Current and future status of ovulation induction in polycystic ovary syndrome

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Great progress has been achieved during the last 20 years in the field of ovulation induction in patients with polycystic ovary syndrome (PCOS). Clomiphene citrate remains the first line of treatment for all anovulatory women with PCOS, since in properly selected patients the cumulative pregnancy rate approaches that in normal women. Human urinary gonadotrophins have been used extensively for ovulation induction, but the development of low-dose regimens has opened a new era in the management of anovulation related to PCOS. This article discusses the main advantages and disadvantages of the principal methods and regimens currently used for ovulation induction in patients with PCOS including clomiphene citrate, gonadotrophins, pulsatile gonadotrophin-releasing hormone (GnRH) and GnRH agonists. It also discusses new drugs discovered recently, particularly recombinant gonadotrophins and GnRH antagonists, and provides some thoughts regarding their use in future protocols. Finally, based on the discovery of new ovarian substances which specifically control luteinizing hormone (LH) secretion, this article develops assumptions on possible implications of these substances in the pathophysiology of PCOS and their potential use in the management of the syndrome.

Key words: clomiphene citrate/gonadotrophins/ovulation induction/polycystic ovary syndrome

Introduction

Anovulation or oligo-ovulation is one of the main characteristics of the polycystic ovary syndrome (PCOS). When patients with PCOS complain of infertility, ovulation induction is the appropriate treatment. Various drugs and treatment regimens have been used for ovulation induction in PCOS, but none of them has become unique in achieving the goals. The reason for the existence of so many treatment regimens is related to the multifactorial pathophysiology of PCOS and consequently to the variability in clinical manifestations and the hormonal milieu. In ~40% of patients with PCOS, luteinizing hormone (LH) is hypersecreted and this may adversely affect the outcome of treatment by increasing the abortion rate (Homburg et al., 1988; Balen and Rose, 1994). Also, in a variety of cases, the ovaries are resistant to treatment particularly to clomiphene even after the administration of relatively high doses. Other problems, such as obesity and resistance to insulin may be relevant, whereas the role of inhibin in the negative feedback effect of the ovaries on follicle stimulating hormone (FSH) secretion remains obscure. It is evident, therefore, that an approach to the treatment of PCOS with the wide range of clinical and endocrinological characteristics, the unclear pathophysiology and the linkage with genetic pathogenesis (Carey et al., 1993), is difficult for several reasons. Certainly, during the last 10–15 years with the availability of new drugs, there has been progress in the management of anovulation related to PCOS which has resulted in a higher success rate and fewer complications, such as miscarriages and the ovarian hyperstimulation syndrome (OHSS). Guidelines on anovulatory infertility including
PCOS in terms of diagnosis and treatment have been recently published (ESHRE Capri Workshop, 1996).

This article aims to describe the conventional methods used for the treatment of anovulation related to PCOS and to provide some strategies for the future, based on the new technology and the development of recombinant gonadotrophins and gonadotrophin-releasing hormone (GnRH) antagonists.

Clomiphene citrate

Clomiphene citrate is used very extensively for ovulation induction in PCOS. This is a triphenylethylene derivative with oestrogenic properties, but mainly acting as an antioestrogenic compound, which is thought to displace endogenous oestrogen from the oestrogenic receptors at the hypothalamic–pituitary system and which blocks the ovarian negative feedback effect on gonadotrophin secretion (for review, see Adashi, 1984). As a consequence, an increase in the secretion of FSH and particularly LH occurs which then stimulates follicle development. In normal women, clomiphene citrate increases the frequency rather than the amplitude of gonadotrophin pulses (Kerin et al., 1985), while in patients with PCOS an increase in the amplitude has been reported (Kettel et al., 1993). When clomiphene citrate is given to normal women for 5 days, FSH concentrations increase during the period of administration and decline after the end of the treatment (Shaw, 1976). 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 (Roseff et al., 1989; Messinis et al., 1993). Clomiphene is specific not only for the treatment of anovulation related to PCOS, but also for any other disorder of ovulation resulting from the absence of the intercycle type of FSH increase and which clinically presents as oligomenorrhea or amenorrhea responsive to progesterone administration with withdrawal bleeding [World Health Organization (WHO) group II]. In these conditions, folliculogenesis is not entirely arrested, as in cases of hypothalamic amenorrhea, but a degree of oestrogen production continues which primes the endometrium to the action of progesterone. When patients with PCOS are treated with clomiphene citrate, a distinction from other PCO-like conditions must be attempted. Most studies published so far present data of ovulation induction in patients belonging to the WHO group II, thus making an assessment of the effectiveness of clomiphene citrate in PCOS difficult. Even when the term PCOS is used, however, there is no consensus regarding the diagnostic criteria, which have been clarified only recently (Adams et al., 1986). According to these, the basis for the diagnosis of PCOS is the ultrasonic appearance of the ovaries in women with oligomenorrhoea or amenorrhoea and hirsutism in addition to at least one biochemical abnormality, i.e. elevated LH, LH/FSH ratio or increased testosterone (Adams et al., 1986; Franks, 1989; 1995). Others, however, are using only the clinical characteristics and biochemical criteria, without taking into account the ultrasound findings (Yen, 1980; Kettel et al., 1993).

Usually, clomiphene citrate is given to anovulatory women for only 5 days. This period is adequate to open the FSH window and to induce the appropriate hormonal changes for follicle recruitment and selection (Shaw, 1976), although continuous administration of clomiphene citrate for longer than 5 days has been reported (O’Herlihy et al., 1981; Lobo et al., 1982a; Garcia-Flores and Vazquez-Mendez, 1984; Fluker et al., 1996). Such an approach, i.e. prolonged treatment with clomiphene citrate, however, may not be very appropriate based on the results of a study which have shown that when clomiphene citrate is given to normal women for 15 instead of 5 days, gonadotrophin changes show a peculiar pattern (Messinis and Templeton, 1988). In particular, basal LH concentrations show an uninterrupted increase as long as clomiphene citrate is administered to the women and decline only after the end of the treatment, while basal FSH concentrations after a significant increase during the first 5 days of treatment decline thereafter, despite the continuation of clomiphene citrate administration. In that respect, tonic concentrations of LH which are high in several patients with PCOS, become even higher during the prolonged administration of clomiphene citrate and may have a detrimental effect on the outcome of treatment (Homburg et al., 1988; Balen and Rose, 1994). On the other hand, FSH concentrations remain high for a certain period of time regardless of the administration of clomiphene citrate for 5 or 15 days. A further reason why prolonged treatment with clomiphene citrate may not have any advantages over the 5 day treatment is that, with the exception of one study (Garcia-Flores and Vazquez-Mendez, 1984), all other studies in which clomiphene citrate was given to patients for more than 5 days showed that the pregnancy rate per patient did not exceed 27% (O’Herlihy et al., 1981; Lobo et al., 1982a; Fluker et al., 1996). Regarding the day of onset of clomiphene citrate administration, this can be any time between days 2 and 5 following spontaneous or progestagen-induced withdrawal bleeding. It seems that there is no difference between an onset on days 2 and 5 (Wu and Winkel, 1989); however, day 2 may have an advantage over day 5 in that ovulation occurs earlier and this may be closer to the
normal profile of FSH increase for follicle recruitment and selection. The daily dose of clomiphene citrate which is recommended for the first treatment attempt is 50 mg. This dose can be increased up to a total daily dose of 150 mg, although a total daily dose of 250 mg has been used (Gorlitsky et al., 1978).

