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Mono-ovulatory cycles: a key goal in profertility programmes

ESHRE Capri Workshop Group[1]

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Mono-ovulatory cycles for women are optimal because singleton pregnancies have a better outcome than multiples. Multiple births began to increase in the 1950s after the first appearance of effective ovulation induction for the treatment of anovulation. Since the 1980s when ovulation induction and IVF were more broadly applied to the treatment of unexplained and persistent infertility, there has been an unprecedented rise in multiple births. Strategies to achieve mono-ovulation during treatment of anovulatory patients are distinct from those for the treatment of ovulating patients who have unexplained and persistent infertility. Anovulatory patients with hypogonadotrophic hypogonadism can be treated with exogenous pulsatile GnRH, which restores normal gonadotrophin secretion, ovulation rates and conception rates. The multiple pregnancy rate is not increased with GnRH treatment. In patients with normogonadotrophic anovulation, attention should be given to diet and exercise before any other interventions are considered. Pharmacological induction of ovulation can be achieved with antiestrogen, gonadotrophin or pulsatile GnRH treatment; antiestrogen is the first choice with gonadotrophin more widely used for clomiphene citrate (CC)-resistant patients. Obesity and polycystic ovaries are common in this group, so that gonadotrophin and GnRH treatment are associated with lower responses compared with hypogonadotrophic hypogonadism, and higher multiple pregnancy rates. Low dosage protocols are being tested that may lower the multiple birth rates. The role of drugs enhancing sensitivity to insulin, e.g. metformin, remains undetermined. Laparoscopic ovarian diathermy achieves conception rates that are equivalent to gonadotrophin treatment, with fewer multiple births. Augmenting normal ovulation processes for couples with unexplained and persistent infertility is less effective. Pregnancy rates are statistically significantly higher with CC but the size of the increase is not clinically important. CC with intrauterine insemination is associated with a clinically important effect on conception. Achieving mono-ovulation is more difficult in assisted reproductive technology cycles because success depends on maintaining the level of FSH above the threshold level longer than normal in order to increase the number of mature follicles. Milder stimulation for IVF and IVF in the untreated cycle show great potential, however, especially in view of the trend toward transfer of a single embryo in assisted reproductive treatment cycles.

Introduction

Despite guidelines from scientific societies on the current practice of ovarian stimulation, the multiple pregnancy rate remains high. The goal of higher pregnancy rates in stimulated cycles and the need for numerous oocytes to allow the transfer of several embryos in IVF and ICSI cycles are powerful forces leading to multiple birth rates that are unprecedented in human history.

Multiple births are a serious complication of profertility treatments, because perinatal mortality is seven times higher among triplets and five times higher among twins (Bergh *et al*., 1999; Fisk and Trew, 1999). The chance of a triplet pregnancy resulting in a baby with cerebral palsy is 47-fold, and for a twin pregnancy 8-fold, that of a singleton pregnancy (Petterson *et al*., 1993). Reducing high order multiple births by multi-fetal reduction is not acceptable to some couples and this invasive procedure does not improve the outcome of triplet pregnancy (Leondires

et al., 2000). Multiple births also impose additional financial, emotional and logistical burdens on families and health-service providers (ESHRE Capri Workshop Group, 2000). Together, these are solid arguments for proposals that would minimize the burden of iatrogenic multiple births by aiming to achieve mono-ovulation whenever the ovulatory process is modified by treatment, whether it be for anovulation, augmentation of ovulatory cycles or assisted reproduction.

Physiology of mono-ovulation

Less than 1% of millions of primordial oocytes present in the ovaries during fetal life are ovulated. Throughout life the numbers are drastically reduced by a process of atresia (McGee and Hsueh, 2000). Every day, ~10–20 follicles leave the resting pool of primordial follicles to commence folliculogenesis. The factors regulating recruitment of primordial follicles are poorly understood but the fact that the proportion increases as the total pool of follicles declines with age suggests that some inhibiting factors may be involved (Baird, 1999). Recruitment and the initial stages of follicle growth occur independently of gonadotrophins but once an antrum is formed (~0.2 mm diameter) the follicles become dependent on FSH and LH.

