Reviews

Clomiphene citrate and ovulation induction*

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Abstract

Clomiphene can be used to treat anovulation due to hypothalamus or pituitary gland dysfunction, and it normalizes the luteal phase in stimulated patients. It can be used to estimate ovarian follicle reserve, and may be predictive of ovulation in women aged ≥35 years or with failed IVF. Contraindications include risk of congenital anomalies, chronic liver disease and visual disorders. Clomiphene may impair fertility through its effects on cervical mucus and in causing various endometrial dysfunctions. However, if clomiphene is administered in 50 mg doses, side-effects are avoided and efficacy is similar to that of a 100 mg dose, although daily dosages of 200 mg/day over 5 days can induce ovulation in ~70% of treated patients. Gonadotrophin concentrations increase up to days 5–9 when follicles are selected, and clomiphene is effective in patients with polycystic ovary syndrome (PCOS). Fifty percent of normal patients conceive, a value perhaps biased by the antagonistic effects of clomiphene on cervical mucus in some women. Clomiphene is valuable for IVF, and is used by some clinics in combination with HMG or recombinant FSH. Resistance to clomiphene can develop, and human chorionic gonadotrophin may be needed to induce ovulation in clomiphene cycles. Corticosteroids and human menopausal gonadotrophin (HMG) can be combined with clomiphene for stimulation, its combination with HMG long having been a standard protocol in assisted reproduction. PCOS patients may become insulin resistant, a condition improved by the administration of metformin. Other adverse effects include multiple pregnancies, an increase in the rate of multiple births, ovarian hyperstimulation and unsubstantiated claims of ovarian cancer.

Keywords: *anovulatory infertility, clomiphene, GnRH agonist, IVF, ovulation induction, polycystic ovary syndrome*

Introduction

Clomiphene citrate (clomiphene), is the most commonly used drug for ovulation induction, since it is inexpensive, highly effective and user-friendly. It was first synthesized in 1956 and has been commercially available since 1961. It constitutes the treatment of choice in hyperandrogenic chronic anovulation and other forms of anovulation with an adequate oestrogen reserve. It is also used in the functional assessment of the gonadal axis, may be combined with gonadotrophins in the therapy of selected cases of controlled ovarian hyperstimulation, and can be used with or without gonadotrophins in assisted reproductive techniques.

Structure and action of clomiphene

Chemical structure and pharmacokinetics

From the chemical standpoint, clomiphene is a tri-phenylene derivative with structural similarities to diethylboestrol. Clomiphene has two isomeric forms, cis and trans, which in the current nomenclature correspond to zuclomiphene and enclomiphene respectively (**Figure 1**). The action of zuclomiphene is mainly anti-oestrogenic, whereas enclomiphene has oestrogenic effects. The commercial preparation is a racemic mixture that contains 40% zuclomiphene and 60% enclomiphene.

Clomiphene binds to the oestrogen receptor, but contrary to what happens with oestradiol, its binding is more prolonged (Adashi *et al.*, 1980). This results in a decrease of the oestrogenic effect; nevertheless, clomiphene has a certain agonistic effect, especially in hypo-oestrogenic states.

Following oral intake of clomiphene, absorption is fast, although the drug has a long half-life. Up to 50% of a dose of clomiphene can be found 5 days after administration and the drug and its metabolites are detected in faeces as long as 6 weeks after ingestion (**Figure 1**).

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Mechanism of action

Two or 3 days after starting clomiphene administration in the follicular phase of the ovarian cycle, the pulse frequency of LH increases, suggesting that the main action of the drug is to increase pulsatile secretion of gonadotrophin-releasing hormone (GnRH) by the hypothalamus (Sir *et al.*, 1989).

Clomiphene could also have a direct oestrogenic effect on the gonadotrophs, enhancing sensitivity to GnRH. As a consequence of the effects mentioned above, there is an increase in plasma concentration of gonadotrophins and in the number of follicles recruited. There is a resulting increase in plasma concentrations of oestradiol before ovulation and of progesterone during the luteal phase. Between 30 and 35% of patients who ovulate with clomiphene do so with a follicular rupture diameter that is larger than expected, as compared with spontaneous cycles. Moreover, clomiphene has a direct oestrogenic effect on the ovary, resembling that described for the pituitary gland. It sensitizes granulosa cells in the follicle to the action of gonadotrophins and up-regulates aromatase activity. Its effects on the cervix and endometrium are mainly anti-oestrogenic (**Figure 2**). The different modulating effects (agonistic or antagonistic) shown by clomiphene on the effectors of the genital tract might be due to the different populations of α- or β-oestrogen receptors in those tissues (Goldstein *et al.*, 2000) (**Figure 2**).