The results during ovulation induction with clomiphene citrate vary considerably among different studies. Ovulation rates of 40–80% have been reported (Rust et al., 1974; Garcia et al., 1977; Shepard et al., 1979; Lobo et al., 1982b; Nunley et al., 1985). This variation may be related to the variable criteria used for the selection of patients. Women with PCOS who do not respond to clomiphene citrate by ovulation tend to be overweight and the main reason for their anovulation seems to be the lack of follicle maturation, while their pituitary shows an appropriate response with increasing FSH secretion (Polson et al., 1989a). It seems that during clomiphene citrate treatment the response of gonadotrophins, follicle development and growth and maturation of the endometrium tend to be consistent across consecutive cycles (Check et al., 1995; Opsahl et al., 1996). The pregnancy rate also varies widely among different studies and is always lower than the ovulation rate (Lunenfeld and Insler, 1978; Nunley et al., 1985). The lower pregnancy rate in relation to the ovulation rate is attributed to the anti-oestrogenic effects of clomiphene citrate which can be exerted not only on the hypothalamic–pituitary system but also on other sites, such as the cervical mucus, the endometrium and possibly the oocyte, although this is difficult to assess. At least one study has shown that in-vitro fertilization (IVF) of human oocytes obtained from normally cycling women is not affected by increasing the dose or the duration of pretreatment with clomiphene citrate (Messinis et al., 1986), while 15% of patients treated with the high dose of 150–200 mg per day can become pregnant (Gorlitsky et al., 1978). Other studies, however, have shown deleterious effects of clomiphene citrate on human oocytes with an increased rate of chromosomal abnormalities (Wramsby et al., 1987) and a decreased potential of in-vitro development of human embryos (Oelsner et al., 1987). These effects may explain the relatively increased rate of abortion reported during clomiphene citrate treatment, although rates approaching those of the normal population have been reported (Gysler et al., 1982; Hammond et al., 1983). Another possible reason for the low success rate during ovulation induction with clomiphene citrate in PCOS is the increase in basal LH secretion induced by clomiphene citrate which may also account for the increased abortion rate.

Despite these considerations, in properly selected patients with no other causes of infertility apart from anovulation, cumulative pregnancy rates as high as 60–75% after 6 months of treatment (Gysler et al., 1982; for review, see Kelly and Jewelewicz, 1990) and nearly 93–100% after an extension of the treatment to 10 or 12 months have been reported (Hull et al., 1979; Hammond et al., 1983). In a series of 77 properly selected patients, we found a cumulative pregnancy rate at 6 months of treatment of 69% and a take-home baby rate (success rate) of 62% (unpublished data). All these patients had anovulation related to PCOS and the diagnostic criteria used were oligomenorrhea or amenorrhea responsive to progesterone and slight hirsutism, elevated LH and/or androgens and the characteristic appearance of the ovaries by ultrasound. It is interesting that the rate of complications with clomiphene citrate, including multiple pregnancies and the OHSS, is generally very low.

It is evident from these studies that the pregnancy rate in patients with PCOS treated with clomiphene citrate is determined by two factors, i.e. the proper selection of patients and the duration of treatment. Exclusion of all other causes of infertility and extension of treatment up to a period of 6–10 months are recommended. However, the recently increasing concern about the ‘theoretical’ risk of ovarian cancer related to such treatment, raises questions regarding the safety of the prolonged administration of clomiphene citrate. A period of 6 months is considered as safe, although an increased risk of ovarian cancer was reported only when clomiphene citrate treatment exceeded the period of 12 months (for review, see Bistow and Karlan, 1996). According to one study, only 7.8% of women having one or more infertility factors in addition to anovulation became pregnant during the period following the first 6 months of treatment with clomiphene citrate (Gysler et al., 1982). Since on several occasions anovulatory women are treated with clomiphene citrate despite the presence of other causes of their infertility, it is reasonable to suggest that clomiphene citrate treatment should not exceed the period of 6 months unless the patients are properly selected. In general, when clomiphene citrate fails to induce ovulation or conception after treatment for a period of 6 months, the situation is considered as clomiphene resistance or failure and other treatment alternatives should be tried.

Combinations of clomiphene citrate with other drugs have also been used, more frequently in the past than nowadays. These include dexamethasone as in cases with high androgens (Lobo et al., 1982c) or even bromocriptine, human chorionic gonadotrophin (HCG) for follicle rupture.
strategy of the use of a low-dose regimen of urinary gonadotrophins. Due to the increased risk of ovarian response, the dose is increased every 5–6 days in a step-wise manner according to the FSH threshold theory (Brown, 1978). One has to consider that the ovaries of patients with PCOS are more prone to develop multiple follicles when treated with exogenous gonadotrophins than those of patients with hypothalamic amenorrhoea. Nevertheless, even with the conventional regimen, ovulation is induced in the majority of patients with PCOS and a reasonable pregnancy rate is achieved (Tsapoulis et al., 1978; Wang and Gemzell, 1980), but both multiple pregnancy rate and the risk of the OHSS are high (Wang and Gemzell, 1980). In comparison with the results of treatment of patients with hypothalamic amenorrhoea, the pregnancy rate and particularly the success rate in patients of WHO group II, treated with the classical regimens of HMG, are significantly lower (Messinis et al., 1988).

Over the last 10 years, the policy of treatment of PCOS patients with the use of human gonadotrophins has changed particularly in relation to the selection of the proper gonadotrophin regimen. Due to the increased risk of multiple follicular development when polycystic ovaries are stimulated with exogenous gonadotrophins, a new strategy of the use of a low-dose regimen of urinary gonadotrophins for ovulation induction in these cases was proposed. It was the time when both the criteria for diagnosis and selection of patients with PCOS were re-evaluated with the use of the ultrasound technology and the urinary preparations of gonadotrophins containing FSH with <1 IU LH became available. The first study, however, which used ‘pure’ FSH was that by Kamrava et al. (1982) in which two women with PCOS were treated with a chronic low-dose regimen of FSH (40 IU per day). During the treatment period, basal LH concentrations declined and both patients conceived. Another study comparing a preparation of ‘pure’ FSH with HMG in two different cycles of five patients with PCOS revealed no difference in the endocrine profile induced by the two preparations apart from higher concentrations of serum oestradiol in the HMG cycles (Venturoli et al., 1984). However, LH concentrations showed a greater fall in the FSH than in the HMG cycles. The beneficial effect of FSH on the biochemical imbalance in PCOS was subsequently shown by Seibel et al. (1984). A further study from the same group compared the chronic low-dose regimen of FSH given to 10 patients with a conventional HMG regimen given to 13 patients (Seibel et al., 1985). The conception rate was 20 and 30% respectively with no cases of OHSS in the FSH cycles, in comparison with four cases between FSH and LH seen in several patients with this syndrome. Subsequent studies, however, showed no advantage of ‘pure’ FSH over the use of HMG in terms of safety and effectiveness (Hoffman et al., 1985; Larsen et al., 1990; Sagle et al., 1991). More recently a ‘highly purified’ preparation of urinary FSH became available by manipulating further ‘pure’ FSH with the removal of the non-FSH proteins, so that it can be injected s.c. So far no published studies have presented results of the use of this preparation in ovulation induction in patients with PCOS. Our preliminary unpublished data have shown no difference between ‘highly purified’ and ‘pure’ FSH in this syndrome (Table I). What seems to be interesting, therefore, is the low dose and the slow administration rather than the type of the gonadotrophin (Buvat et al., 1989; Sagle et al., 1991). Although initially FSH was injected in a pulsatile way (Franks et al., 1985; 1988; Polson et al., 1987), it was proven later that the daily i.m. administration was equally effective (Polson et al., 1989b).
A starting dose of 75 IU FSH per day has been adopted in the low-dose regimens which in some cases is even lower, i.e. 37.5 IU. This initial dose is kept constant for ~7–14 days. In case of inadequate ovarian response, assessed by oestradiol measurement and ultrasound, the dose is increased by 37.5 IU per day every 7 days up to a maximum of 225 IU (three ampoules) per day. Once a follicle of 12–13 mm is seen in the ovary by ultrasound, the same dose of FSH is maintained up to the day of HCG administration. The main advantage of such protocols over the classical HMG protocols is that monofollicular development is achieved in ~75% of the cycles together with a reasonable pregnancy rate and a lower risk of multiple pregnancies and the OHSS (Meldrum, 1991). The use of low-dose gonadotrophins has opened a new era in the management of anovulatory infertility related to PCOS.