It is essential that the number of ovulatory follicles is that which is optimal for that species (Baird, 1987). Polytocous species like pigs and rats can carry many fetuses during pregnancy and successfully rear multiple offspring. In women, singleton pregnancies have a better outcome than twins. The perinatal mortality and morbidity of triplets and higher order multiples is ⩾5-fold greater than singletons (ESHRE Capri Workshop Group, 2000).

In the natural cycle, final selection of the ovulatory follicles does not occur until the last 2 weeks of folliculogenesis (Fauser and van Heusden, 1997; McGee and Hsueh, 2000). As the levels of estradiol, progesterone and inhibin A decline during regression of the corpus luteum, the concentration of FSH and LH rise. When this intercycle rise of FSH exceeds a certain critical level it 'activates' the largest healthy antral follicles (2–5 mm) present in the ovaries at this time (Brown, 1978). This 'cyclic' recruitment involves inducing differentiation of certain key functions of the granulosa cells including aromatase enzyme and LH receptors. Once activated, the chosen follicle suppresses the secretion of FSH by secreting increasing amounts of estradiol and inhibin A. Thus the dominant ovulatory follicle ensures that the hormonal environment will be hostile to the recruitment of additional follicles. The ovulatory follicle can sustain its growth by several paracrine mechanisms which increase its sensitivity to FSH, that is, an increase in insulin-like growth factors (IGF)-I and -II. At the same time because the granulosa cells acquire increased LH receptors the follicle can use LH as well as FSH for gonadotrophic support (Campbell *et al*., 1999).

The number of ovulatory follicles can be increased by either increasing the number of near-synchronized small antral follicles present in the ovaries at the time of selection or by maintaining the level of FSH above the threshold level for longer (Baird, 1987). All methods of 'controlled' ovarian stimulation involving antiestrogens or gonadotrophins use the latter strategy (Fauser *et al*., 1999).

Ovarian stimulation is a form of infertility treatment for two different categories of women: those with chronic anovulation and those suffering from other forms of infertility. Modern regimens of treatment for ovulatory infertility, the first indication, are designed to achieve the development of a single follicle in the majority of cycles (Baird, 1993). In the second indication, multiple ovulation (alone or in combination with other assisted reproductive technology strategies) is programmed with the aim to increase the generic chance of pregnancy. This group comprises >40% of infertile couples for whom no specific treatments are available because they have unexplained infertility or infertility due to other causes that persist after specific treatment.

Induction of mono-ovulation in anovulatory patients

Pituitary stimulation in hypogonadotrophic hypogonadism

Hypogonadotrophic hypogonadism, or World Health Organization (WHO) Type I anovulation, can be treated in a physiological manner with pulsatile GnRH or by replacement, using gonadotrophin treatment. Pulsatile GnRH is discussed in this section on hypogonadotrophic hypogonadism and gonadotrophin treatment is discussed in the following section on normogonadotrophic anovulation.

Patients with primary hypogonadotrophic amenorrhoea usually present with virtually absent endogenous GnRH secretion; a primary pituitary disorder is rare, and normal gonadotrophin secretion can be restored by the administration of exogenous pulsatile GnRH in the great majority of cases. The treatment of primary hypogonadotrophic amenorrhoea results in high ovulation rates (80–95% per treatment cycle), and pregnancy ensues in ~30% of cycles. The occurrence of multiple pregnancy is rare in these patients, possibly due to the lack of pituitary gonadotrophin priming at the outset of treatment (Filicori *et al*., 1994).

For several biological reasons, pulsatile GnRH treatment is the most specific method of ovulation induction for this condition if it is available.

