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Oral Agents for Ovulation Induction

Ovulasyon İndüksiyonunda Oral Ajanlar

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ABSTRACT

Infertility due to ovulation disorders is 25% of all infertility causes. The most common cause of ovulation disorders is patients with normogonadatropic normogonadism, which is group II according to the World Health Organization anovulation classification and mostly consists of patients with polycystic ovary syndrome which affects 6-20% of women of reproductive age. Oral ovulation induction agents are a suitable option only for patients in this group. The purpose of the ovulation induction is to stimulate the ovaries for monofollicular development. Oral agents used in ovulation induction can be divided into two groups, selective estrogen receptor modulators and aromatase inhibitors as first-line agents, and metformin and inositols as second-line agents. The aim of this review is to compare the use and efficacy of the primary oral ovulation induction agents, clomiphene citrate and letrozole, and also to reveal the contributions of the adjuvant drugs metformin and inositol. It is seen that letrozole is superior to clomiphene citrate in polycystic ovary syndrome and is currently preferred as the first-choice drug worldwide. Metformin alone increases the ovulation rate compared to placebo in women with polycystic ovary syndrome, but should not be used as first-line therapy for anovulation. Similarly, when inositol is used alone, it does not increase the pregnancy rate.
Keywords: Anovulation; polycystic ovary syndrome; clomiphene citrate; letrozole; metformin; inositol.

ÖZ

Ovulasyon bozukluklarına bağlı infertilite, tüm infertilite nedenlerinin %25'ini oluşturmaktadır. Ovulasyon bozukluklarının en sık nedeni, Dünya Sağlık Örgütü anovulasyon sınıflamasına göre grup II olan ve çoğunlukla üreme çağındaki kadınların %6-20'sini etkileyen polikistik over sendromlu hastalardan oluşan normogonadatropik normogonadizmli hastalardır. Oral ovulasyon indüksiyon ajanları sadece bu gruptaki hastalar için uygun bir seçenektir. Ovulasyon indüksiyonunun amacı, yumurtalıkları monofoliküler gelişim için uyarmaktır. Ovulasyon indüksiyonunda kullanılan oral ajanlar, birinci basamak ajanlar olarak selektif östrojen reseptör modülatörleri ve aromataz inhibitörleri ve ikinci basamak ajanlar olarak metformin ve inositoller olmak üzere iki gruba ayrılabilir. Bu derlemenin amacı, birincil oral ovulasyon indüksiyon ajanları olan klomifen sitrat ve letrozolün kullanım ve etkinliklerini karşılaştırmak ve ayrıca adjuvan ilaçlar olan metformin ve inositolün katkılarını ortaya koymaktır. Polikistik over sendromunda letrozolün klomifen sitrata göre daha üstün olduğu ve günümüzde dünya çapında ilk seçenek ilaç olarak tercih edildiği görülmektedir. Polikistik over sendromlu kadınlarda, tek başına metformin plaseboya kıyasla ovulasyon oranını arttırır, ancak anovulasyon için birinci basamak tedavi olarak kullanılmamalıdır. Benzer şekilde, inositol tek başına kullanıldığında gebelik oranını artırmamaktadır.
Anahtar kelimeler: Anovulasyon; polikistik over sendromu; klomifen sitrat; letrozol; metformin; inositol.

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INTRODUCTION

Ovulation disorders constitute 25% of infertility cases (1). In women during the ovulation period, menstruation occurs approximately once a month (24-38 days). Unpredictable menstrual cycles are observed in oligo-anovulatory women (from 39 days to six months). Even if ovulation occurs, this is not enough to ensure pregnancy. The basis of ovulation induction is to ovulate and the first step here is to find the cause of the ovulation problem. The World Health Organization (WHO) divided anovulation into three groups, in Table 1 (2). The only area where oral agents can be used for ovulation induction is anovulatory patients in WHO group II, the majority of whom are patients with polycystic ovary syndrome (PCOS). PCOS is the most common endocrine pathology in women of reproductive age. Depending on diagnostic criteria, this disorder affects 6-20% of women of reproductive age (3,4), and is the most common cause of oligo-anovulation (80%). It was first described by Stein and Leventhal in 1935. Multiple morbidities, including infertility, metabolic syndrome, obesity, impaired glucose tolerance, type 2 diabetes mellitus, cardiovascular risk, depression, obstructive sleep apnea, and endometrial cancer are associated with PCOS (5-7) Therefore, the first step in treatment is lifestyle changes. Lifestyle interventions consist of many components, including healthy diets, physical activity, reduced sedentary behavior, and behavioral strategies (8). In the treatment of infertility, oral ovulation induction agents, as well as lifestyle changes, constitute the first-line treatment. Women who fail to ovulate or fail to conceive after first-line treatment options are often referred to gonadotropin therapy. Laparoscopic ovarian puncture, evaluated in well-designed studies, may be an alternative to gonadotropins. In vitro fertilization is the last option for couples who cannot conceive after all these treatments. The purpose of the ovulation induction is to stimulate the ovaries for monofollicular development. Monofollicularity reduces the two main risks of induction of ovulation: ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy (9). More than one follicle should not be targeted in ovulation induction (10).