The development of a single pre-ovulatory follicle during treatment with a chronic low-dose gonadotrophin regimen is related to the low dose of FSH which creates in blood an appropriate threshold value. The ‘FSH threshold’ concept in individual patients with PCOS has been more thoroughly investigated recently (for review, see Schoemaker et al., 1993). According to this, the actual concentration of FSH in serum is more important than the dose. Another factor, which also plays a role in the selection of a single dominant follicle during treatment with low-dose FSH, is the negative feedback effect of the ovaries on the secretion of FSH from the pituitary, as has been demonstrated for endogenous LH (Kamrava et al., 1982). This negative effect on FSH secretion was investigated in a recent study during treatment of PCOS patients with low-dose FSH according to the above mentioned FSH threshold concept (van der Meer et al., 1996). A significant decline in serum FSH concentrations was seen during treatment with FSH; however, this was not seen in another group of patients also treated with FSH but in combination with a GnRH agonist. Monofollicular development was induced in that study in 80% of the FSH cycles but in only 22.2% of the combined cycles. From a theoretical point of view, the concept of the FSH threshold is interesting. However, in practical terms, any protocol using a low-dose FSH regimen, as has been described above, is expected to result in monofollicular development in the majority of the cycles and in a good pregnancy rate. It should be emphasized that it is the low dose of HMG or FSH which prevents the recruitment and selection of multiple follicles, although the endogenous feedback effect may also play an important role.

The protocols of low-dose gonadotrophins described above use the step-up principle of HMG or FSH administration. However, a step-down mode of administration has been also used successfully (for review, see Fauser et al., 1993). According to this, a dose of two (150 IU) to three ampoules (225 IU) HMG is used for 1–2 days which then is decreased once or twice during the treatment by half or one ampoule per day and this dose is then maintained constant up to the day of HCG administration. In these protocols, the starting dose of FSH or HMG can be higher in women with a high body mass index, since maximum concentrations of FSH in blood are thought to be dependent on body weight (Mannaerts et al., 1993), although this is in dispute (McClure et al., 1992). It seems, however, that obese women with PCOS require higher doses of HMG or FSH than lean women (Dale et al., 1993; Franks and Hamilton-Fairley, 1994). The principle of the step-down protocol is based on experience gained from ovulation induction with the conventional HMG protocols in patients with hypothalamic–pituitary amenorrhoea and represents the physiological sequence of FSH changes during the intercycle period of recruitment and selection of the dominant follicle as well as during the rest of the follicular phase (Roseff et al., 1989; van Santbrink et al., 1995a). A step-down regimen may initially result in the recruitment of more than one follicle; however, after the reduction of FSH dose, these follicles lose their ability for further growth and the majority of them become atretic (Lolis et al., 1995). Both the step-up and step-down protocols of FSH or HMG administration provide similar results in terms of monofollicular development and pregnancy rate (van Santbrink et al., 1995b). A combination of the two protocols has been recently reported (sequential protocol), according to which treatment starts as a step-up protocol.
Figure 1. Characteristics of endogenous luteinizing hormone (LH) surge induced in four women (a, b, c, d) with polycystic ovary syndrome (PCOS) during treatment with a low-dose follicle stimulating hormone (FSH) regimen (starting dose 75 IU per day, increments 37.5 IU per day) (unpublished data). LH values have been normalized to the time of onset of the LH surge (time 0). The first value of LH that exceeded 180% of the mean of the previous four values was taken to indicate that the surge had started and the time of the previous sample was considered the time of onset of the surge. The peak value of LH and the duration of the LH surge in normal menstrual cycles in this Laboratory varies between 20 and 120 mIU/ml and 48 and 72 h respectively. Patient (c) conceived a viable pregnancy without the administration of human chorionic gonadotrophin (HCG) and despite the relatively low peak of LH. The four patients, were treated in the Department of Obstetrics and Gynaecology, University of Aberdeen, UK in collaboration with Professor Allan Templeton.

and when the leading follicle reaches the size of 14 mm in diameter, the dose of FSH is reduced by half (step-down) (Hugues et al., 1996). Although with this combination the number of medium-sized 14–15 mm follicles can be reduced in comparison with the step-up protocol, similar pregnancy rates have been reported (Hugues et al., 1996).

In most studies using a low-dose gonadotrophin protocol, HCG is injected to rupture the pre-ovulatory follicle. The administration of HCG may not be necessary, since pregnancies have occurred occasionally even without the injection of this gonadotrophin (Claman et al., 1986). An endogenous LH surge can occur during ovulation induction with FSH in patients with PCOS and the characteristics of the surge in cycles with mono-ovulation can be similar to those in normal women during the normal menstrual cycle (Figure 1) (unpublished data). In general, if three or more follicles are >16 mm in diameter, HCG must be withheld and the patients should be advised to avoid intercourse in order to eliminate the risk of multiple pregnancy and OHSS. Whether luteal support of the cycles induced with low-dose HMG or FSH is required has not been clarified (Franks et al., 1985; Polson et al., 1987), although this is not a policy in most centres using this method (Sagle et al., 1991; Shoham et al., 1991a; Homburg et al., 1995a).

To avoid confusion when comparing the results of treatment with a low-dose HMG or FSH protocol between different studies, a distinction between various terminologies used, regarding the treatment regimen, should be made. For instance, in the ‘classical’ low-dose gonadotrophin regimen the starting dose is 75 IU and each of the increments is 37.5 IU. In the first published study, however, the starting dose was only 40–50 IU and each increment was 50 IU per day (Seibel et al., 1984). Such a low starting dose varying from 37.5 to 52.5 IU per day has been recently re-evaluated (White et al., 1996). Other protocols also use a low starting dose of 75 IU for 7–10 days, but in case of no ovarian response the dose is increased by 75 IU instead of 37.5 IU per day. The latter is named by some authors a ‘conventional’ regimen (Shoham et al., 1991a; Homburg et al., 1995a) and by others an ‘intermediate’ regimen (Kim et al., 1994). On the other hand, the term ‘conventional’ is also given to protocols used for ovulation induction in patients with hypothalamic amenorrhoea in which, however, the starting dose of HMG is 150 IU.
In most studies published so far, in which a low-dose protocol was used, the treatment was applied to the patients for a period of <6 months. The pregnancy rate per treated cycle varies widely from 17% (Homburg et al., 1995a) to 45% (Shoham et al., 1991a) with most studies reporting a pregnancy rate within these limits (Polson et al., 1987; Buvat et al., 1989; Dale et al., 1993; Mizunuma et al., 1991; Sagle et al., 1991). It should be emphasized, however, that in the majority of the studies the number of patients treated was small, for instance the study by Shoham et al. (1991a) included only six patients treated in 11 cycles. In one of the largest series of 100 patients with PCOS treated with low-dose gonadotrophins (Hamilton-Fairley et al., 1991), the cumulative pregnancy rate at 6 months was 55%. A similar cumulative pregnancy rate at 6 months (57%) was also achieved in a more recent study in which 225 patients with PCOS were treated with HMG in 934 cycles (White et al., 1996). What is interesting with the use of a low-dose regimen is the low risk of multiple pregnancies and the OHSS. In a comparative study (Homburg et al., 1995a), OHSS was seen in five out of 48 cycles (10.9%) treated with the ‘conventional’ FSH regimen and in none out of 59 cycles treated with a low-dose regimen. Similarly, the multiple pregnancy rate was 33.3% in the ‘conventional’ regimen with no multiple pregnancies in the low-dose regimen, but the miscarriage rate can be as high as 32% and is higher in obese than lean women (Hamilton-Fairley et al., 1991). This is despite the significant decline of LH concentrations during treatment with FSH (Polson et al., 1987; Sagle et al., 1991; Shoham et al., 1991a), which, however, may not be sufficient to overcome the deleterious effects of LH on the outcome of treatment.