When hMG is given to hypogonadotrophic subjects, FSH levels are elevated throughout the follicular phase and peak before ovulation. This pattern is in stark contrast to the endocrine events of the normal menstrual cycle when elevated FSH levels are present in the peri-menstrual transition (late luteal and early follicular phase) and decline thereafter until just before the pre-ovulatory surge. Although LH levels progressively increase during the follicular phase of the spontaneous menstrual cycle, LH is usually low in the hMG-induced follicular phase. In the spontaneous cycle, the dynamic interplay between LH and FSH levels provides an optimal stimulus for follicular recruitment at first and for the selection of the dominant follicle later. Conversely, the tonically elevated FSH levels seen typically in the hMG-induced follicular phase may predispose patients to develop ovarian hyperstimulation and multiple conception.

With pulsatile GnRH administration, the pituitary retains its ability to modify gonadotrophin output in response to negative and positive feedback stimuli. Thus, the physiological events leading to the maturation and ovulation of a single dominant follicle are preserved (Filicori *et al*., 1991).

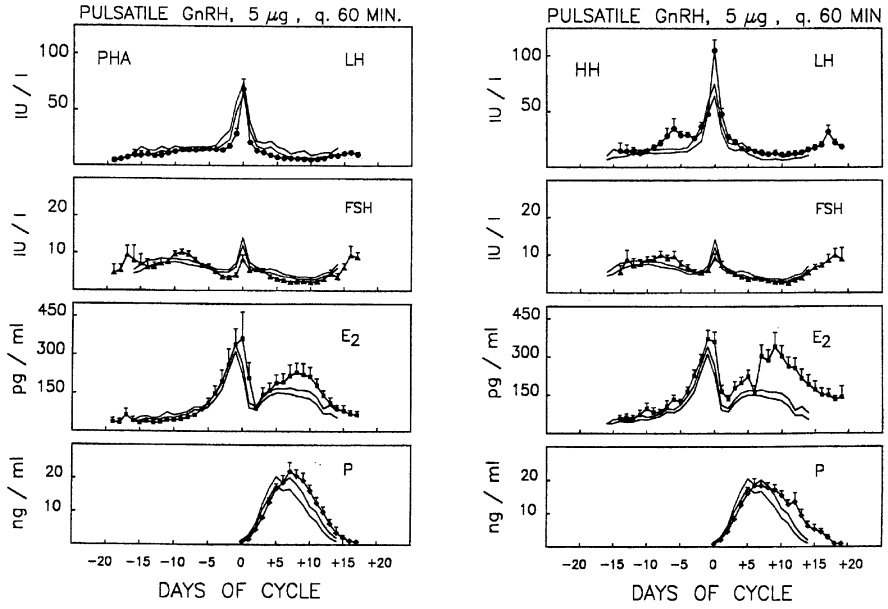

Figure 1. The endocrine patterns in the luteal phase in GnRH-induced cycles in patients with primary hypogonadotrophic amenorrhoea or hypogonadotrophic hypogonadism. Reproduced from Filicori, M., Flamigni, C., Meriggiola, M.C., Ferrari, P., Michelacci, L., Campaniello, E., Valdiserri, A. and Cognini, G. (1991) Endocrine response determines the clinical outcome of pulsatile gonadotrophin-releasing hormone ovulation induction in different ovulatory disorders. *J. Clin. Endocrinol. Metab.*, **72**, 965–972, with permission of the copyright holder, The Endocrine Society.

The endocrine dynamics of the midcycle pre-ovulatory surge in GnRH cycles are also noteworthy. Pre-ovulatory estradiol levels peak at 300–450 pg/l, thus indirectly confirming that a single ovarian follicle has achieved maturity. The midcycle LH surge occurs spontaneously after the estrogen peak and a modest increment of progesterone concentrations. These endocrine events faithfully recapitulate the pre-ovulatory dynamics of the normal menstrual cycle and assure that the ovulation signal occurs when optimal follicular maturity is achieved and not at a time artificially chosen by the physician. Furthermore, the spontaneous gonadotrophin surge avoids the need for pre-ovulatory administration of hCG.

The endocrine events of the luteal phase in GnRH-induced cycles (Figure 1) in patients with primary hypogonadotrophic amenorrhoea or hypogonadotrophic hypogonadism are also strikingly similar to those of spontaneous cycles. Estradiol and progesterone levels are normally elevated, and a late luteal phase rise of FSH is present; if pulsatile GnRH is continued for a second consecutive cycle, the normal dynamics of the luteal–follicular transition ensue. The duration of the GnRH cycle luteal phase may be longer than normal by a couple of days, but spontaneous menses occur in non-pregnant cycles in spite of continued GnRH stimulation. The corpus luteum can safely be supported by means of hCG treatment.