Indications and uses

Indications

The main indication for clomiphene is to induce ovulation in patients with anovulation due to hypothalamic and pituitary dysfunction. Oestrogen concentrations in these patients are >50 pg/ml, especially in those with polycystic ovary syndrome (PCOS), oligomenorrhoea and secondary amenorrhoea of various aetiologies with low or normal concentrations of gonadotrophins and prolactin. Clomiphene has also been proposed for the treatment of short luteal phase, and to assess and diagnose the function of the hypothalamus–pituitary–ovarian axis.

Evaluation of ovarian follicle reserve

Numerous researchers have proposed various functional tests aimed at predicting a woman's fertility potential. The most interesting of these tests are the baseline measurement of FSH on day 3 of the menstrual cycle, baseline FSH plus oestradiol on day 3 of the menstrual cycle, the clomiphene citrate test, GnRH-agonist stimulation test and the measurement of inhibin B. These tests are intended to evaluate the number of ovarian follicles and the quality of the oocyte, and the best characterized and most sensitive of them so far is the clomiphene citrate test. This was first described in 1987, and was used to evaluate ovarian reserve in women aged ≥35 years. This functional test involves measuring serum FSH on day 3 of the menstrual cycle, followed by a new assessment on day 10 after the administration of clomiphene at a dose of 100 mg/day, from day 5 to day 9 (**Figure 3**). Conception rates are low when the sum of FSH values is >26 IU/l (Loumaye *et al.*, 1990). The sensitivity of the test is 26%, which is much higher than the isolated measurement of FSH. The specificity for both tests reaches 96%. The predictive success of the clomiphene citrate test depends directly on the population studied, since its highest positive predictive value of 98% is observed in the evaluation of patients scheduled for IVF. Its value declines dramatically to 87% when used in a population of women aged ≤33 years (Barnhart and Osheroff, 1999).

In summary, there is evidence supporting the predictive value of this test in a population of women with high risk of failure in assisted reproductive medicine. This high-risk group includes women >35 years of age, who respond poorly to ovarian stimulation, and women who have failed with previous IVF cycles (**Figure 3**).

Contraindications

The administration of clomiphene is contraindicated during pregnancy, since it can cause congenital abnormalities. It is also contraindicated in chronic liver disease, since it is mainly cleared through the liver, and in the presence of functional ovarian cysts, which could grow larger. It is also contraindicated when there is a history of visual disorders (blurred vision and scotomas) during or after previous therapy with clomiphene. Its use in women who are infertile through non-ovulatory factors remains controversial. Furthermore, it is not recommended in ovulatory patients because its administration may paradoxically cause disorders leading to a decrease in fertility. Such disorders include a reduction in the amount and quality of cervical mucus, abnormal development

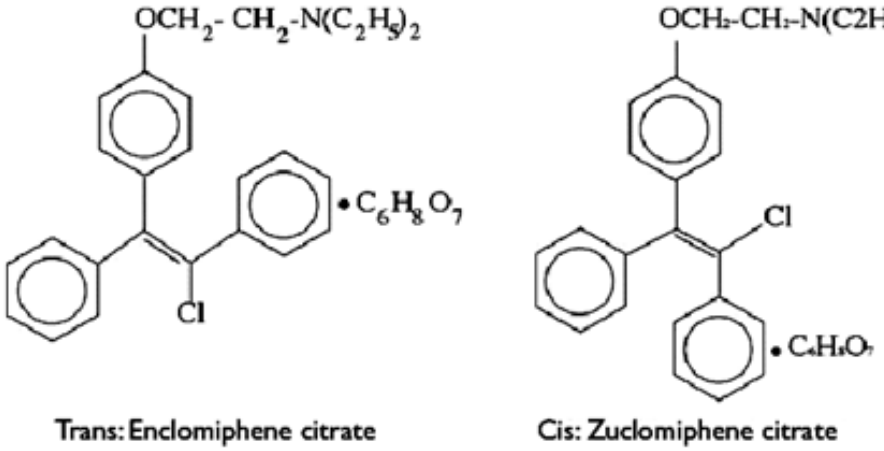

Figure 1. Clomiphene citrate.

or dysfunction of the endometrium, reduction in adhesion proteins (integrins, mainly subunit β_3), decline in glandular density and an increase of vacuolated cells in the endometrium of ovulatory women (Palomino *et al.*, 1998; Sereepapong *et al.*, 2000).

These observations suggest that the use of clomiphene in ovulatory patients would not be beneficial, since it would reduce their fecundity index.

Forms of use and outcomes

Clomiphene is preferably administered in 50 mg doses for 5 days from days 3–5 of a spontaneous or induced menstrual flow (**Figure 4a**). Starting with higher doses fails to provide any advantages for the following two reasons. First, the administration of clomiphene at doses of 50 or 100 mg a day results in similar pregnancy rates. Second, the incidence of side-effects is dose-dependent, with adverse reactions first being observed with initial doses of 50 mg/day. Starting therapy with higher doses (100 mg/day) could result in more severe reactions. In hyper-reacting women (those who have developed ovarian cysts or hyperstimulation in previous cycles), lower doses (12.5–25 mg) may prevent hyperstimulation. In hyporeacting patients, such as obese women, induction is started with a dose of 100 mg a day. The clomiphene dose may be increased progressively up to a maximum of 200 mg/day for 5 days. Fewer than 50% of hyporeacting patients will ovulate at that dose. Approximately 50% of normal women ovulate with a dose of 50 mg, and an additional 20% will ovulate with 100 mg/day, the overall ovulation rate ranging from 70 to 85%.