The importance of the continuation of ovulation induction treatment in patients with PCOS for a period longer than six cycles has already been emphasized during ovulation induction with clomiphene citrate where the pregnancy rate approaches 100% at 10 or 12 months of treatment (Hull et al., 1979; Hammond et al., 1983). This high pregnancy rate however, does not seem to be related to clomiphene citrate itself but to the uninterrupted mode of treatment. We recently treated prospectively a group of 55 anovulatory patients because of PCOS with clomiphene citrate for six cycles (unpublished data). Those who failed to become pregnant were then treated without interruption with a low-dose regimen of HMG for another six cycles. The diagnostic criteria of PCOS included oligomenorrhoea or amenorrhoea responsive to progesterone administration and hirsutism, elevated LH and/or androgens and the characteristic echogenicity of the ovaries. The starting daily dose of clomiphene citrate was in all cases 50 mg from cycle days 2 to 6 following a spontaneous or a progestagen-induced menstrual period. In case of no response with ovulation, the daily dose was increased by 50 mg per day every two cycles up to a total daily dose of 150 mg per day. If ovulation occurred, however, the particular dose was kept constant up to a total period of six consecutive cycles. Monitoring of clomiphene citrate treatment for assessment of ovulation was performed only by measuring serum progesterone concentrations on cycle days 21 and 28, while ultrasound scans of the ovaries were not used. With this treatment, presumptive ovulation

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### Table II. Ovulation induction in 55 women\(^a\) with anovulatory infertility due to polycystic ovary syndrome (PCOS) with clomiphene citrate and low-dose human menopausal gonadotrophin (HMG) over a period of 12 consecutive cycles. Figures in parentheses are percentages.

<table>
<thead>
<tr>
<th></th>
<th>Clomiphene citrate</th>
<th>Low-dose HMG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>55</td>
<td>20</td>
<td>55</td>
</tr>
<tr>
<td>Treatment cycles</td>
<td>211 (77.8)</td>
<td>73</td>
<td>284</td>
</tr>
<tr>
<td>Ovulatory cycles</td>
<td>164 (77.8)</td>
<td>66 (90.4)</td>
<td>230 (80.9)</td>
</tr>
<tr>
<td>Patients with ovulation</td>
<td>51 (92.7)</td>
<td>20 (100)</td>
<td>55 (100)</td>
</tr>
<tr>
<td>Patients with pregnancy</td>
<td>35 (63.6)</td>
<td>15 (75.0)</td>
<td>50 (90.9)</td>
</tr>
<tr>
<td>Pregnant/treated cycle (%)</td>
<td>16.5</td>
<td>20.5</td>
<td>17.6</td>
</tr>
<tr>
<td>Pregnant/ovulatory cycle (%)</td>
<td>21.3</td>
<td>22.7</td>
<td>21.7</td>
</tr>
<tr>
<td>Twin pregnancies</td>
<td>1 (2.8)</td>
<td>1 (6.6)</td>
<td>2 (4.0)</td>
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<tr>
<td>Spontaneous abortions</td>
<td>4 (11.4)</td>
<td>1 (6.6)</td>
<td>5 (10.0)</td>
</tr>
<tr>
<td>Ectopic pregnancies</td>
<td>1 (2.8)</td>
<td>1 (6.6)</td>
<td>2 (4.0)</td>
</tr>
</tbody>
</table>

\(^a\)All 55 women were initially treated with clomiphene citrate for six cycles. Then, 20 women who failed to respond to clomiphene citrate were then treated with HMG for another six cycles (unpublished data).
higher in the conception cycles (72.6
cycles (25.7
cycles), monitoring of treatment was performed only
in case of no ovarian response (no follicles >10 mm by
7–10 days, then increasing every 7 days by 37.5 IU per day
total of 73 cycles. The starting dose of HMG was 75 IU for
treated without a break with a low-dose HMG protocol in a
6 months treatment with clomiphene citrate, were then
recommended starting dose.

The 20 patients who did not become pregnant during the
6 months treatment with clomiphene citrate, were then
treated without a break with a low-dose HMG protocol in a
total of 73 cycles. The starting dose of HMG was 75 IU for
7–10 days, then increasing every 7 days by 37.5 IU per day
in case of no ovarian response (no follicles >10 mm by
ultrasound). Monitoring of treatment was performed only
by the use of ultrasound (Shoham et al., 1991b). In all
women, 5000 IU of HCG were given i.m. when the largest
follicle was 18–20 mm in diameter. All 20 patients
ovulated during treatment with HMG and 15 of them
conceived (75%) (Table II). Monofollicular development
occurred in 72.6% of the cycles. The combination of the
results of the two treatment regimens, i.e. clomiphene
citrate and HMG, demonstrated that 50 out of the 55
patients eventually conceived and the cumulative pregnancy rate was 63.6% at 6 months and 90.9% at 12
months (Figure 2). Overall, the abortion rate was rather low
(10.0%) as were the multiple (4.0%) and ectopic pregnancy
rates (4.0%). There were no cases of severe OHSS. These
results, in conjunction with previous data (Hammond et al.,
1984), demonstrate that apart from the proper selection of
patients, an important issue in the management of
anovulation in PCOS may not be the drugs themselves, i.e.
clophene citrate or HMG, but the uninterrupted and
prolonged duration of treatment. In all aspects, however, it
is strongly recommended the first choice of treatment
should be clomiphene citrate in patients with PCOS
starting with the lowest daily dose, while for those who fail
to ovulate and/or become pregnant, a low-dose regimen of
FSH or HMG is a good alternative.

The use of growth hormone as an adjuvant to HMG in
order to increase the ovarian response for IVF is
controversial (Ibrahim et al., 1991; Hughes et al., 1994).
Regarding the PCOS, the addition of growth hormone to a

Figure 2. Cumulative pregnancy rate over 12 consecutive cycles in
55 women with polycystic ovary syndrome (PCOS) treated with (○)
clophene citrate and (●) low-dose human menopausal gonado-
trophin (HMG; unpublished data). Clomiphene citrate was given to
all 55 women for the first six cycles and HMG for another six cycles
to 20 of them who did not respond to clomiphene citrate.