Pelvic ultrasound performed in the course of pulsatile GnRH ovulation induction shows that maturation of a single dominant follicle occurs in the majority of cycles; furthermore, the development of numerous (>3) large follicles (>15 mm diameter) is rare. The occurrence of frank ovarian hyperstimulation is practically non-existent. Although ultrasound and biochemical signs of excessive ovarian stimulation, such as enlarged ovaries and high E levels, may develop in a few patients, none of the complications that often require hospital admission after hMG ovulation induction (e.g. oliguria, ascites) have been reported to occur, and the incidence of multiple pregnancy is limited. Furthermore, even continuation of pulsatile GnRH (hCG should be avoided) does not lead to serious problems. Close endocrine monitoring with daily estradiol determinations is superfluous with this procedure. Thus pulsatile GnRH would appear to be the ideal candidate for outpatient ovulation induction.

Secondary hypogonadotrophic hypogonadism in underweight women

Pulsatile GnRH can be effectively employed in patients with weight loss and/or anorexia nervosa (Marshall *et al*., 1979; Braat *et al*., 1991). Results in these patients overlap on treatment outcome obtained in other subjects with different forms of hypogonadotrophic hypogonadism (Braat *et al*. 1991). The combined use of naloxone and pulsatile GnRH suggests that opiates could be involved in the pathogenesis of reduced gonadotrophin secretion in these patients (Giusti *et al*., 1988).

Pituitary stimulation in secondary hypogonadotrophic hypogonadism due to hyperprolactinaemia

When hypogonadotrophic anovulation is caused by hyperprolactinaemia, pulsatile GnRH administration will restore ovulation and normal gonadotrophin and steroid secretion. This finding indirectly confirms that deranged reproductive function in this disorder depends upon altered hypothalamic GnRH secretion. Of course in the presence of chronic anovulation due to an excessive prolactin secretion the best approach is restoring ovulatory cycles with a long-term treatment with

prolactin-lowering drugs (ESHRE Capri Workshop Group, 1995; Crosignani, 2002).

Restoring ovarian function in normogonadotrophic anovulation

In nearly 90% of anovulatory patients, serum gonadotrophin concentration is not significantly decreased and the normal ovulatory processes are inhibited by inherited or acquired endocrine and/or metabolic disturbances. In these patients a polycystic ovary is frequently found (Laven *et al*., 2002).

Another frequent finding in patients with normogonadotrophic anovulation is excessive body weight (Grodstein *et al*., 1994). A body mass index of >27 kg/m^2 is an important factor for decreased fertility (Barbieri, 2001). Not only the excessive amount but also the distribution of body fat is clearly related to irregular menstrual cycles, oligomenorrhoea and hirsutism (Hartz *et al*., 1984) and fecundity (Zaadstra *et al*., 1993). In addition, obesity is associated with an increased rate of miscarriage (Pettigrew and Hamilton-Fairley, 1997).

Overweight not only impairs spontaneous ovulation but also affects the ovarian response in pharmacologically induced cycles (Hamilton-Fariley *et al*., 1992; Filicori *et al*., 1994; Imani *et al*., 1998) or in assisted reproduction treatment cycles (Crosignani *et al*., 1994; Fedorcsak *et al*., 2000).

Ovulation induction in women with normogonadotrophic anovulation can be achieved by methods which release the ovary from some inhibitory process (reduction of insulin drive, ovarian wedge resection or diathermy) or by methods which stimulate the ovary, either by enhancing endogenous gonadotrophins or by administering exogenous gonadotrophins.