The purpose of this review is to describe oral ovulation induction agents, mainly clomiphene citrate (CC), letrozole, and as adjuvant agents metformin and inositol, and also compare their efficacies.

Oral agents used in ovulation induction can be divided into two groups: i) First-line agents as a) selective estrogen receptor modulators (SERMs) and b) aromatase inhibitors; and ii) Second-line agents (insulin-sensitizing drugs) as a) metformin and b) inositols.

FIRST-LINE AGENTS

Selective Estrogen Receptor Modulators (SERMs)

SERMs are competitive inhibitors of estrogen binding to estrogen receptors (ERs) and all have mixed agonist and antagonist activity depending on the target tissue. There are three agents in this group; tamoxifen, raloxifene, and clomiphene (11). The antagonist effect of tamoxifen is particularly evident in breast cancer. Although its primary use is in the treatment of breast cancer, recent reviews suggest that tamoxifen and CC both have similar ovulation and pregnancy rates (12,13). Raloxifene is particularly used in the treatment of osteoporosis, it does not appear to have endometrial agonistic effects; unlike tamoxifen, it does not increase the risk of uterine cancer. CC is a nonsteroidal triphenylethylene derivative, most commonly used as an ovulation stimulant in fertility treatment. The commercially available form of clomiphene is the dihydrogen citrate salt, namely CC. It contains two stereoisomers: zu-clomiphene (38%, cis-isomer) and en-clomiphene (62%, trans-isomer), which is the more potent isomer with greater antiestrogenic activity and responsible for inducing follicular development (14). Clomiphene is cleared through the liver and excreted in the feces. More than half of the clomiphene is excreted after five days, but traces of radioactivity from labeled clomiphene are visible in the stool for up to six weeks after administration. Although this observation raises concerns about fetal clomiphene exposure, most studies suggest that the frequency of congenital malformations does not increase (15). On the other hand, there is some evidence for a possible relationship between CC exposure and fetal malformations, especially neural tube defects and hypospadias, which required to investigate further (16,17). Although CC has no progestational, corticotropic, androgenic, or antiandrogenic effects and does not affect adrenal or thyroid function, a meta-analysis of women taking CC (and other fertility drugs) has shown an increased risk of thyroid cancer (18).

Table 1. Anovulation classification according to World Health Organization (WHO)

WHO group I, Hypogonadotrophic hypogonadism
- Idiopathic
- Kallmann's syndrome (isolated gonadotrophin deficiency and anosmia)
- Functional hypothalamic dysfunction (e.g. excessive weight loss such as in anorexia nervosa, exercise, stress, drugs, iatrogenic)
- Pituitary tumor, pituitary infarct (e.g. Sheehan's syndrome)
WHO group II, Normogonadotrophic normogonadic ovarian dysfunction
- Polycystic ovary syndrome (PCOS)
WHO group III, Hypergonadotrophic hypogonadism (ovarian failure)
- Genetic (e.g. Turner's syndrome)
- Autoimmune causes
- Infection (e.g. mumps oophoritis)
- Iatrogenic (e.g. surgical menopause, post-radiotherapy, or chemotherapy)
- Idiopathic
- Other endocrinopathies, such as hyperprolactinemia, thyroid dysfunction, and other conditions of androgen excess such as congenital adrenal hyperplasia and androgen-secreting adrenal and ovarian tumors