GnRH agonist plus HMG regimen did not influence the
clinical outcome suggesting that the use of growth
hormone in these patients is not justified (Homburg et al.,
1995b). However, co-administration of the somatostatin
analog, octreotide, with HMG has resulted in lower LH
and oestradiol concentrations, smaller number of follicles,
reduced number of cycles abandoned and reduced risk of the
OHSS (Prelevic et al., 1995).

Pulsatile GnRH

The hypothalamic decapeptide, GnRH, applied in a
pulsatile way has been successful more often in the past
and to a lesser extent today for ovulation induction in
patients with hypothalamic amenorrhoea (Shoham et al.,
1990). Based on this success, the use of pulsatile GnRH has
been also extended to cases belonging to WHO group II of
anovulation; this includes patients with PCOS. The idea
behind it was that the pulses of exogenous GnRH could
override the abnormal pattern of endogenous GnRH
secretion and induce ovulation. Although the initial results
were optimistic (Ory et al., 1985; Burger et al., 1986),
subsequent studies demonstrated that the use of pulsatile
GnRH in patients belonging to the WHO group II and
PCOS was less successful in terms of ovulation and
pregnancy rate than in patients with hypothalamic
amenorrhoea. In a study of 48 patients resistant to
clophene citrate, ovulation was induced with the use of
pulsatile GnRH in only 52% of the cycles (Eshel et al.,
1988). Obesity, hyperandrogenism and LH hypersecretion
were factors adversely affecting the treatment. Out of 384
cycles surveyed by Shoham et al. (1990), the ovulation rate
per treated cycle was 50.7% and the pregnancy rate only
14.6%. However, when the pregnancy rate was corrected for ovulation, it was raised to 28.7% (Shoham et al., 1990). It is suggested, therefore, that the difficulty in PCOS during treatment with pulsatile GnRH is to achieve ovulation, i.e. the ovaries are rather refractory to such treatment. The miscarriage rate was also high in that study, i.e. 45% (Shoham et al., 1990). The reasons for this high abortion rate are not clear, but impaired luteal function has been implicated (Filicori et al., 1991).

Pulsatile GnRH is injected through the s.c. or the i.v. route via a portable infusion pump. The frequency of administration is one pulse every 60–120 min and the dose per pulse is usually lower with the i.v. than the s.c. route ranging between 5 and 40 µg. After the injection of each GnRH pulse, stimulation of FSH and LH secretion from the anterior pituitary occurs. Patients with PCOS always have higher concentrations of LH than patients with hypothalamic amenorrhoea and in ~40% of them LH is even higher than in normal women (Balen and Rose, 1994). In these patients, the use of GnRH in a pulsatile way stimulates a further increase of LH secretion (Burger et al., 1989). A comparison between a low-dose FSH regimen and pulsatile GnRH in patients with PCOS demonstrates great differences in terms of LH response to treatment. An example is given in Figure 3, in which one woman with PCOS and high basal LH concentrations was treated with both regimens in two different cycles (unpublished data). During treatment with FSH, LH concentrations declined significantly as a result of the negative feedback effect of increasing serum oestradiol concentrations and monofollicular ovulation occurred, while during treatment with pulsatile GnRH at the dose of 15 µg per pulse s.c., a further increase of LH occurred followed by a degree of desensitization and no follicle development was apparent. Patients with PCOS demonstrate a rapid frequency of LH pulses compared with those in the early to mid-follicular phase of the menstrual cycle (Kazer et al., 1987). When this frequency is reduced after the exogenous administration of oestradiol and progesterone, a selective secretion of FSH occurs resulting in follicle development and normalization of LH/FSH ratio (Christman et al., 1991). In practical terms, this may have an impact on the treatment, since by lowering the frequency of the pulses of s.c. GnRH, a decrease in the ratio of LH/FSH and a preferential increase in FSH occur (Hurwitz et al., 1986).

A recent study has analysed the data from 600 consecutive cycles treated with pulsatile GnRH in patients with anovulation of various aetiologies (Filicori et al., 1994). The highest pregnancy rate was achieved in patients with hypothalamic amenorrhoea (22% per treated cycle) and the lowest in patients with hyperandrogenism including patients with PCOS (13% per treated cycle). An abortion rate of 45% in the PCOS group was significantly higher than in patients with hypothalamic amenorrhoea (25%). This study also confirmed that pulsatile GnRH treatment is less successful in obese patients with high LH, testosterone and insulin concentrations. However, an advantage of treatment with pulsatile GnRH is that the risk of multiple pregnancies and OHSS is low (Filicori et al., 1994).

Combinations of pulsatile GnRH with other drugs in the treatment of PCOS have been reported. For example, a better ovulatory response is achieved when pulsatile GnRH is supplemented with clomiphene citrate or FSH (Eshel et al., 1988; Homburg et al., 1990a; Tan et al., 1996). Also, pretreatment of PCOS patients with a GnRH agonist seems to induce a more favourable endocrine profile in terms of LH secretion and ovulation induction during pulsatile GnRH administration, but has no effect on pregnancy and abortion rates (Surrey et al., 1989; Filicori et al., 1991, 1994). It should be mentioned that the pulsatile administration of GnRH requires a frequent, every 4–5 days, refilling of the pump while skin reactions are not uncommon requiring frequent changes of the site of the needle. For all these reasons and the low success rate, pulsatile GnRH is used less frequently today for ovulation induction in PCOS.

**GnRH agonists plus human gonadotrophins**

GnRH agonists act on the pituitary and induce down-regulation of GnRH receptors and desensitization of the secretion of gonadotrophins. After a flare-up effect which lasts for a few days, serum FSH and LH concentrations become almost undetectable (for review, see Schmutzler and Diedrich, 1990). When a GnRH agonist is used in patients with PCOS the flare-up effect on LH secretion lasts for a longer period than in normally menstruating women due to the higher sensitivity of the anterior pituitary to exogenous GnRH in the PCOS women (Fleming et al., 1985; Cheung and Chang, 1995). The idea behind the use of GnRH agonists in patients with PCOS is that the reduction of the high basal LH concentrations may improve the outcome of the treatment with human gonadotrophins. The use of GnRH agonists in PCOS was first introduced by Fleming et al., (1985) in Glasgow more than 10 years ago. The results demonstrated a pregnancy rate as high as 64%, but, the initial studies were retrospective, non-randomized and uncontrolled (Fleming et al., 1985; 1988; Charbonnel et al., 1987). Subsequent prospective studies demonstrated that GnRH agonists prevent premature luteinization in HMG-treated cycles.
Figure 3. Luteinizing hormone (LH), oestradiol and progesterone values in response to either a low-dose regimen of follicle stimulating hormone (FSH) or to pulsatile gonadotrophin-releasing hormone (GnRH) (15 µg per pulse s.c.) in an anovulatory woman with polycystic ovary syndrome (PCOS) treated with the two regimens in two different cycles (unpublished data). A normal profile with ovulation was achieved during treatment with FSH and an exaggerated LH response during treatment with pulsatile GnRH followed by desensitization and no follicle development. This patient was treated in the Department of Obstetrics and Gynaecology, University of Aberdeen, UK in collaboration with Professor Allan Templeton.

and induce a pregnancy rate per treated cycle similar to, or even higher than, that with the conventional gonadotrophin regimens (Dodson et al., 1987; 1989). However, patients pretreated with the agonist require more ampoules of HMG and days of treatment and show an increased risk of OHSS (25 versus 8%) (Homburg et al., 1990b). Retrospective data (Fahri et al., 1993; Homburg et al., 1993a) have shown that cumulative pregnancy rate and live birth rate at 4 months can be as high as 69 and 64% respectively during treatment with a GnRH agonist plus HMG compared with 47 and 32% respectively during treatment with HMG alone. Furthermore, in the non-randomized, comparative study of Homburg et al. (1993a), pretreatment with the GnRH agonist significantly reduced the miscarriage rate (16.7%), when compared with the use of HMG alone (39.1%).