The method that proposes starting patients on clomiphene on day 5 of the menstrual cycle is empirical, yet, it can be reasonably justified on the basis of the following observations on ovarian physiology. Clomiphene induces an increase in gonadotrophins on days 5–9 of the cycle, i.e. when the follicle is selected. Follicular maturation beginning at that time is characterized by the development of a preovulatory follicle, the elevation of circulating oestrogens, a preovulatory surge of LH and discharge of LH leading to ovulation with high post-ovulatory levels of progesterone (**Figure 4b**).

Cycles induced with clomiphene in patients presenting with PCOS differ from those of ovulatory women, since the former show higher oestrogen and progesterone concentrations (Kettel *et al.*, 1993). The early administration of clomiphene could theoretically produce multiple follicular maturation, generating a higher incidence of multiple pregnancies. Nevertheless, no differences have been observed in ovulation, pregnancy, or miscarriage rates with clomiphene administered from days 2, 3, 4 or 5 of the cycle in the usual protocols for ovulation induction. Following the final dose of clomiphene on day 9 of the cycle, the LH surge may occur at any time between 5 and 12 days after the last dose. It occurs most frequently at 6–7 days after the last dose, especially with clomiphene from days 5–9 of the cycle (**Figure 4a**). This observation is important in the planning of coitus. Couples are told they must have sexual intercourse for a week, starting on day 5 after the last clomiphene tablet.

Only 40–50% of ovulatory patients become pregnant. The discrepancy observed between the ovulation index and the fecundity index could be due to many factors. These include the coexistence of a male factor, the existence of other causes of infertility or an anti-oestrogenic effect of clomiphene on some effectors of the reproductive tract. This effect is known to arise in cervical mucus and its interaction with spermatozoa, in tubal transport of ova, and in the function and synchronization of the endometrium (Palomino *et al.*, 1998).

Ovulatory response and pregnancy predictors

Predictors of the ovulatory response to clomiphene are body mass index, the free androgen index, ovarian volume (Imani *et al.*, 1998), and low concentrations of IGF-BP-I. On the other hand, pregnancy predictors with clomiphene are age and the severity of the cycle disorders, i.e. better responses occur in women of a younger age and with maintained oligomenorrhoea or amenorrhoea, suggesting that FSH threshold (amount of FSH required to stimulate the follicular maturation and the ensuing ovulation) and oocyte quality are specifically regulated (Imani *et al.*, 1999) (**Figure 4**).

Clinical treatments using clomiphene

Use of clomiphene citrate in IVF

Clomiphene citrate (clomiphene) was one of the first drugs used in the initial protocols for ovulation induction for IVF and embryo transfer, either alone or in combination with urinary gonadotrophins (Quigley *et al.*, 1984). However, in the last 10 years, clomiphene has lost popularity, having been replaced by the combination of GnRH agonists (GnRHa) and gonadotrophins in almost all centres in which IVF is performed, including our own.

In spite of the 20 years that has elapsed since the birth of a child through IVF techniques, there continues to be no consensus among researchers regarding the best scheme for ovarian stimulation capable of providing optimal ovarian responses together with successful outcomes. One of the critical factors impinging on the routine use of IVF is the high cost of drugs, especially those schemes using recombinant FSH and GnRHa. This factor, together with the high costs of the new techniques that are becoming increasingly frequent,

Table 1. Results of IVF with clomiphene citrate (clomiphene) or gonadotrophin-releasing hormone agonist (GnRHa), associated with human menopausal gonadotrophin (HMG).

	HMG+ clomiphene	*HMG*	*GnRHa + HMG*
Cycles started (*n*)	1063	395	689
Cycles cancelled (%)	23.5	35.2	10.9
Oocytes per aspirate (*n*)	6.4	7.4	11.1
Embryos per aspirate (*n*)	3.9	4.1	5.9
No. pregnancies per aspirate (%)	58 (21.8)	28 (16.4)	30 (22.5)
No. of births per aspirate (%)	44 (17.3)	23 (12.9)	21 (18.1)