Diet and exercise

The first methods of induction of ovulation to advise are those designed to release the ovary from the high insulin drive present in many slim and most obese patients with polycystic ovary syndrome (PCOS) (Dunaif, 1997). Initially one attempts to correct metabolic disturbances by changes to lifestyle, most particularly by the patient adopting a programme of aerobic exercise (20–30 min aerobic exercise sufficient to cause sweating, three times per week) together with a diet low in calories and fat and favouring carbohydrates with low glycaemic index (Ludwig, 2002).

Weight loss can restore ovulation and fertility. Bates and Whitworth (1982) reported 10 pregnancies achieved in 13 PCOS patients who had lost 15% of body weight through a diet. In another study (Kiddy *et al*., 1992) 24 obese women with PCOS were prescribed a 1000 kcal, low fat diet and 13 subjects lost >5% of their starting body weight; five of them conceived. A few years later Clark *et al*. (1998) showed that weight loss can re-establish ovulation in obese anovulatory patients, or improve their response to ovulation induction. In a series of 67 anovulatory women, 90% resumed ovulation after weight loss, 78% conceived (33% spontaneously) and 67% achieved a live birth. These data have since been reconfirmed in a larger number of patients, in a study where weight loss was maximized through exercise and psychological support (Clark *et al*., 2000).

In a recent study carried out in 27 overweight PCOS patients with oligo-amenorrhoea, a weight loss of 5% was achieved by 25 women, 18 resumed regular cycles, 15 had spontaneous ovulation and 10 of them became pregnant. Interestingly a parallel reduction of ovarian volume and lower number of ovarian microfollicles was observed (Crosignani *et al*., 2002).

Thus, several studies show that lifestyle modification causing loss of fat increases (mono-)ovulation. Although the exact metabolic mechanisms have been difficult to define, as little as 5% weight loss can restore cycles.

Drugs enhancing sensitivity to insulin

Metformin is a biguanide which is used for the regulation of glucose levels in non-insulin-dependent diabetes, since it reduces liver production of glucose and enhances its peripheral metabolism (Bailey, 1992; Bailey and Turner, 1996). Additionally, it lowers the levels of LH, insulin and androgens in the plasma (Diamanti-Kandaraki *et al*., 1998).

It is important to recognize that the insulin resistance that characterizes perhaps ~40% of women with PCOS is due to the extra-splanchnic action of insulin on glucose disposal (Dunaif *et al*., 1992). However, the effectiveness of treatment with metformin is due in large part to its inhibition of hepatic gluconeogenesis (Kirpichnikov *et al*., 2002)—which is not disturbed in PCOS as it is in diabetes. Thus the benefit to be expected in patients with PCOS from treatment with metformin is necessarily less than predicted from experience gained in the treatment of diabetes. Insulin sensitization by other drugs, most particularly the newer glitazones, has little to offer the patient with anovulatory infertility since these medications are thought to be fetotoxic.

Recent studies have indicated that metformin and other insulin-sensitizing drugs normalize the menses, increase spontaneous ovulation and increase pregnancy rates in anovulatory patients wishing to conceive. In some studies the overweight patients registered the best results (Morin-Papunen *et al*., 1998; Nestler *et al*., 1998; Velazquez *et al*., 1998; Moghetti *et al*., 2000; Heard *et al*., 2002). The effect on miscarriage rates remains uncertain, although untreated hyperinsulinaemia and insulin resistance may be independent risk factors predisposing to first trimester miscarriages (Regan *et al*., 1989). In a retrospective comparison the miscarriage rate was significantly reduced in women with PCOS when metformin treatment was continued throughout pregnancy (Glueck *et al*., 2001), but in a prospective study the miscarriage rate among metformin-treated patients was 35% (Heard *et al*., 2002).