The reason for the high rate of the OHSS during treatment with GnRH agonists is difficult to explain. It seems, however, that this is not related to the values of LH but rather to the increased sensitivity of the polycystic ovaries to exogenous gonadotrophins. In all these studies using GnRH agonists the dose of gonadotrophins was
scheduled in the context of a ‘conventional’ regimen, i.e. a starting dose of either 150 IU per day (Dodson et al., 1987; 1989) or only 75 IU as in low-dose regimens but with increments of 75 IU every 5 days (Homburg et al., 1990b; 1993a). Nevertheless, the use of a low-dose regimen of FSH in combination with a GnRH agonist does not eliminate the risk of multiple follicular development, since monofollicular growth is less frequent with this regimen (14%) than with the use of FSH alone (44%) (Scheele et al., 1993). Multiple follicular development during the use of a GnRH agonist is not avoided even when the starting dose of FSH is as low as 37.5 IU (Buckler et al., 1993). With this in mind and the rather high cost of such a treatment, it is doubtful whether GnRH agonists in combination with gonadotrophins constitute a better treatment modality than gonadotrophins alone for ovulation induction in patients with PCOS. As mentioned above, treatment with a regimen containing a GnRH agonist may result in a reduced rate of miscarriage probably because of the decrease in LH concentrations (Homburg et al., 1993a). Therefore, elevated LH values before the onset of treatment is probably the only indication for the use of GnRH agonists in ovulation induction programmes in PCOS, provided that treatment with clomiphene citrate and HMG has failed. One should take into account, however, that the study by Homburg et al., (1993a) is a retrospective analysis of data and that the literature lacks a prospective randomized controlled trial to evaluate the effect of a GnRH agonist on the miscarriage rate. An important issue during ovulation induction with the combination of a GnRH agonist and human gonadotrophins is the function of the corpus luteum. A recent study has shown a short luteal phase of 8.8 days on average in GnRH agonist plus HMG treated cycles (Donderwinkel et al., 1993). Several studies, however, support the luteal phase with the injection of extra HCG or the administration of progesterone (Dodson et al., 1989; Abdel Gadir et al., 1992; Homburg et al., 1993a).

Over the last few years, GnRH agonists have been proposed as surrogates for the endogenous LH surge during ovulation induction with HMG. This includes the administration of one or two doses of a GnRH agonist during the pre-ovulatory period, instead of the administration of HCG, to rupture the follicle in those cases with multiple follicular development and thus at a high risk for the OHSS. The idea behind this is that HCG has a long half-life and therefore may exert a prolonged stimulation of the corpora lutea increasing thus the risk of OHSS, while the use of a GnRH agonist stimulates endogenous LH secretion in a pattern similar to the midcycle LH surge. The latter is adequate to induce follicle luteinization and rupture without affecting the corpora lutea for long. This treatment has been applied to anovulatory patients, including PCOS, as well as to patients during induction of multiple follicular development for IVF (Emperaire and Ruffie, 1991; Imoedemhe et al., 1991; Tulchinsky et al., 1991). The results have been controversial, as the OHSS is not prevented in all cases and the luteal phase may be defective (Gerris et al., 1995; Schmidt-Sarosi et al., 1995). An alternative to the pre-ovulatory administration of a GnRH agonist for prevention of the OHSS is the use of native GnRH as a single i.v. injection of 200 µg (Blumenfeld et al., 1994) or the selective reduction of follicles by aspiration through an ultrasound-guided needle (Ingersler, 1991).

The administration of a GnRH agonist as a pretreatment before the use of pulsatile GnRH in PCOS has already been mentioned. The normalization of LH concentrations achieved with the GnRH agonist stimulates during the pulsatile GnRH a profile of LH closer to the normal (Filicori et al., 1991; 1994). This may result in a better ovulation rate, but the clinical performance is not improved.

Other methods

Ovarian cauterization

A surgical approach to the management of anovulation in patients with PCOS was in the past the wedge resection of the ovaries. This was performed through laparotomy, but due to the high rate of post-operative adhesions it has recently been abandoned and substituted with the laparoscopic approach. The studies performed in this area have been recently reviewed by Donesky and Adashi (1995). The main advantages of this method over gonadotrophin therapy, as they are outlined in that review, include the lower cost, the possibility for ovulation in consecutive cycles as a result of one treatment attempt, no risk of OHSS and multiple pregnancies and a lower abortion rate, while the disadvantages include the still existing possibility of intrapelvic adhesions despite the use of fine laparoscopic techniques, the possibility of ovarian atrophy and the potential, although speculative, for inducing ovarian cancer. In terms of ovulation and pregnancy rates, electrocautery seems to be equally effective as HMG or FSH, with cumulative pregnancy rates at 6 months of 52.1, 55.4 and 38.3% respectively (Abdel Gadir et al., 1990). The possibility, however, exists that following electrocautery women unresponsive to clomiphene citrate may become responsive and that the abortion rate during treatment with HMG becomes lower after electrocautery than when HMG is combined with a GnRH agonist (Abdel Gadir et al., 1990; 1992). The invariable occurrence of ovulation post-operatively seems
to be related to the decrease in LH concentrations and particularly LH amplitude (Rossmanith et al., 1991; Abdel Gadir et al., 1993). Electrocautery of the ovaries by diathermy or laser may be useful for those patients who fail to respond to clomiphene citrate and are unwilling to proceed with gonadotrophin therapy.

**IVF and embryo transfer**

IVF can be applied successfully as a last resort to those patients who fail to respond to extensive treatment with clomiphene citrate or human gonadotrophins (Radwanska et al., 1988; Homburg et al., 1993b). With this method, a cumulative pregnancy rate of 82% at 6 months has been reported in a series of 68 women with PCOS who previously failed to respond to other therapeutic means (Homburg et al., 1993b). A recent review of the main studies published so far confirms that the pregnancy rate is comparable to that in women with tubal infertility (Buyalos and Lee, 1996). However, a high rate of cancellation of embryo transfer has been reported in patients with PCOS (Kodama et al., 1995). Also, the increased ratio of LH:FSH seems to adversely affect oocyte maturity and to increase the possibility for miscarriage (Tarlatzis et al., 1995).

A promising approach to the management of patients with PCOS in the context of IVF is the recently developed technique of in-vitro maturation of immature oocytes obtained from polycystic ovaries (Trounson et al., 1994). Fertilization and pregnancies after embryo transfer have been reported; however, further improvement is required before it becomes a routine method for treatment of infertility in patients with PCOS (Barnes et al., 1996). A distinction should be made between the ovaries of women with PCOS and the ovaries of otherwise normal women but with polycystic appearance. The latter group of women responds to HMG with a greater number of follicles and oocytes and a higher percentage of mature oocytes than women with PCOS and women with normal-appearing ovaries (Wong et al., 1995). Women in such a group could be used as excellent oocyte donors.