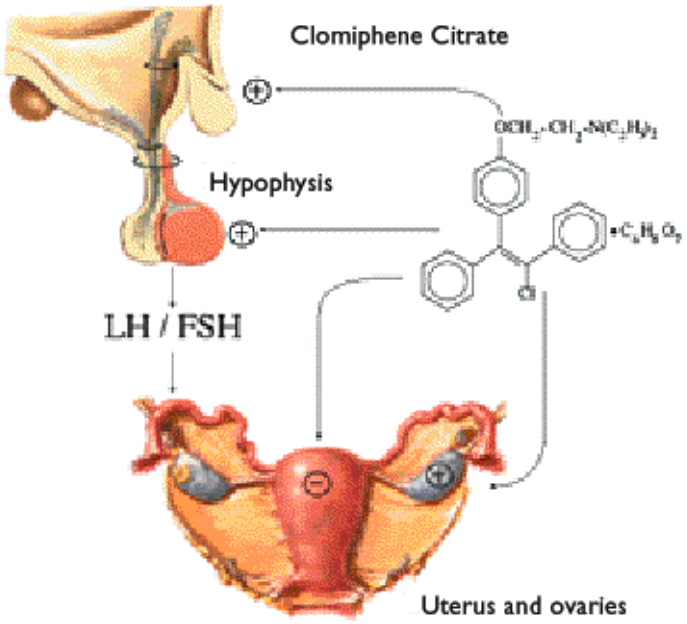

Figure 2. Sites of action of clomiphene citrate.

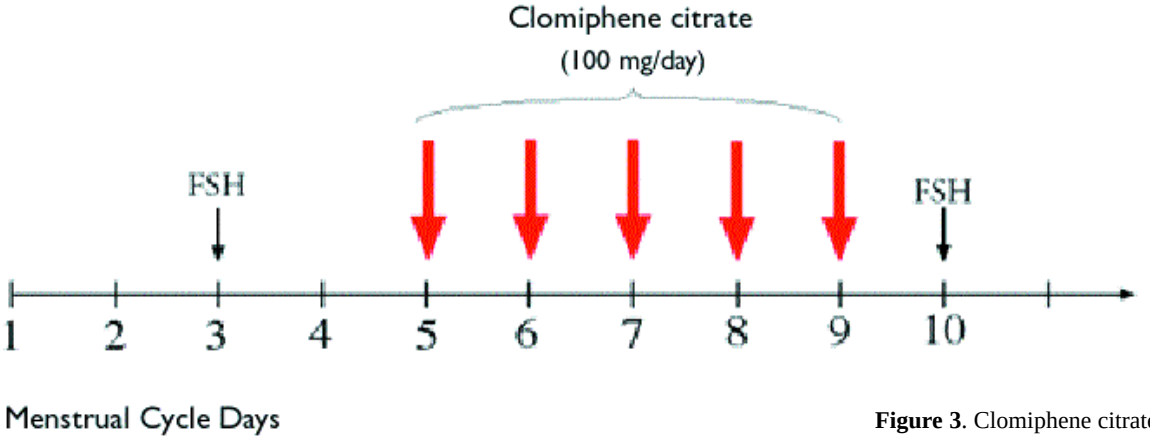

Figure 3. Clomiphene citrate test.

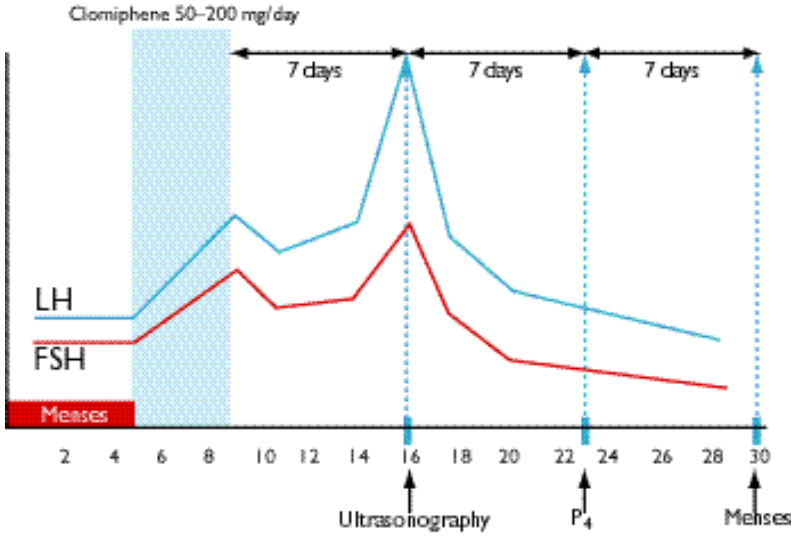

Figure 5. Monitoring of clomiphene citrate.

a.

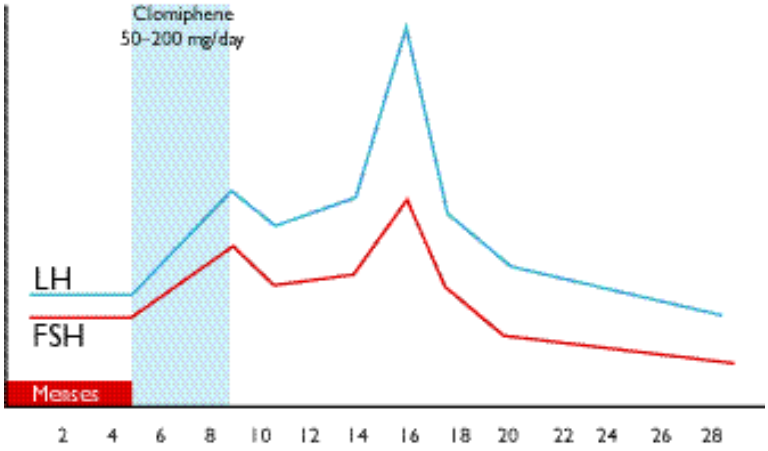

Figure 4a. Form of use of clomiphene.

b.