Five randomized controlled trials compared metformin and placebo in CC-resistant PCOS patients who received CC treatment with metformin or placebo (Nestler *et al*., 1998; Ng *et al*., 2001b; Kocak *et al*., 2002; Sturrock *et al*., 2002; Vandermolen *et al*., 2002). The ovulation rates generally were higher with metformin and CC than with placebo and CC, but the trials with pregnancy as an outcome involved only 127 patients and 18 pregnancies overall (Table I). None of the individual trials had sufficient power to evaluate differences in pregnancy or live birth rates. Only one trial has compared the

Table I. Randomized controlled trials involving clomiphene citrate (CC) administration after metformin or placebo pre-treatment

Authors	CC dose (mg)	CC cycles	Events/number randomized			
			Metformin + CC		Placebo + CC	
			Ovulation	Pregnancy	Ovulation	Pregnancy
Nestler *et al.* (1998)	50	1	19/21		2/25	
Vandermolen *et al.* (2001)	Up to 150	Up to 6	9/12	6/12	4/15	1/15
Ng *et al.* (2001b)	100	1	4/10	1/10	7/10	2/10
Kocak *et al.* (2002)	100	1	21/28	3/28	4/27	0/27
Sturrock *et al.* (2002)	50–100	3	5/12	3/12	4/14	2/14

Table II. Randomized controlled trials to evaluate the efficacy of clomiphene citrate (CC)

Authors	Patients	Ovulation (%)		Pregnancy (%)	
		CC	Control	CC	Control
Cudmore and Tupper (1966)	62	48.4	41.9	20.0	12.9
Johnson *et al.* (1966)	22	61.5	22.2	11.1	5.3
Connaughton *et al.* (1974)	46	83.3	36.4	33.3	9.1
Garcia *et al.* (1985)	71	57.9	9.1	14.3	1.5
Total	201				
Average difference (random effects)		37.3		12.0	
No. needed to treat (95% CI)		3 (2, 7)		12.08 (5, 26)	

metformin and CC option with gonadotrophin, the established treatment for CC-resistant anovulation. Although this trial also was underpowered for a comparison of pregnancy rates, 5/30 (17%) women randomized to metformin and CC conceived compared with 7/30 (23%) in the gonadotrophin group (George *et al*., 2003).

The use of metformin is generally well-tolerated, although occasional gastrointestinal disturbances occur which diminish after 2 weeks of administration. No known teratogenicity occurs in animal models, but there is insufficient experience in human pregnancy to rule out an adverse effect on fetal and neonatal outcomes from use during pregnancy.

In summary, metformin is a promising treatment, but its role among other treatments for normogonadotrophic anovulation has not been established. Larger trials with live birth as the outcome are needed among anovulatory patients to compare metformin with CC as primary treatment and with gonadotrophin in CC-resistant patients. It would be useful if these studies were designed to evaluate independently the treatment effects among obese and non-obese patients.

Laparoscopic ovarian diathermy

Surgical treatment also may release the ovary from some inhibitory process, allowing monofollicular ovulation. Bilateral ovarian wedge resection has been replaced by the modern-day equivalent, laparoscopic ovarian diathermy (LOD) (Donesky and Adashi, 1995). The mechanisms by which these procedures enhance ovulation are unknown; they are thought to involve modification(s) of ovarian–pituitary feedback relationships (Balen and Jacobs, 1994). Whatever the mechanism, the result is a satisfactory rate of mono-ovulation. The indication for LOD is correction of anovulatory infertility in slim women with raised serum LH concentrations (i.e. almost the opposite of that for metformin). The risks are ovarian damage and peri-ovarian adhesions; the advantages are that LOD is a simple procedure which usually results in prolonged resumption of ovulation and avoids ovarian hyperstimulation. The potential for ovarian damage, however, implies that in most cases pharmacological treatment should be considered first.

Pharmacological induction of ovulation in normogonadotrophic patients

In patients with normogonadotrophic anovulation who do not ovulate following use of diet and exercise programmes to control insulin secretion, the next step is ovarian stimulation. The majority of these patients will have polycystic ovaries. Ovarian stimulation in this group can be accomplished by enhancing endogenous gonadotrophin secretion with drugs acting on the hypothalamo-pituitary axis (antiestrogens or pulsatile GnRH) or by the use of drugs acting directly on the ovary (gonadotrophins).

Antiestrogens

The antiestrogens involved in the induction of ovulation are CC and tamoxifen (ESHRE Capri Workshop Group, 1995; 1997). While tamoxifen is mainly used for the prevention and treatment of breast cancer, for 6–8 weeks, CC is preferred to

induce ovulation in anovulatory infertility and to augment ovulation in some cases of unexplained or persistent infertility. Multiple births can be associated with any of these clinical applications of CC.

