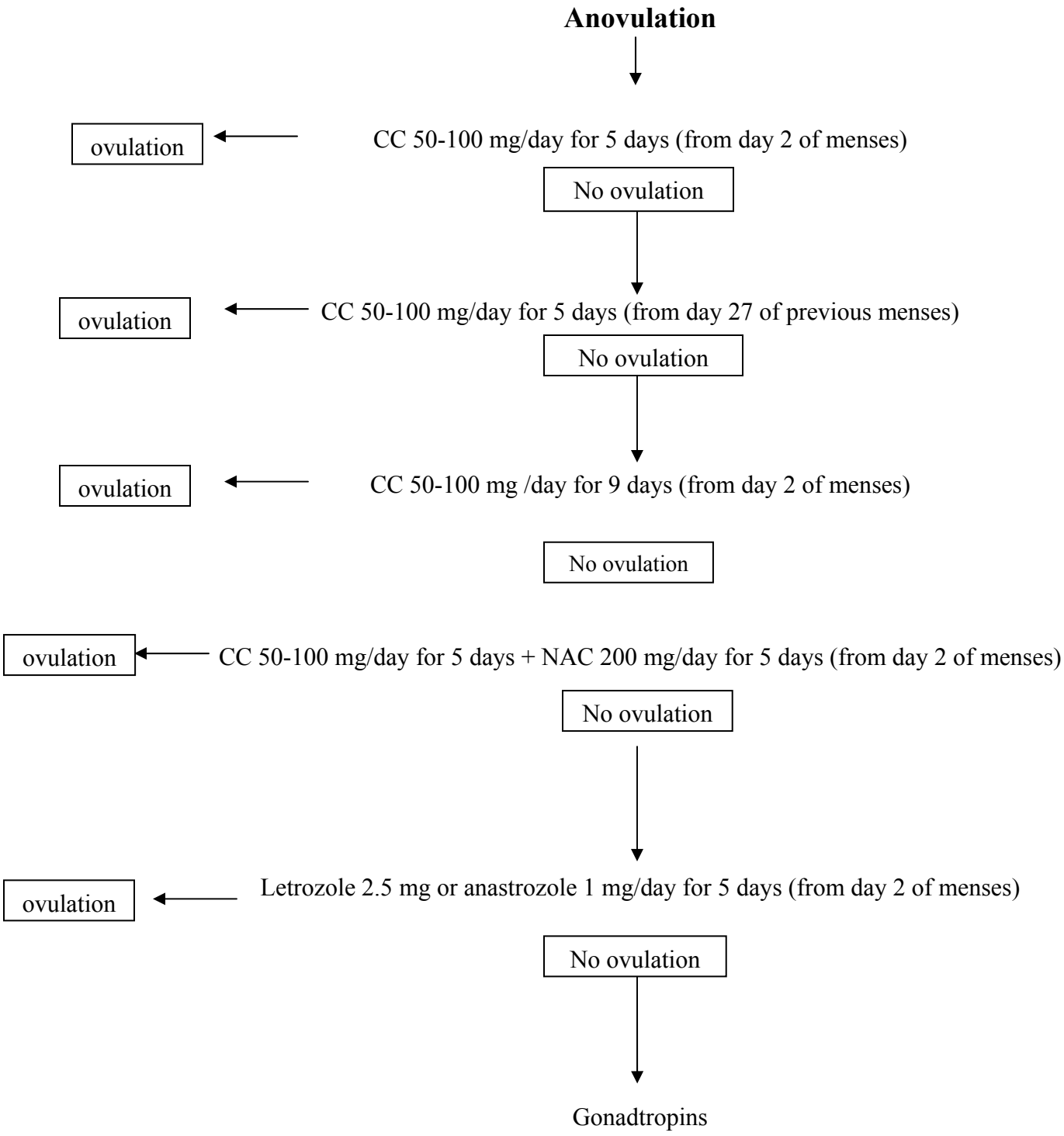

Figure (1) Suggested flow chart for ovulation induction

Implications for future research

Clomiphene citrate (CC) is an old drug that should be revisited. CC has been widely accepted, for more than 40 years, as the standard treatment for most of ovulation problems. However, there are paradigms of ovarian stimulation protocols developed in the early days, which are still widely applied today. This thesis showed that manipulation of the time of starting CC and possibly be the duration of treatment may give a better outcome and reduce the incidence of clomiphene-resistance with no increase in the side effects of CC on the cervix or endometrium. Further research is needed to:

- Compare the CC with and without gonadotropins for stimulation protocols before intrauterine insemination.
- Compare the CC-based mini-stimulation protocols with gonadotropins-only protocols during IVF programs.
- Evaluate the role of CC in friendly-stimulation protocol during IVF programs for poor responders.

In this context, large, well designed RCTs are needed to revise the old protocols of CC and introduce new regimens for ovulation induction in view of our better understanding of the physiology of ovulation. RCTs should follow the consolidated standards of reporting trials (CONSORT guidelines and power calculation). Trials should be of sufficient duration to report live birth as their primary outcome and should ideally report all outcomes, in particular incidence of multiple pregnancy and miscarriage.

There should be some problems with performing such trials on CC. The vast majority of clinical trials are performed by pharmaceutical companies that must do these studies to get drug approval. Many of the most helpful medicines now in routine use emerged from drug-company-sponsored clinical trials. It is patently unfair to suggest that a manufacturer-sponsored clinical trial is inherently biased. However, critics of the system believe that the drug industry's money has a corrupting influence on the clinical trial process. As expected, companies focus more on maximizing profits than on maximizing public health. Companies sometimes put marketing ahead of science when reporting clinical trial results. The pendulum has swung too far one way, and it is sometimes difficult to have it returning.

NAC is a promising new adjuvant to CC. The development of new molecular tools in the fields of genomics, proteomics, and pharmacogenomics provides new horizons on ovarian and endometrial physiology. These novel techniques will have a major impact on our understanding of the patient to patient variation in response to ovarian stimulation. NAC is a new promising adjunct to CC and may be to other oral agents used for ovulation induction such as aromatase inhibitors or metformin. All the studies done on NAC, referred to in this thesis, point to potentially beneficial clinical effects. The actual mechanisms underlying its positive outcome, however, are still obscure. Dose-determining studies for NAC are still lacking.

A well-designed large randomized controlled trial that compare NAC/CC protocol versus metformin/CC for induction of ovulation in PCOS is needed. The role of metformin either alone or in combination with CC in PCOS is still controversial despite of many trials done. Lord et. al (2003) demonstrated in meta-analysis of 13 trials were included for analysis, including 543 women with polycystic ovary syndrome that metformin is an effective treatment for anovulation in women with polycystic ovary syndrome. Its choice as a first line agent seems justified, and there is some evidence of benefit on variables of the metabolic syndrome. More recently, Legro et al. (2007) randomly assigned 626 infertile women with the polycystic ovary syndrome to receive clomiphene citrate plus placebo, extended-release metformin plus placebo, or a combination of metformin and clomiphene for up to 6 months. They concluded that clomiphene is superior to

metformin in achieving live birth in infertile women with the polycystic ovary syndrome, although multiple birth is a complication. The difference in live birth rate was remarkable between the two groups (22.5% vs. 7.2%). Combination of both drugs gives slight increase in live birth rate (22.6%).

What is the proper place of aromatase inhibitors as an ovulation induction agent? Many studies showed a beneficial effect for aromatase inhibitors (AIs) to induce ovulation. However, the results of comparative trials, in this thesis, presented different results. We call for a meta-analysis for the results of the previously released trials to show if the AIs alone such as letrozole and anastrozole has provided advantages over the cheaper traditional protocols of ovulation induction using CC. Meta-ananlysis is strong in revealing structural flaws and sources of bias in primary research and in posing promising research questions for future study. Meta-analysis can make use of its methods to focus on the conflicts of interest and likely sources of bias and make known what precautions should be taken by would-be consumers. Nevertheless, meta-analysis cannot exceed, however, the limits of what is reported by primary researchers. It is particularly challenged to quantify the size of a common effect of treatment across reported trials because of (1) the clinical diversity of the trials and (2) the myriad of potential differences among patients with varying characteristics within the trials. Without access to the original data of reported trials, meta-analysis cannot overcome the bias of underpowered trials toward overstatement of the size of main treatment effects, nor the tendency for such trials to falsely conclude there were no statistically significant adverse events.