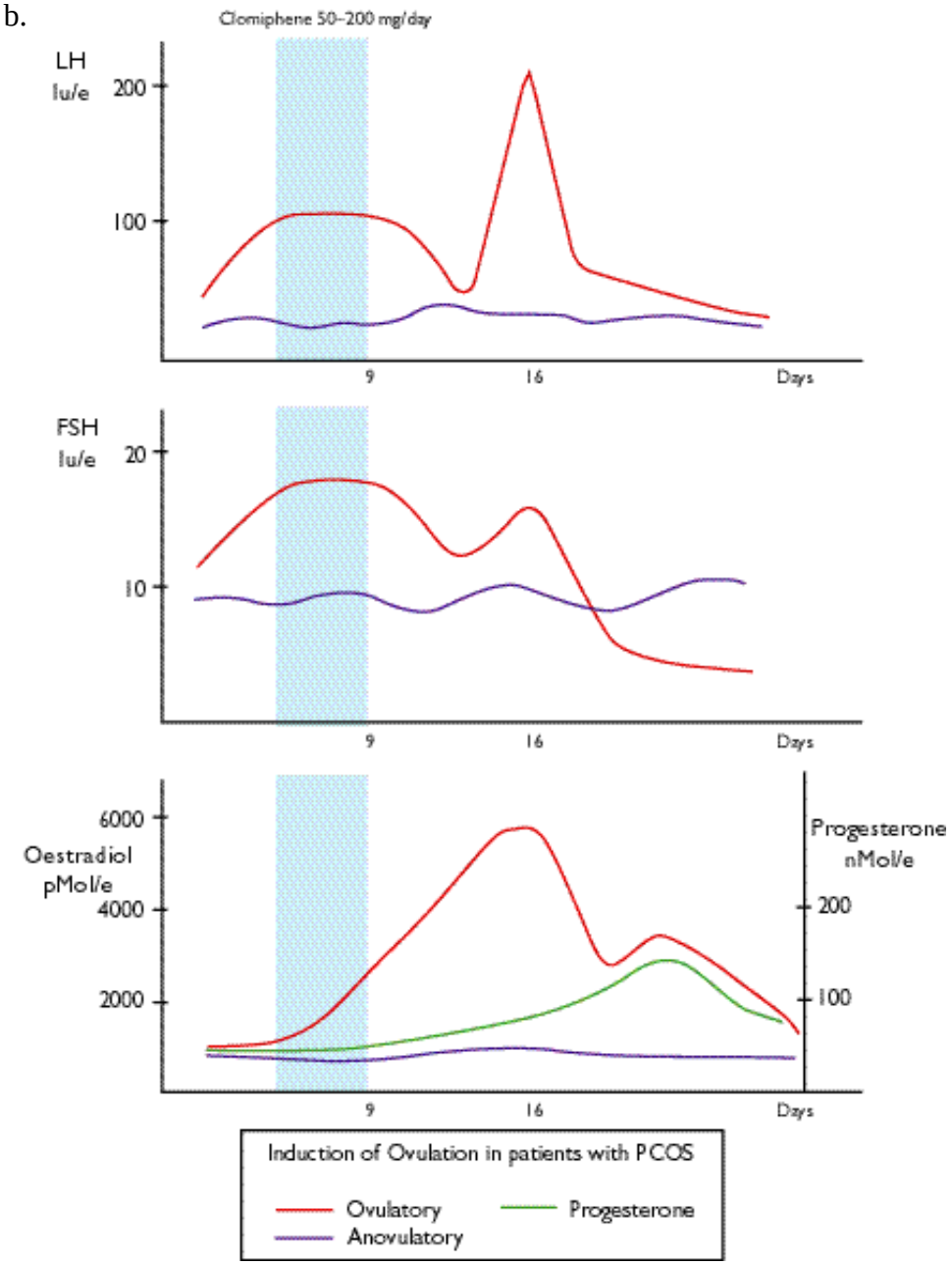

Figure 4b. Ovulation induction with clomiphene citrate in patients with polycystic ovary syndrome.

such as intracytoplasmic sperm injection, makes it very expensive for many infertile couples needing IVF. In view of this situation, it is necessary to reassess ovulation induction schemes, to reduce costs while avoiding any negative impact, to the greatest possible extent, on success rates. Clomiphene is cheaper to use and it is safe, but its usage in the new IVF schemes requires complete evaluation. Some investigators believe that the use of clomiphene in IVF schemes is not justified, in view of its adverse effects on the endometrium, which could influence the outcomes of treatment. At present, clomiphene is used in some IVF programmes in combination with human menopausal gonadotrophin (HMG), prior to the use of GnRHa.