Four randomized controlled trials which compared CC and placebo treatment of anovulatory women all featured crossover designs. Although the protocols were not typical of those in current use, the average likelihood of ovulation was 2-fold higher with CC (Cudmore and Tupper, 1966; Johnson *et al*., 1966; Connaughton *et al*., 1974; Garcia *et al*., 1985). On average there would be one additional ovulation for every three anovulatory women receiving CC treatment rather than placebo (95% CI 2, 7) (Table II).

Based on the first phase experience only (in the trials where it was separately reported), overall pregnancy rates were 19 and 6% in CC and placebo users respectively. In a random effects model, the typical result would be one additional pregnancy for every eight women treated with CC rather than placebo (95% CI 5, 26). None of the four studies mentioned multiple pregnancy.

The likelihood of ovulation and conception in anovulatory women with CC treatment with current regimens has been carefully evaluated in studies from Rotterdam. Among 201 anovulatory women, 99 (49%) ovulated while receiving the initial dosage (50 mg for five days), 44 (22%) at the next dosage (100 mg), and 13 (6.5%) at the final dosage (150 mg), making a total of 156 (78%) (Imani *et al*., 1998). Of the 156 ovulating women, 51% conceived and 73 women had live births (36% of 201 women) (Imani *et al*., 1999).

Although CC is preferred, tamoxifen appears to have similar effects on ovulation and would be useful in patients with allergic or other reactions. In a randomized controlled trial involving 95 women, ovulation rates per cycle with CC and tamoxifen were 45 and 44% per cycle respectively and pregnancy rates were 7 and 9% respectively (Boostanfar *et al*., 2001). Cyclophenil, another triethylene derivative, does not appear to be useful for the induction of ovulation (Yong *et al*., 1992; Acharya *et al*., 1993).

CC treatment does not require monitoring by costly methods. Ultrasound scanning after the treatment can easily diagnose multiple gestations, which are relatively infrequent (8–10%) despite the fact that multifollicular development is common in CC-treated cycles.

Pulsatile GnRH

In normogonadotrophic anovulation, pulsatile LH release is usually altered, and restoration of a physiological pattern of pulsatile LH secretion with exogenous GnRH results in the resumption of ovulation in 90% and pregnancy in >25% of treatment cycles (Homburg *et al*., 1989; Martin *et al*., 1990; Filicori *et al*., 1994). Nevertheless, in these patients exogenous GnRH stimulation often causes brisk gonadotrophin secretion; follicular phase LH and FSH levels are significantly higher than in normal spontaneous cycles. This excessive gonadotrophin secretion may result in the maturation of more than a single dominant ovarian follicle; thus the multiple pregnancy rate is higher than that in GnRH-treated hypogonadotrophic patients.

In normogonadotrophic anovulation with polycystic ovaries, the frequency and/or amplitude of LH pulses is already excessive. Consequently, further gonadotrophin stimulation with pulsatile GnRH cannot be expected to be very effective in this disorder; the ovulation rate is only 40–50% and the pregnancy is $\leqslant$16% per cycle (Homburg *et al*., 1989; Filicori *et al*., 1994).

To improve the response in PCOS, a reversible hypogonadotrophic condition before the initiation of pulsatile GnRH could be induced (Filicori *et al*., 1988; 1989) involving pituitary suppression for 6–8 weeks with a GnRH analogue. Even with this improvement, however, pulsatile GnRH is rarely used for normogonadotrophic anovulation with or without polycystic ovaries (Shoham *et al*., 1990).

Gonadotrophins

Gonadotrophin therapy is the first line of treatment for patients with CC-resistant anovulation, and also for many who ovulate during CC treatment but do not conceive. For the best possible response to gonadotrophin treatment, it is preferable to ensure that each patient has achieved optimal metabolic and endocrine status prior to the use of ovarian stimulants. It is possible that new neurally active drugs that control feeding behaviour, combined with aerobic exercise and insulin sensitizers, may reduce the number of patients with normogonadotrophic anovulation and polycystic ovaries needing medications such as gonadotrophins which act directly on the ovary, but direct evidence for the effectiveness of such methods is not available.