Well-designed cost-effectiveness trials are still required for the use of AIs. A well-designed randomized controlled trial is needed to compare combined CC/FSH with AIs/FSH for ovulation induction in unexplained infertility with and without IUI. AIs have theoretical advantages in securing monofollicular growth, reducing serum estrogen and subsequently improving endometrial receptivity and implantation. These effects can be optimally utilized in IVF/ICSI programs especially in PCOS and poor ovarian responders. More well-designed large randomized controlled trials are required to prove these valuable effects of AIs on the outcome of IVF.

Samenvatting

Het doel van dit proefschrift was om een aantal belangrijke onbeantwoorde vragen te beantwoorden met betrekking tot orale medicatie (zoals clomifeen citraat en aromatase remmers) die gebruikt worden bij ovulatie-inductie behandeling.

Hoofdstuk 1
In hoofdstuk 1 wordt een korte introductie gegeven over de fysiologie van ovulatie, de ontwikkeling van de ovulatie-inductie behandeling en de orale medicatie die gebruikt wordt om ovulatie te bewerkstelligen. Aan het einde van het hoofdstuk wordt het doel van dit proefschrift besproken.

Hoofdstuk 2
Al meer dan 40 jaar is clomifeen citraat (CC) het geneesmiddel van eerste keus bij een ovulatie-inductie behandeling. Er zijn echter weinig klinische gegevens beschikbaar die als basis dienen voor het startmoment van CC en hoelang het gecontinueerd moet worden. Wij hebben de hypothese getoetst dat wanneer CC gestart wordt in de luteale fase van de voorafgaande cyclus, het aantal rijpe follikels toeneemt, het endometrium beter opbouwt, en de kans op ovulatie en zwangerschap toeneemt in vergelijking met het huidige standaard behandelschema. Aan het huidige onderzoek namen in totaal 212 vrouwen deel (438 cycli) met polycysteus ovarium syndroom (PCOS). Patiënten in de groep die in de voorafgaande cyclus starten met CC kregen dagelijks 100 mg CC, in de laatste 5 dagen van de behandeling gecombineerd met medroxy progesterone acetate (MBA) (110 patiënten, 227 cycli). Patiënten in de standaard groep die laat met de CC starten, kregen vanaf cyclusdag 3 van de menstruatie dagelijks 100 mg CC gedurende 5 dagen (102 patiënten, 211 cycli). Er werd geen significant verschil gevonden in kans op ovulatie tussen beide groepen. Het totaal aantal follikels en het aantal follikels ≥14 mm en ≥18 mm gedurende de stimulatie was significant groter ($P<0.05$) in de vroege start groep. Het endometrium op de dag van toedienen van de hCG was significant dikker in de vroege start groep (9.1 ± 0.23 vs. 8.2 ± 0.60 mm). Serum E2 en progesteron waarden waren niet significant verschillend in beide groepen ($P>0.05$). Zwangerschap trad op in 23 van de 110 cycli in de vroege start CC groep (20.9%) en in 16 van de 102 cycli in de late start CC groep (15.7%), het verschil was niet statistisch significant ($P>0.05$). Het aantal miskramen was in beide groepen gelijk. Uit deze resultaten concludeerden wij dat vroege start van CC in patiënten met PCOS leidt tot meer follikel groei en dikker endometrium wat zou kunnen leiden tot hogere zwangerschapscijfers.