CC acts primarily on the hypothalamus, which appears to bind to and deplete hypothalamic ERs, thereby blocking the negative feedback effect of circulating endogenous estradiol. This causes an increase in hypothalamic gonadotropin releasing hormone (GnRH) pulse frequency and an increase in serum concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH). High FSH and LH stimulate follicular development in the ovaries (19). Antiestrogen effects are evident in the endometrium and cervix. The normal increase in uterine volume and endometrial thickening that occurs during spontaneous menstrual cycles are largely absent during clomiphene-induced cycles, despite higher estradiol levels (20,21). This may explain why pregnancy rates are relatively low, while ovulation rates are so high in women on a clomiphene cycle. A recent study showed that ovarian stimulation with CC delays endometrial maturity and may impair the implantation process, possibly due to mismatch (22). Data on the effect of clomiphene on cervical mucus are unclear. In a meta-analysis, a deleterious effect was seen only at doses ≥100 mg/day (23).

Since CC is competitively bound with estrogen, estrogen must be present in the environment for its effectiveness and the hypothalamic-pituitary axis must be intact.

The diagnosis of unexplained infertility (10-30%) and its etiology may remain unknown despite intensive evaluation of both male and female partners (24). The efficacy of human chorionic gonadotropin (hCG) trigger, timed intercourse and intrauterine insemination (IUI) approaches compared to the expectant approach in patients for whom CC is indicated are as follows.

- Anovulatory/oligo-ovulatory women:
  IUI has no benefit over timed intercourse
- Unexplained infertility:
  CC+timed intercourse = expectant approach
  CC+hCG trigger < expectant approach
  CC+IUI > expectant approach

Clomiphene Citrate Treatment Protocols

Before starting treatment, necessary evaluations should be made to rule out causes other than anovulatory infertility.

Standard Protocol: 50 mg/day orally for 5 days, typically on day 5 of the cycle (can be started on days 2-5). If there is no response, the dose is increased to 100 mg/day in the next cycle. Maximum recommended doses are 100 mg/day by the US Food and Drug Administration (FDA), and 150 mg/day by the American College of Obstetricians and Gynecologists (ACOG). Once ovulation has occurred, the same dose should be continued for four to six cycles. Lower doses (12.5-25 mg daily) may be used in women with clomiphene sensitivity or who develop persistently large ovarian cysts (25).

- The total daily dose should be taken once to maximize the effect.
- Sexual intercourse is recommended for one week, starting 5 days after the last dose.
- Pregnancy usually occurs in 3-6 cycles. Routine basal ultrasonography (USG) is not recommended.
- If an endogenous LH increase is detected for ovulation, routine hCG is not recommended, if any. Pregnancy rates are highest when the dominant follicle is 23-28 mm in diameter on USG for hCG trigger (26).
- No dosage adjustment is required in renal impairment, but should not be used in patients with liver disease.

Alternative Protocols

Longer Courses: Instead of the classic 5-day course, some CC-resistant anovulatory women may respond to longer CC courses (7 to 8 days). There is limited data on this practice (27).

Stair-step Protocol: CC can be initiated at any time during the stable follicular phase when there is no suspicion of pregnancy (predominant follicle, absence of the corpus luteum, and can be judged by three linear endometrial appearances on ultrasound). 50 mg CC is given for 5 days. 2-3 days after the last dose, it is checked whether a dominant follicle is formed by ultrasound examination. If there is no dominant follicle, 100 mg CC is started immediately after seven days of the last dose, without causing withdrawal bleeding with progestins (28). A summary of the main features and results of previous studies using the CC stair-step protocol is shown in the Table 2, derived from Horowitz and Weissman (29). According to the available data, it has been reported that the pregnancy and live birth rates in cycles starting with spontaneous bleeding or progesterone withdrawal bleeding are lower than in anovulatory cycles in which CC is initiated without progestin interruption bleeding (30).

Half of the patients ovulate with 50 mg CC, 20-25% with 100 mg, and 10% with 150 mg. As the age, body mass index (BMI), insulin resistance, and free androgen index increase, the ovulation rate decreases. There is no point in continuing as pregnancy rates per cycle remain unchanged after six months of treatment. Failure to conceive after six maximum ovulation therapy cycles indicates the need to further evaluate potential infertility factors or switch to another treatment strategy. The rate of multiple pregnancy with CC is 7-10%. CC does not increase the abort rate (31) and the risk of ectopic pregnancy.