The theoretical advantages of combining HMG and clomiphene are the strength of the luteal phase supported by clomiphene, a lesser need for HMG and higher concentrations of progesterone in the luteal phase, which often renders luteal support unnecessary. **Table 1** shows the results obtained from IVF cycles between 1983 and 1990, at the New Orleans Fertility Institute, comparing HMG/clomiphene, HMG alone and GnRHa/HMG (Dickey *et al.*, 1998). Clinical pregnancies and birth rates were higher with HMG/clomiphene than with HMG alone, and the results were similar to those obtained with GnRHa/HMG. The main advantage seen with GnRHa/HMG was that fewer cycles were cancelled, which is probably related to the beneficial effect of GnRH agonists, in reducing premature luteinization caused by an early LH surge. As a result, more oocytes are obtained, and that in turn makes it possible to generate more cryopreserved embryos with this therapy.

As clomiphene is only used at doses between 50 and 150 mg/day, more than one follicle may progress to the preovulatory state, and an average number of two or three oocytes are aspirated from each woman (Marrs *et al.*, 1984). The combination of clomiphene and gonadotrophins yields a better ovarian response, with more oocytes and with better morphology, as compared with clomiphene alone (Quigley *et al.*, 1984). This observation could be explained by several mechanisms. When clomiphene is administered for 5 days during the early follicular phase (days 3–7 of the cycle), it not only stimulates the secretion of FSH, but also LH, which may result in relatively high concentrations of LH during the follicular phase (Messinis and Templeton, 1986). This could cause early luteinization of granulosa cells, and so alter the outcomes of pregnancy (Stanger and Yovich, 1985).

A new strategy for the use of clomiphene in combination with GnRH antagonist for ovulation induction has been proposed recently (Olivennes *et al.*, 2000; Reissmann *et al.*, 2000). The physiological rationales supporting this association are mainly based on the ability of GnRHa to reduce high concentrations of LH generated by clomiphene. Antagonists, contrary to the action of GnRHa, competitively block GnRH receptors at the pituitary level, preserving the post-receptor mechanism of the latter intact. Nevertheless, it is necessary to conduct further studies, including an adequate number of patients, in order to validate the bases of these new schemes.

A study using the down-regulation of the hypothalamus–pituitary–ovarian axis with oral contraceptives in patients with clomiphene-resistant PCOS showed that the LH surge can be prevented or reduced in the cycle following the discontinuation of oral contraceptives (Branigan and Estes, 1999). This observation enabled researchers to design the 'minimal stimulation study', using clomiphene at 100 mg/day for 8 days starting on day 3 of the cycle in a group of 36 candidates for IVF. These patients had undergone down-regulation with oral contraceptives 2 months prior to ovulation induction. No LH surge was observed in this group of patients. Their average number of mature oocytes recovered was 3.2, with a 90% fertilization rate and an average number of 2.5 embryos transferred, and a 32.8% pregnancy rate per aspiration. These outcomes were similar to those obtained in IVF stimulated cycles, and this schedule has the advantages of being cheap and simple, while presenting low risks for the patients (Branigan and Estes, 2000).

Association with other drugs

There is a group of patients, estimated as between 10% and 20%, in whom doses of 150 mg clomiphene citrate per day for 5 days do not result in ovulation after three or more attempts, and/or who fail to conceive after 4–6 months of this induction protocol. This group of patients is said to be clomiphene resistant. When this phenomenon occurs, clomiphene may be administered with other drugs, after evaluating the individual patient characteristics (body mass index, hirsutism, acanthosis nigricans, galactorrhoea), together with a baseline functional assessment including ultrasonography, total testosterone, sex hormone binding globulin, free androgen index, DHEA-S, prolactin and progesterone in the luteal phase. This is followed by the evaluation of the cervical mucus, endometrial thickness and follicular size during the follicular follow-up.

Depending on the aetiology of the condition, several drugs can be administered with clomiphene citrate, including the following. The first is human chorionic gonadotrophin (HCG), which is used when anovulation persists in spite of good follicular development and adequate oestrogen production. It is administered at doses of 5000–10,000 IU, when the dominating follicle reaches a diameter of ≥18 mm and the concentration of oestrogens exceeds 200 pg/ml. HCG can also be added in cases of luteal failure.

Corticosteroids are especially indicated in hyperandrogenic chronic anovulation, preferably of adrenal origin, using 0.5–1.0 mg dexamethasone q.h.s, which leads to a 70–90% increase in the ovulation rate. Dopaminergic agonists are indicated in chronic anovulation associated with hyperprolactinaemia. Pituitary gonadotrophins are used when ovulation does not occur, but moderate follicular development is achieved.