**Others**

One of the biochemical characteristics of the PCOS is the resistance to insulin and the subsequent hyperinsulinaemia even in non-obese women (Dunaif et al., 1992). Hyperinsulinaemia may play a role in the increased androgen production by the polycystic ovaries, while it also decreases the sex hormone binding globulin (Barbieri et al., 1986; Nestler et al., 1991). Administration of drugs which reduce insulin, such as diazoxide or metformin, to women with PCOS decreases free testosterone concentrations through a reduction in ovarian cytochrome P450c17a activity (Nestler et al., 1989; Velazquez et al., 1994; Nestler and Jakubowicz, 1996). Ovarian cyclicity with regular ovulation and pregnancy have been reported after treatment with metformin (Velazquez et al., 1994), however, further study is required to establish the effectiveness of these compounds as ovulation inducing agents in PCOS. Obese women with PCOS may also be helped by losing weight. Even a 5% loss of body weight reduces insulin and androgens with subsequent restoration of normal menstruation and ovulation (Kiddy et al., 1992).

**Future perspectives**

As has already been mentioned one of the main problems in patients with PCOS is the hypersecretion of LH combined with the increased sensitivity of the anterior pituitary to exogenous GnRH and the ovaries to exogenous gonadotrophins. These features have an impact on the outcome of treatment including ovulation and success rate. Although GnRH agonists dramatically reduce basal LH secretion and prevent premature luteinization, they increase multiple pregnancy rate and the risk of OHSS. It is clear, therefore, that GnRH agonists cannot be used as a first line of treatment and even when an indication exists, such as in cases with high LH concentrations, the rate of complications can be high. Also, the duration of treatment with these drugs is longer and the cost is higher. On the other hand, since the pathophysiology of PCOS is unknown, an aetiological approach to the treatment is not possible. In an attempt to overcome these problems, new treatment modalities have been proposed and these will be discussed below together with some future perspectives.

**Recombinant gonadotrophins**

The use of urinary gonadotrophins containing FSH with minimal LH were introduced for the treatment of PCOS in the early 1980s. As has been mentioned above, the introduction of such preparations in the treatment of PCOS was based on the fact that LH contained in the HMG could be added to the already increased endogenous LH, but this potentiated the detrimental effects of LH on the outcome of treatment. The use of FSH in low-dose protocols was proven to be effective with a low risk of the OHSS; however, FSH did not have any advantages over the use of HMG. In fact, in most infertility centres HMG, which nowadays contains FSH and LH in the ratio of 3:1 was adopted instead of FSH. Recently, rFSH produced with the use of DNA technology has become available in the form of two preparations. The first established pregnancies with the use of each of the two preparations of rFSH in patients...
of the WHO group II or PCOS were published recently (Donderwinkel et al., 1992; Hornnes et al., 1993; Van Dessel et al., 1994). A normal increase in serum oestradiol and inhibin concentrations was seen during treatment with rFSH (Hornnes et al., 1993). This is consistent with in-vitro data demonstrating a similar dose-dependent stimulating effect of rFSH to that produced by urinary FSH on the production of oestradiol and progesterone from human granulosa cells obtained from women during a spontaneous menstrual cycle (Mason et al., 1993). A partial analysis of the results of a multicentric, prospective, randomized study of treatment of women belonging to WHO group II with a chronic low-dose regimen of rFSH has shown a pregnancy rate per initiated cycle of 18% versus 11% with 'pure' FSH (Loumaye et al., 1995). In another study including 22 PCOS patients with a history of severe OHSS after treatment with a conventional HMG regimen in combination with a GnRH agonist (Aboughar et al., 1996), 14 patients were treated with rFSH and eight with HMG in a classical low-dose protocol. The pregnancy rate was similar in the two groups (20 and 15.4% respectively), but the abortion rate was high (25 and 50% respectively). However, no cases of OHSS were seen in the two groups. It is clear from these preliminary data that rFSH can be as effective as urinary FSH or HMG in ovulation induction in PCOS.

An interesting approach to the ovulation induction in PCOS is expected to be the use of various isohormones of FSH. During the normal menstrual cycle there are changes in the molecule of FSH, mainly related to glucosylation, under the effect of the ovarian steroids. For instance, FSH secreted during the reproductive life is mainly basic with a short half-life (Wide, 1982). During the normal menstrual cycle, as oestradiol concentrations increase in blood after the selection of the dominant follicle FSH becomes more basic with a more rapid clearance (Wide, 1982). This results in a quick reduction of serum FSH concentrations below a threshold value that prevents the development of multiple follicles. If FSH isoforms were available for clinical use, theoretically they could be used for ovulation induction in PCOS starting with an acidic and long-acting form of FSH to recruit follicles and continuing with a basic one of a shorter half-life to maintain the growth of only a single follicle. Alternatively, depending on the duration of the activity of the FSH isohormones, only a single injection of a long-acting FSH might be adequate to recruit and select a dominant follicle in patients with PCOS by creating an FSH window similar to that during the administration of clomiphene citrate. The production of such FSH isoforms is expected to become a reality through recombinant DNA technology and in fact a long-acting FSH preparation is expected to be available soon.

Recently, recombinant LH (rLH) was also produced and has been used experimentally. Such a preparation may be useful in clinical terms as a surrogate of the endogenous LH surge instead of the use of HCG during ovulation induction. So far, attempts have been made to mimic the midcycle LH surge in monkeys with the injection of rLH or pituitary LH (Chandrasekher et al., 1994). Also, in a hypogonadal woman who responded to rFSH with follicle growth but no steroidal production, the addition of rLH to the rFSH regimen induced normal steroidogenesis (Hull et al., 1994). These preliminary results provide evidence that rLH may be useful in ovulation induction programmes instead of the use of HCG, which has a long half-life. In that respect, rLH might be useful in supporting the luteal phase or in cases which are at high risk for OHSS. Another possibility might be the use of rLH in the context of rFSH-induced folliculogenesis, thus enabling only a small cohort of follicles to achieve pre-ovulatory maturation.

**GnRH antagonists**

GnRH antagonists are produced from the GnRH decapeptide by amino acid substitutions at positions 1, 2 and 6. Although these products have been available for several years, their use in clinical practice is limited. The reasons for this are related to the short half-life of the initial products and, therefore, the necessity for multiple injections and to the stimulation of histamine release from mast cells which produce allergic reactions. Recently, some products with longer half-life and minimal or no stimulation of histamine production became available and have reached clinical experimentation. GnRH antagonists bind to the GnRH receptors at the pituitary and by competing with endogenous GnRH prevent the stimulation of LH secretion. They differ from GnRH agonists in that they do not stimulate endogenous gonadotrophin secretion, but induce an immediate and rapid decrease of gonadotrophin release.

Experiments with GnRH antagonists have been carried out in women during the normal menstrual cycle (Fluker et al., 1991; Hall et al., 1991; Dubourdieu et al., 1994; Leroy et al., 1994). The use of the GnRH antagonist, cetrorelix, during the mid-follicular phase induced a rapid decline in LH and oestradiol and to a lesser extent in FSH values and postponed the endogenous LH surge for 6–17 days (Leroy et al., 1994). At the same time, the size of the dominant follicle remained stable before it increased further during the days after the injection of the antagonist (Leroy et al., 1994).
1994). If multiple doses of the antagonist are given, gonadotrophin concentrations remain low throughout the treatment. The antagonists have, so far, been used in women undergoing IVF in order to block the endogenous LH surge and prevent premature luteinization (Frydman et al., 1991; Olivennes et al., 1994; 1995). Since during treatment with antagonists GnRH agonists are not used, it is expected that the duration of treatment will be shorter and require a smaller number of FSH ampoules. This, however, remains to be confirmed since theoretically the reduction of endogenous LH in late follicular phase may decelerate the growth of the follicle which at that stage of the cycle is also LH-dependent (Hillier et al., 1991). Higher doses of FSH may then be required to promote follicle growth. In fact, a recent study has shown that induction of multiple follicular development in monkeys pretreated with a GnRH antagonist for 3 months required a longer period of treatment with rFSH than rFSH plus LH, although more oocytes completed meiosis to metaphase II and fertilized in vitro in the rFSH group (Zelinski-Wooten et al., 1995). Other points to be clarified are the optimal dose of the antagonists for use in ovulation induction programmes and whether luteal phase support is required.