The most common side effect of CC is ovarian enlargement. Less frequently, hot flashes may be associated with hypoestrogenism at the hypothalamic level. The side effects of CC are not dose dependent.

Table 2. Outcomes of the studies comparing the CC stair-step and traditional protocols (29*)

	Stair-step Protocol			Traditional Protocol		
Design	**n**	**Time to Ovulation**	**Ovulation Rate**	**n**	**Time to Ovulation**	**Ovulation Rate**
Retrospective	31	23-35 days	64% (100 mg) 74% (≤150 mg)	H	55-88 days	22% (100 mg) 35.5% (≤150 mg)
RCT	30	20.5±2 days	43%	30	48.6±4 days	33.3%
RCT	30	13.65±6.7 days	66.7%	30	32.8±20.4 days	50%
Retrospective	43	23±0.9 days	88%	66	47.5±6.3 days	39%

*: derived from Horowitz and Weissman (29), CC: clomiphene citrate, RCT: randomized controlled trial, H: historical control

Aromatase Inhibitors

Aromatase enzyme takes place in the last step of estrogen synthesis. It catalyzes the demethylation of carbon 19 of androgens to produce phenolic 18-carbon estrogens. Aromatase is the only member of the family 19 of P450 super enzymes called CYP19 (32), which is found in tissues such as the ovary, adipose tissue, breast, brain, liver, and muscle. Numerous aromatase inhibitors have been developed (Table 3). The third generation, nonsteroidal aromatase inhibitors commonly used today are letrozole and less commonly anastrozole (33).

Letrozole is currently the only registered indication for breast cancer (34). Letrozole potently inhibits aromatase activity (reduces endogenous estrogen synthesis by 97-99%) competitively and reversibly (35). Letrozole (2.5 mg once daily) has a plasma half-life of 41~48 hours. Letrozole metabolism may be markedly increased in patients with hepatic insufficiency (34). The aromatase inhibition effect starts after 2 days.

Letrozole's main mechanism of action for ovulation induction is that it reduces circulating estrogen levels significantly by decreasing the estrogen synthesis. This prevents negative feedback (central effect) in the hypothalamic-pituitary-gonadal axis (36). Also, because the conversion of androgen substrates to estrogen is inhibited, transient accumulation of intraovarian androgens can increase follicular sensitivity (peripheral effect) through amplification of FSH receptor gene expression (37). Typically letrozole is taken on a daily basis on the 3~7th day of the menstruation. During this period, follicle sizes are 6-8 mm and contain high levels of androgen receptors. Therefore, the increase in androgen levels during this period promotes granulosa cell mitosis and the induction of FSH receptors (38).

The negative effects of CC on the endometrium and possibly the cervix have made the use of multi-follicular growth aromatase inhibitors increasingly popular (39). Because letrozole does not inhibit negative feedback of estrogen to the hypothalamic-pituitary-gonadal axis, it generally induces single follicle development and prevents multiple pregnancies. It is also indicated for patients who cannot tolerate the side effects of CC and who cannot tolerate fertility-preserving treatment and assisted reproductive techniques in cancer patients.

Treatment Protocols of Letrozole

Prior to starting the treatment, thyroid stimulating hormone (TSH), prolactin, and FSH values should be checked to differentiate the patients who will not respond to letrozole, and hysterosalpingography (HSG) and spermiogram tests should be performed to exclude other factors causing infertility.

Standard Protocol: Typical treatment of letrozole consists of 2.5 mg daily taken during days 3~7 of menstrual for a 5-day course. If there is no response, dosage is increased to 7.5 mg/day in the next cycle.

Table 3. Aromatase inhibitors

1st generation	2nd generation	3rd generation
Aminoglutedimide	Fadrozole	Letrozole
	Formestane	Anastrazole
		Exemestane

Alternative Protocols

Single Dose: A single dose of 20 mg can be taken on the 3rd day of the cycle but related data is limited.

Stair-step Protocol: If ovulation does not occur, the sequential dose can be increased to 5 mg and 7.5 mg, respectively, without waiting for withdrawal bleeding. This protocol is widely used by many clinicians (40).