Hoofdstuk 3
In hoofdstuk 3 worden de resultaten besproken van een gerandomiseerd gecontroleerd onderzoek naar het effect op zwangerschapskans van verlengen van CC therapie (langer dan de standaard 5 dagen) in vergelijking met gonadotrofine therapie. Aan de studie namen 318 vrouwen (802 cycli) met clomifeen-resistente PCOS deel. Patiënten in de CC groep werden behandeld met dagelijks 100 mg CC vanaf cyclusdag 2 gedurende 9 dagen (160 patiënten, 405 cycli). Patiënten in de gonadotrofine groep injecteerden dagelijks 75 IU hMG intramusculair vanaf cyclusdag 3 gedurende 5 dagen. Het aantal patiënten met een ovulatie was significant hoger in de gonadotrofine groep (57.6% vs. 28.1%). Het totaal aantal follikels tijdens de stimulatie was eveneens significant hoger in de gonadotrofine groep (6.7 ± 0.3 vs. 4.1 ± 0.4). De dikte van het endometrium op de dag van de hCG toediening was significant beter in de gonadotrofine groep (10.2 ± 0.6 vs. 8.2 ± 0.3 mm). Het serum progesteron was significant hoger in de gonadotrofine groep. Zwangerschap trad op in 47 van de 405 cycli in de CC groep (11.3%) en in 80 van de 397 cycli (20.1%) in de gonadotrofine groep ($P<0.05$). Het verlengen van de duur van het gebruik van CC resulteerde in lichte toename van de kans op ovulatie en zwangerschap en er werden geen bijwerkingen gerapporteerd. Uit deze studie kan geconcludeerd worden dat deze therapie

economische, effectiviteits- en veiligheidsvoordelen heeft en het als alternatieve keuze kan worden gezien voordat er overgegaan wordt op duurdere of geavanceerdere behandelingen.

Hoofdstuk 4
Er zijn een aantal voorlopige artikelen over de rol van *N*-acetyl cysteine (NAC) met CC bij ovulatie inductie behandeling gepubliceerd, maar de rol van deze adjuvante therapie is niet op grote schaal onderzocht. In hoofdstuk 4 demonstreren wij de resultaten van een cross-over trial die CC met NAC vergelijkt met alleen CC voor ovulatie-inductie behandeling bij patiënten met PCOS. Vijfhonderd en drieënzeventig PCOS patiënten werden alleen behandeld met CC gedurende 1 cyclus, waarvan 470 patiënten eveneens werden behandeld met CC en NAC in een volgende cyclus. Patiënten kregen de eerste cyclus 50 mg CC tweemaal daags en de volgende cyclus vanaf cyclusdag 3 CC met NAC 1200 mg/dag oraal gedurende 5 dagen. Primaire uitkomstmaten waren aantal rijpe follikels, serum E2, serum progesteron en dikte van het endometrium. Secundaire uitkomstmaat was het optreden van een zwangerschap. De kans op ovulatie verbeterde significant na het toevoegen van NAC (52.1% versus 17.9%). Hoewel er meer rijpe follikels in de NAC groep waren (2.19 ± 0.88 versus 3.29 ± 0.93), was het verschil niet statistisch significant. De gemiddelde E2 waarden (pg/ml) op tijdstip van de hCG injectie, serum progesteron waarden (ng/ml) op cyclusdag 21-23 en de dikte van het endometrium waren significant verbeterd in de NAC groep. De zwangerschapskans was 11.5% in de NAC groep. Insuline resistentie werd gevonden bij 260 patiënten (55.4%). Er was geen significant verschil tussen de insuline-resistentie groep (N=260) en de niet insuline-resistentie groep (N=210) met betrekking tot de kans op ovulatie, aantal follikels, serum E2 (pg/ml), serum progesteron (ng/ml), dikte van het endometrium (mm) of kans op zwangerschap. Concluderend kunnen we zeggen dat N-Acetyl cysteine bewezen effectief is als adjuvante therapie bij CC in een ovulatie inductie behandeling bij PCOS patiënten.