It is possible to administer clomiphene and HMG sequentially, which permits substantial reductions in the dose of HMG required to produce ovulation. Progesterone is indicated when the administration of clomiphene produces a deficient luteal phase. It is used as pure progesterone in the luteal phase (72 h after ovulation) at doses of 12.5 mg i.m. or 25 mg b.i.d. as vaginal suppositories, or orally in the form of micronized progesterone. Ethynyl oestradiol is used at low doses to improve the quality of the cervical mucus and the endometrial thickness, with a consequent increase in pregnancy rates (Gerli *et al.*, 1999).

Hyperinsulinaemia must be corrected. Anovulatory women who present with polycystic ovary syndrome and hyperinsulinaemia are more resistant to clomiphene therapy. The best treatment for such patients, who are usually obese, is weight reduction, since both hyperinsulinaemia and hyperandrogenism diminish when there is weight loss. Weight reduction should represent at least 5% of the initial weight. This metabolic improvement is linked to an increase of the ovulation and pregnancy rates. It has been shown that when the body mass index drops under 27, both insulin and free testosterone concentrations fall, so improving fecundity.

It is reasonable to think that patients with PCOS and obesity are likely to be insulin resistant, so we recommend checking the baseline glucose concentrations and the concentrations after challenge with 75 g glucose, together with assays of insulin plasma concentrations in baseline and post-challenge conditions. If these plasma concentrations are >15 mIU/ml at baseline or >60 mIU/ml post-challenge, the patient is considered to be insulin-resistant and is started on metformin. Metformin improves insulin sensitivity, but its primary effect is a significant reduction of liver gluconeogenesis, hence decreasing the production of glucose by the liver. Metformin therapy reduces hyperinsulinaemia, both the baseline concentrations and the LH-stimulated concentrations, and also produces a fall in free testosterone concentrations in women with PCOS who are overweight. A recent study (Nestler *et al.*, 1998) showed that the ovulation response to clomiphene is enhanced by up to 90% in these women with the addition of metformin, as compared with 8% with placebo and clomiphene.

The use of oral contraceptives has been described in the cycle prior to IVF with minimal stimulation. Oral contraceptives are administered two cycles before the use of clomiphene, and decrease the LH surge, providing similar outcomes in terms of pregnancy rates when compared with the cycles in which GnRHa and gonadotrophins are used. The cost of treatment can be significantly reduced by the use of this protocol (Branigan and Estes. 2000).

Functional evaluation of clomiphene administration

The objective is to assess follicular development, establishing whether or not ovulation occurred and determining the competence of the luteal phase. This can be achieved quite simply through vaginal ultrasound and the measurement of progesterone in the luteal phase. Moreover, ultrasound allows detection of a multifollicular response, to establish the occurrence of cystic follicles, the presence of non-ruptured luteinized follicles and the thickness of the endometrium.

One possible practical scheme is the so-called 7–7–7 scheme, which consists of the following steps: 7 days after the last clomiphene tablet, follicular development is evaluated with a cervical mucus test and vaginal ultrasound, including oestrogen concentrations in selected cases. Mid-luteal progesterone concentrations are determined 7 days later. Finally, 7 days afterwards, the occurrence or absence of menses is recorded, in attempt to detect a short luteal phase or an early pregnancy (**Figure 5**).

Adverse effects

Adverse effects include multiple pregnancies in up to 6–8% of cases; most of these are twins, but triplets have been reported in 1%, quadruplets in 0.3% and quintuplets in 0.1% of clomiphene-induced pregnancies. Furthermore, there is a 23% rate of missed abortions, probably associated with early resumption of oocytic meiosis due to hypersecretion of LH. Ovarian hyperstimulation syndrome is observed in 13% of cases treated with clomiphene, but it is usually mild. Other minor effects include visual disorders, flushes, nausea and breast discomfort. Ovarian cancer is an adverse effect associated with the use of drugs to induce ovulation (Rossing *et al.*, 1994), although recent papers report no association between ovarian or breast cancer and the use of clomiphene (Potashnick *et al.*, 1999). On the basis of these initial studies (Rossing *et al.*, 1994), the United Kingdom Committee for Medical Safety recommended the use of clomiphene for 6 consecutive cycles only.

Conclusions

Clomiphene citrate continues to be the most commonly used drug to induce ovulation in the treatment of normogonadotrophic anovulatory infertility with normal oestrogen concentrations. Its popularity is due to its low cost, scarce adverse reactions and easy monitoring. However, its ability to stimulate follicular stimulation is limited and it can induce high LH concentrations. Together with other adverse aspects in reproductive function, this limits the use of clomiphene in the development of an optimum scheme in IVF programmes. Consequently, clomiphene citrate is the recommended drug for the treatment of infertility associated with PCOS, where the main objective is to obtain development of a single follicle and a reduction in multiple pregnancies.