In a prospective randomized controlled trial comparing letrozole and CC for ovulation induction in women with PCOS in India in 2020, while there was no significant difference between the groups for mean endometrial thickness, a significant difference was found in favor of letrozole for monofollicular development. In addition, pregnancy rates were found to be 42% in letrozole and 20% in CC, with a significant difference. The gestation period was also found to be significantly shorter in the letrozole group (41).

In a meta-analysis by Tsiami et al. (42), they reviewed 26 randomized controlled trials over 13 years and found that those given letrozole were more likely to ovulate than those given CC.

Letrozole has been used off-label in the treatment of patients with anovulation. The reason it was not approved is related to its potential teratogenic effect. However, letrozole has a short half-life period (48 hours) in contrast to CC (2 weeks), so it is cleared before ovulation; therefore, it is unlikely to be teratogenic. In the study of Akbari Sene et al. (43), CC and letrozole were evaluated in terms of congenital fetal anomaly risk, and no difference was found between them.

For ovulation sufferers, letrozole generally increases their chances of getting pregnant. It provides monofollicular development in most cycles, thus reducing multiple pregnancies and OHSS may be the first treatment option for unexplained infertility (44).

In a randomized controlled trial in which the combination of letrozole and CC, and only letrozole was given for ovulation induction in patients with PCOS, researchers concluded that the combination of letrozole and CC was associated with a higher ovulation rate compared to letrozole alone (45).

SECOND-LINE AGENTS (INSULIN-SENSITIZING DRUGS)

Metformin

Metformin is an orally active, water-soluble biguanide used in type 2 diabetes mellitus, it is antihyperglycemic and does not cause hypoglycemia. It increases insulin sensitivity in peripheral tissue, where it inhibits hepatic glucose production and increases glucose uptake and use in muscle tissue. Reducing hyperinsulinemia in PCOS may normalize endocrine, metabolic, and reproductive functions, and leading to the resumption of ovulation (46). Practice committee of the American Society for Reproductive Medicine (ASRM) noted that metformin alone increased the ovulation rate in women with PCOS compared to placebo. However, it should not be used as a first-line ovulation induction agent due to the its lower efficacy than CC and letrozole (47).

Inositol

Inositols are a family of biomolecules that are important in regulating vital cellular functions, signal transduction, energy transmission, and ion channel

physiology, and serve as structural components of cell membranes (48). It mainly has two stereoisomers, Myo-inositol and D-chiro-inositol; they are incorporated intracellularly into insulin's second messengers, inositol phosphoglycans, and some effects of insulin are mediated by these inositol phosphoglycan mediators (49). D-chiro-inositol and its combination have been shown to improve metabolic, hormonal, and reproductive aspects of PCOS (50). In a meta-analysis evaluating the use of inositol in infertile PCOS patients, no clear information could be obtained that it increased pregnancy rates (51).

CONCLUSION

There are many studies and meta-analyses evaluating the effectiveness of CC and letrozole in the literature. In addition, it is also included in combinations with different adjuvant agents. There are many studies showing the effects of insulin-sensitizing drugs when given alone or as an adjuvant. As a result of the examination of all these studies, the following information is obtained:

- Letrozole appears to improve live birth and pregnancy rates compared to CC in subfertile women with anovulatory PCOS.
- There is high-quality evidence that rates of OHSS are comparable with letrozole or CC (52).
- Letrozole is a better alternative for ovulation induction in anovulatory women with PCOS due to the higher pregnancy rates and shorter gestational period (41).
- Fewer or a similar multiple pregnancy rates are obtained with letrozole compared to CC (41,52).
- There is no clear information about the negative effect of CC on cervical mucus. There is even a study showing that letrozole has a more negative effect (53).
- The letrozole stair-step protocol elicited a shorter ovulation time and higher ovulation, clinical pregnancy, and live birth rates than the CC stair-step protocol (54).
- Correction of hyperinsulinemia with metformin improves the spontaneous ovulation but not the live birth rates (55).

As a result, letrozole has proven superior over CC for WHO group II patients. Despite the advantage of letrozole in PCOS patients; in terms of mild oligoasthenospermia, early-stage endometriosis, and unexplained infertility conditions, letrozole and CC results were similar.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

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