Hoofdstuk 5
In hoofdstuk 5 beschrijven we een gerandomiseerd, dubbelblind, gecontroleerd onderzoek naar het verschil tussen clomifeen citraat plus NAC versus alleen clomifeen citraat voor het vergroten van de kans op ovulatie bij onverklaarde infertiliteit. We rekruteerden 404 patiënten in de onderzoeksgroep (clomifeen citraat plus *N*-acetyl cysteine groep) en 400 patiënten in de controlegroep (clomifeen citraat groep). Patiënten in de onderzoeksgroep starten op cyclusdag 2 met CC (50 mg tabletten) tweemaal daags met *N*-acetyl cysteine (1200 mg/dag oraal) gedurende 5 dagen. Patiënten in de controlegroep werden behandeld met CC met suikerpoeder. De primaire uitkomstmaten waren aantal en grootte van de follikels, serum E2, serum progesteron en endometriumdikte. Secundaire uitkomstmaat was het optreden van zwangerschap. Er was geen statistisch significant verschil tussen beide groepen in het aantal follikels >18 mm, gemiddelde E2 waarden, serum progesteron waarden en dikte van het endometrium. Zwangerschapscijfers waren vergelijkbaar in beide groepen (22.2% vs. 27%). Ook het aantal miskramen was gelijk in beide groepen (6.7% in de onderzoeksgroep vs. 7.4% in de controle groep). Concluderend kunnen we zeggen dat *N*-Acetyl cysteine als adjuvante therapie bij CC ineffectief is in het vergroten van de kans op ovulatie bij patiënten met een onverklaarde infertiliteit en dat het niet kan worden aanbevolen in een dergelijke situatie.

Hoofdstuk 6
Een aantal gerandomiseerde onderzoeken hebben aromatase remmers en CC vergeleken voor ovulatie inductie behandelingen. Echter het aantal patiënten in deze onderzoeken was klein hetgeen de validiteit van de conclusies beïnvloedt. Hoofdstuk 6 presenteert een prospectief gerandomiseerd onderzoek dat het effect van letrozol (5 mg) en CC (100 mg) vergelijkt voor ovulatie-inductie behandeling bij PCOS patiënten. Er werden 438 infertiele vrouwen (1063 cycli) met PCOS geïncludeerd. Patiënten werden gerandomiseerd voor een behandeling met 5 mg

letrozol dagelijks (218 patiënten, 545 cycli) of 100 mg clomifeen citraat per dag (220 patiënten, 518 cycli) gedurende 5 dagen en beginnend op dag 3 van de menstruatie. Getimede coïtus werd geadviseerd 24 tot 36 uur na de hCG injectie. Aantal follikels, serum oestradiol, serum progesteron, endometriumdikte, aantal zwangerschappen en aantal miskramen waren de uitkomstmaten. Het aantal follikels was significant groter in de CC groep (6.8±0.3 versus 4.4±0.4). De endometriumdikte op het moment van de hCG injectie was significant dikker in de CC groep (9.2±0.7 mm versus 8.1±0.2 mm). Het aantal dagen dat nodig was om follikels van 18 mm of groter te verkrijgen was significant langer in de letrozol groep (12,1 ± 1,3 versus 8,8 ± 2,9 dagen). Ovulatie trad op in 365 van de 540 cycli (67.5%) in de letrozol groep en bij 371 van de 523 cycli (70.9%) in de CC groep, het verschil was echter niet statistisch significant. Serum E2 en progesteron waarden waren significant hoger in de CC groep. Het zwangerschapscijfer per cyclus was 15.1% in de letrozol groep en 17.9% in de CC groep, maar niet statistisch significant. Concluderend kunnen we zeggen dat deze studie geen voordeel heeft aangetoond voor het gebruik van letrozol ten opzichte van CC als eerste keus ovulatie-inductie behandeling bij PCOS patiënten.