References

Adashi EY, Hsueh AJW, Yen SSC 1980 Alterations induced by clomiphene in the concentrations of estrogen receptors in the uterus, pituitary gland and hypothalamus of female rats. *Journal of Endocrinology* **87**, 383–392.

Barnhart K, Osheroff J 1999 We are overinterpreting the predictive value of serum follicle-stimulating hormone levels. *Fertility and Sterility* **72**, 8–9.

Branigan EF, Estes MA 1999 Treatment of chronic anovulation resistant to clomiphene citrate (CC) by using oral contraceptive ovarian suppression followed by repeat CC treatment. *Fertility and Sterility* **71**, 544–546.

Branigan EF, Estes MA 2000 Minimal stimulation IVF using clomiphene citrate and oral contraceptive pill pretreatment for LH suppression. *Fertility and Sterility* **73**, 587–590.

Dickey RP, Taylor SN, Rye PH, Lu PY 1998 A role for clomiphene in the 21st century? *Human Reproduction* **13**, 2361–2362.

Gerli S, Gholami H, Manna A *et al.* 1999 Use of ethinyl estradiol to reverse the antiestrogenic effects of clomiphene citrate in patients undergoing intrauterine insemination: a comparative, randomized study. *Fertility and Sterility* **73**, 85–89.

Goldstein SR, Siddhanti S, Ciaccia AV, Plouffle L Jr 2000 A pharmacological review of selective oestrogen receptor modulators. *Human Reproduction Update* **6**, 212–224.

Imani B, Eijkemans MJC, Te Velde ER *et al.* 1998 Predictors of patients remaining anovulatory during clomiphene citrate induction in normogonadotropic oligomenorrheic infertility. *Journal of Clinical Endocrinology and Metabolism* **83**, 2361–2365.

Imani B, Eijkemans MJ, Te Velde ER *et al.* 1999 Predictors of chances to conceive in ovulatory patients during clomiphene citrate induction of ovulation in normogonadotropic oligomenorrheic infertility. *Journal of Clinical Endocrinology and Metabolism* **84**, 1617–1622.

Kettel LM, Berga SL, Roseff SJ *et al.* 1993 Hypothalamic-pituitary-ovarian response to clomiphene citrate in women with polycystic ovary syndrome. *Fertility and Sterility* **59**, 532–538.

Loumaye E, Billion JM, Mine JM *et al.* 1990 Prediction of individual response to controlled ovarian hyperstimulation by means of a clomiphene citrate challenge test. *Fertility and Sterility* **53**, 295–301.

Marrs RP, Vargyas JM, Shangold GM *et al.* 1984 The effects of time of initiation of clomiphene citrate on multiple follicle development for human *in vitro* fertilization and embryo replacement procedures. *Fertility and Sterility* **41**, 683–685.

Messinis IE, Templeton A 1986 Urinary oestrogen levels and follicle ultrasound measurements in clomiphene-induced cycles with an endogenous luteinizing hormone surge. *British Journal of Obstetrics and Gynaecology* **93**, 43–49.

Nestler J, Jakubowitz D, Evans W, Pascuali R 1998 Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. *New England Journal of Medicine* **338**, 1876–1880.

Olivennes F, Ayoubi JM, Fanchin R *et al.* 2000 GnRH antagonist in single-dose applications. *Human Reproduction Update* **6**, 313–317.

Palomino AW, González R, Boric R *et al.* 1998 Expresión de integrinas de epitelio endometrial en ciclos estimulados con citrato de clomifeno. *Libro IX Reunión de Resúmenes anual de la Sociedad Chilena de Fertilidad*, p. 54.

Potashnick G, Lerner-Geva L, Genkin L *et al.* 1999 Fertility drugs and the risk of breast and ovarian cancers: results of a longterm follow-up study. *Fertility and Sterility* **71**, 853–859.

Quigley MM, Schmidt CL, Beauchamp PJ *et al.* 1984 Enhanced follicular recruitment in an *in vitro* fertilization program: clomiphene alone versus a clomiphene/human menopausal gonadotropins combination. *Fertility and Sterility* **42**, 25–33.

Reissmann T, Schally AV, Bouchard P *et al.* 2000 The LHRH antagonist cetrorelix: a review. *Human Reproduction Update* **6**, 322–331.

Rossing MA, Daling JR, Weiss NS *et al.* 1994 Ovarian tumors in a cohort of infertile women. *New England Journal of Medicine* **331**, 771–776

Sereepapong W, Suwajanakorn S, Triratanachat S *et al.* 2000 Effects of clomiphene citrate on the endometrium of regularly cycling women. *Fertility and Sterility* **73**, 287–291.

Sir T, Alba F, Devoto L, Rossmanith W 1989 Clomiphene citrate and LH pulsatility in PCO syndrome. *Hormone and Metabolic Research* **21**, 583.

Stanger JD, Yovich JL 1985 Reduced *in vitro* fertilization of human oocytes from patients with raised basal luteinizing hormone levels during the follicular phase. *British Journal of Obstetrics and Gynaecology* **92**, 385–393.