Based on the above considerations, GnRH antagonists could be useful for ovulation induction in patients with PCOS in order to reduce high endogenous LH secretion. A theoretical protocol would be to start the treatment with the GnRH antagonist on days 2 or 3 of the cycle and when LH concentrations become low to continue with a low-dose regimen of urinary or rFSH. However, it might be necessary to continue the treatment with the antagonist up to the point of the selection of the dominant follicle and the enhancement of the ovarian negative feedback effect on LH secretion by the rising oestradiol, unless antagonists with very long half-life are produced for clinical use. An alternative protocol would be to start with a low-dose regimen of FSH and the GnRH antagonist added when the dominant follicle reaches the size of 12–14 mm in diameter, as in the protocols used for IVF. A decrease then in LH concentrations during the late follicular phase may prevent the detrimental effects of this hormone on the outcome of treatment. Whether such a treatment would be useful to all patients with PCOS or only to those with high LH concentrations remains to be investigated.

Inhibin

This dimeric protein which is produced by the granulosa cells of the follicle has been implicated in the pathophysiology of the PCOS. It was initially shown that concentrations of bioactive inhibin in the follicular fluid of patients with PCOS were lower than those of normal controls (Tanabe et al., 1983). From this, a hypothesis was developed that inhibin in the peripheral circulation may suppress endogenous FSH secretion in patients with PCOS and therefore prevent the stimulation of follicle growth and maturation. When immunoassays for measurement of inhibin in blood became available, it was shown that there was no difference in basal and gonadotrophin-stimulated inhibin concentrations between normal and women with PCOS (Buckler et al., 1991; Falcone et al., 1991). On the other hand, during the follicular phase of the cycle, inhibin concentrations do not change significantly apart from a midcycle peak (McLachlan et al., 1987). It is difficult, therefore, to assess whether circulating inhibin plays a role in the control of FSH secretion at that stage of the cycle, although the declining concentrations of immunoreactive inhibin during the late luteal phase may contribute to the intercycle rise of FSH (Roseff et al., 1989). If the role of inhibin during the normal menstrual cycle in terms of FSH secretion is obscure, it is even more unclear in patients with PCOS. Methods measuring dimeric inhibin have recently developed. With these, inhibin A and inhibin B show different patterns of changes during the normal menstrual cycle (Groome et al., 1996). For instance, inhibin A shows a significant increase during the luteal phase concomitant with that of progesterone, while inhibin B increases during the follicular phase. It is not known, however, which of the two inhibins is physiologically more important. However, recombinant inhibin A suppresses pituitary FSH secretion if given to primates during the early follicular phase of the cycle (Molskness et al., 1996).

If inhibin played a role in the pathogenesis of PCOS, a theoretical approach might be to counteract the activity of this substance and consequently to increase basal FSH concentrations in a way similar to that in ewes and heifers (Glencross et al., 1992; Mann et al., 1992). So far, there are no means to do this in women and the production of antibodies against inhibin may have permanent implications. An alternative, also theoretical approach, might be to increase the activity of activins which at least in vitro stimulate the secretion of FSH from pituitary cell cultures. Preliminary data in primates support this assumption, although further experimentation is required (Stouffer et al., 1993). At this moment, manipulation of the activity of inhibin and related peptides in an effort to counteract anovulation in PCOS is not possible.

Gonadotrophin surge attenuating factor

Gonadotrophin surge attenuating factor (GnSAF) is a putative non-steroidal ovarian substance that attenuates the
endogenous LH surge in women undergoing ovulation induction (Messinis and Templeton, 1989; 1991a). This factor acts through the reduction of the pituitary response to GnRH possibly by antagonizing the stimulating effect of oestradiol on the pituitary (Messinis and Templeton, 1991b; Messinis et al., 1994). So far, substances with GnSAF activity have been isolated from rat Sertoli cells and porcine follicular fluid, but differences in the molecular weight and the N-terminal sequences were found between the two species (Tio et al., 1994; Danforth and Cheng, 1995).

Whether GnSAF in human follicular fluid is the same as these substances is not known. Although activity of GnSAF different from inhibin has been detected in human follicular fluid, so far only partially purified fractions of this substance have been obtained (Fowler et al., 1990, 1995; Pappa et al., 1995). A role for GnSAF in PCOS is possible, but only theoretical at the moment. The fact that GnSAF reduces the response of LH to GnRH and that in patients with PCOS this response is usually exaggerated, suggest that it is possible that hypersecretion of LH in PCOS is partly related to a defective production of GnSAF from the granulosa cells.

If purification and characterization of GnSAF from human follicular fluid is completed, this factor may have some applications in various clinical conditions. Regarding PCOS, the development of an assay for measuring GnSAF concentrations will enable researchers to study values in blood as well as in the follicular fluid. On the other hand, preparations of GnSAF may be useful in patients with PCOS by reducing high LH concentrations and normalizing the exaggerated LH response during treatment with pulsatile GnRH.

Conclusions

An aetiological approach to ovulation induction in patients with PCOS is not likely, due to the unknown pathophysiology of the syndrome. However, the choice of drugs for use in individual patients has been clarified during the last few years. Clomiphene citrate remains the most safe and inexpensive method for PCOS and is still used as the first line of therapy. In clomiphene citrate resistant cases, human gonadotrophins in a low-dose protocol, as it has been established during the last 10 years, is the first alternative, resulting in a good pregnancy rate per treated cycle with a low incidence of multiple pregnancy and a low risk of OHSS. An extended period of treatment up to 10 or 12 months either with clomiphene citrate or with clomiphene citrate for 6 months followed by a low-dose HMG regimen in properly selected women is very effective. Pulsatile GnRH exaggerates the abnormal gonadotrophin profile and fails to induce ovulation in nearly 50% of the cases. Its use as a routine treatment today is not justified in patients with PCOS. GnRH agonists in combination with gonadotrophins reduce endogenous LH and the rate of abortion. However, due to the high rate of multiple pregnancies and the increased risk of the OHSS, their use is indicated only for women with high LH concentrations who fail to respond to other treatments. Ovarian cauterization and IVF are effective alternatives for patients failing to conceive with the above treatments. Nevertheless, the risk of the post-operative formation of pelvic adhesions is a limiting factor for cauterization. The advent of new drugs, such as recombinant gonadotrophins and GnRH antagonists, is expected to provide new insight into the management of anovulation in PCOS. So far, the limited experience with recombinant FSH indicates that this preparation is at least equally effective with urinary gonadotrophins. However, the great advantage of the recombinant gonadotrophins is expected to be obtained from the production of FSH isoforms. Their use in PCOS may result in a simplified protocol of ovulation induction, which is closer to the physiological process. On the other hand, GnRH antagonists, by reducing LH, are expected to show their advantages over the GnRH agonists. Finally, it is possible that the purification of ovarian substances which specifically control LH secretion may clarify further the pathophysiology of PCOS and may provide new means for the treatment of this syndrome.

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