Hoofdstuk 7

Hoofdstuk 7 bevat een prospectief gecontroleerd onderzoek waarin de effecten van anastrozol (1 mg) en CC (100 mg) voor de ovulatie inductie behandeling van vrouwen met PCOS worden vergeleken. In het onderzoek werden in totaal 216 infertiele vrouwen (469 cycli) met PCOS geïncludeerd. Patiënten starten met anastrozol (1 mg/dag, 115 patiënten, 243 cycli) op cyclusdag 3 en gebruikten dit gedurende 5 dagen. Een vergelijkbare historische groep van patiënten met PCOS die behandeld waren met CC (100 mg/dag, 101 patiënten, 226 cycli) werd gebruikt als controle groep. Aantal follikels, E2 waarden, progesteron waarden, dikte van endometrium, aantal zwangerschappen en aantal miskramen waren de uitkomstmaten. Het totaal aantal follikels was significant hoger in de CC groep (3.8 ± 0.6 vs. 3.4 ± 0.5). De dikte van het endometrium op het moment van de hCG injectie was significant beter in de anastrozol groep (10.1 ± 0.22 mm vs. 8.2 ± 0.69 mm). De duur van de stimulatie was in beide groepen gelijk. Ovulatie trad op in 165 van de 243 cycli (67.9%) in de anastrozol groep en in 150 van de 226 cycli (68.6%) in de CC groep; niet statistisch significant. De progesteron waarde in het bloed was significant hoger in de CC groep (7.1 ± 1.11 vs. 8.1 ± 0.88 ng/ml). De zwangerschapscijfers en aantal miskramen was in beide groepen gelijk. Concluderend kunnen we zeggen dat anastrozol geassocieerd wordt met significant minder rijpe en groeiende follikels, dikker endometrium en licht hogere zwangerschapscijfers. Anastrozol kan nuttig zijn in situaties waarbij het ontstaan van tweelingzwangerschappen ongewenst is of het risico op ovarieel hyperstimulatie syndroom groot is.

Hoofdstuk 8

In hoofdstuk 8 wordt een prospectief gerandomiseerd gecontroleerd onderzoek gepresenteerd waarin letrozol (2.5 mg) en anastrozol (1 mg) vergeleken worden voor ovulatie inductie behandeling bij clomifeen resistente vrouwen met PCOS. In het onderzoek zijn in totaal 220 infertiele vrouwen (574 cycli) geïncludeerd met CC resistente PCOS. Patiënten werden gerandomiseerd voor een behandeling met dagelijks 2,5 mg letrozol (111 patiënten, 295 cycli) of dagelijks 1 mg anastrozol (109 patiënten, 279 cycli) gedurende 5 dagen waarbij gestart werd op cyclusdag 3. De uitkomstmaten waren aantal follikels, serum E2, serum progesteron, endometriumdikte, aantal zwangerschappen en aantal miskramen. Het totaal aantal follikels was significant groter in de anastrozol groep (5.4 ± 0.4 vs. 5.8 ± 0.4). Het aantal follikels met een afmeting van >14 mm (3.1 ± 0.3 vs. 2.7 ± 0.2) en >18 mm (2.3 ± 0.1 vs. 3.1 ± 0.2) was significant groter in de anastrozol groep. Dikte van het endometrium op het moment van de hCG injectie was significant dikker in de anastrozol groep (9.1 ± 0.2 vs. 10.2 ± 0.7 mm). De duur van de stimulatie

totdat een follikel een afmeting had bereikt van 18 mm was langer in de letrozol groep (12.1 ± 1.3 dagen vs. 8.8 ± 1.9 dagen), maar niet statistisch significant. Ovulatie trad op in 183 van de 295 cycli (62%) in de letrozol groep en in 177 van de 279 cycli (63.4%) in de anastrozol groep en zwangerschap kwam tot stand in 36 van de 295 cycli (12.2%) in de letrozol groep en in 42 van de 279 cycli (15.1%) in de anastrozol groep, maar deze verschillen zijn niet statistisch significant. De conclusie van dit onderzoek is dat er geen significant verschil in zwangerschapscijfers of aantal miskramen tussen anastrozol en letrozol is bij ovulatie-inductie behandeling bij vrouwen met een CC-resistente PCOS.

Hoofdstuk 9

Dit hoofdstuk behandelt de optimale dosis van letrozol voor ovarium stimulatie bij patiënten met een onbegrepen infertiliteit. In totaal werden 179 patiënten willekeurig geworven in dit prospectieve onderzoek waarbij gerandomiseerd werd tussen 2.5, 5 en 7.5 mg letrozol gedurende 5 dagen. Respectievelijk werden in elke dosis groep 58, 61 en 60 patiënten geïncludeerd. Het onderzoek liet een significant hoger aantal follikels zien (totaal, >14 mm en ≥18 mm) op de dag van de hCG injectie in de 7.5 mg groep, geassocieerd met een significant kortere duur van de stimulatie. Echter de zwangerschapscijfers en het aantal miskramen waren in alle drie de groepen gelijk. Uit dit onderzoek kan geconcludeerd worden dat het lijkt dat een hogere dosering van letrozol geen voordeel biedt qua aantal zwangerschappen ten opzichte van de lage (2.5 mg) dosis groep.

Recent zijn de veiligheid van letrozol en andere aromatase remmers die gebruikt worden voor ovulatie-inductie behandeling opnieuw ter discussie gesteld. Hoofdstuk 10 behandelt een prospectief gerandomiseerd gecontroleerd onderzoek naar het verschil in zwangerschapscijfers bij een ovulatie-inductie behandeling met aromatase remmers of CC. In het onderzoek werden 796 infertiele vrouwen (1100 cycli) geïncludeerd en 200 spontaan zwangere vrouwen (298 cycli) als controle groep. Patiënten kregen een behandeling met dagelijks 100 mg CC (420 patiënten, 634 cycli), dagelijks 5 mg letrozol (269 patiënten, 323 cycli) of dagelijks 1 mg anastrozol (107 patiënten, 143 cycli) gedurende 5 dagen, waarbij gestart werd op cyclusdag 3. Getimede coïtus werd geadviseerd 24 tot 36 uur na de hCG injectie. De uitkomstmaten waren optreden van zwangerschap, aantal miskramen en neonatale gevolgen. Zwangerschap trad op in 167 van de in totaal 1398 cycli (11.9%) zonder statistisch significante verschillen tussen de groepen onderling. Het totaal aantal miskramen was 16.1% (variërend van 14.2% in de CC groep tot 19.9% in de anastrozol groep) zonder verschil tussen spontane zwangerschappen of zwangerschappen ontstaan na stimulatie. Er waren in totaal 129 bevallingen over alle groepen. Er was geen statistisch significant verschil tussen zwangerschappen ontstaan na stimulatie en spontane zwangerschappen ten aanzien van gemiddelde zwangerschapsduur, premature bevallingen, geboortegewicht, laag geboortegewicht voor de zwangerschapsduur <10 percentiel of Apgar score bij 5 minuten. Er was 1 casus met een complete schisis (hazenlip) en 1 casus met congenitale hartafwijkingen in de letrozol groep. Er waren 2 casus van talipes equinovarus (klompvoet) in de CC groep en in de groep van de spontane zwangerschappen. Uit dit onderzoek kunnen we concluderen dat aromatase remmers en CC gelijke effectiviteit hebben qua ovulatie inductie behandeling. Beide medicamenten geven gunstige zwangerschapscijfers en een gemiddeld aantal miskramen.

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2008

Curriculum vitae

The author of this thesis was born on February 23, 1964 in Mansoura, Egypt. After finishing high school in 1981, he studied medicine at Mansoura University, Egypt. He was graduated in November 1987 with the honor degree. In 1990, he was appointed as a senior house officer in the department of Obstetrics and Gynecology in Mansoura University hospitals, Egypt. In 1992, he received his Master's degree (MSc) in obstetrics and Gynecology. In the same year, he started to work as an assistant lecturer and registrar of Obstetrics and Gynecology in Mansoura Faculty of Medicine. Subsequently, from 1995 to 1998, he worked as a clinical research fellow in the Minimally Invasive Therapy Unit in the Royal Free Hospital, London. During this period he received his degree of Doctor in Medicine (MD) in endoscopic surgery. He also received his membership of the Royal College of Obstetricians and Gynaecologists (MRCOG) in May 1997. He was appointed in July 1998 as a lecturer and senior registrar of Obstetrics and gynecology in Mansoura Faculty of Medicine and then as an associate professor and consultant in July 2003. In October 2008, he became a professor of Obstetrics & Gynecology in Mansoura University. He is the clinical director of the assisted reproduction unit in Mansoura University Hospital, Egypt. His research activity in the last years has been in the area of reproduction.