Approximately 40% of women with WHO II anovulatory infertility need gonadotrophin therapy, either because they are anovulatory with CC treatment (CC-resistant) or, having ovulated with CC treatment, they do not conceive (CC failure). Around 80–90% of these patients ovulate, depending on cancellation criteria. Cumulative pregnancy rates approach 60% within 6 months (Imani *et al*., 2002). The most important complications are multiple pregnancies and ovarian hyperstimulation syndrome (OHSS).

A difficult challenge in present-day infertility practice is the high rate of multiple pregnancy and ovarian hyperstimulation among women with anovulatory infertility and polycystic ovaries. Thus, ovarian stimulation of patients with normogonadotrophic anovulation should always be preceded by ultrasound evaluation of ovarian morphology. Gonadotrophins should be administered in low doses to women with normogonadotrophic anovulatory infertility and in particular when the ovary has a polycystic appearance. Strict criteria should be employed before the administration of the ovulatory trigger.

Table III shows the results of six studies of conventional dose gonadotrophin therapy (i.e. 150 IU/day) and six studies of low-dose therapy (i.e. 75 IU/day) (Hull, 1992). Although pregnancy rates are higher and miscarriage rates are lower with standard dose than low-dose therapy, the multiple pregnancy

Table III. Comparison of gonadotrophin regimens (Hull, 1992)

Gonadotrophin regimen	Conventional	Low dose
No. of patients	111	243
Pregnancy rate per cycle (%)	23	11
Pregnancy rate per ovulatory cycle (%)	30	15
Miscarriage rate (%)	17	37
Ongoing pregnancy rate per cycle (%)	19	7
Multiple pregnancy rate (%)	23	9

rate was 23% in the standard dose cycles compared with 9% in the low-dose cycles.

A number of alternative protocols of low-dose gonadotrophin stimulation have been devised to try to increase the rate of monofollicular ovulation and so reduce the rate of multiple pregnancy and ovarian hyperstimulation (White *et al*., 1996). Head-to-head comparisons of substantial numbers of patients have not been reported but small doses of gonadotrophin, careful ultrasound surveillance and strict criteria for the administration of hCG may be the most important factors in achieving monofollicular ovulation. There are no comparisons of mixed (LH plus FSH) gonadotrophin preparations versus FSH alone with respect to their efficacy in inducing monofollicular ovulation.

While low gonadotrophin dosage is important, the balance between success or complications also depends on three additional conditions: (i) preparation and dose regimen factors, (ii) patient factors and (iii) cycle management factors.

Preparation and dose regimen factors

Several new low-dose protocols have been devised over the last two decades to address this intrinsic sensitivity of the polycystic ovary to ovarian stimulation (Balen and Jacobs, 1997). The new protocols all attempt to balance the risk of non-response against the risk of over-response.

The conventional low-dose step-up regimen is safe and effective but has an extended stimulation period. The initial daily dose is 50 to 75 IU, and this is increased by 75 IU/day if there is no ovarian response after 2 weeks. The maximum daily dose is usually 225 IU. The step-down dose regimen more closely mimics serum FSH levels during the follicular phase of the normal menstrual cycle. Here the initial daily dose is 150 IU, and this is decreased in two subsequent steps until a final daily dose of 75 IU.

In a randomized comparison of low-dose step-up and step-down dose regimens, the duration of stimulation was significantly reduced with the step-down protocol and there were more mono-follicular cycles and more cycles with serum estradiol levels within the normal range (van Santbrink and Fauser, 1997). This small study lacked the power, however, to allow firm clinical inferences. Indeed, until now, there have been very few high quality and adequately powered studies in the area of ovulation induction.

The chief drawbacks of the step-down approach are: (i) the initial dose of 2 ampoules/day is too high for some patients; (ii) and it is not always clear when the initial dose should be reduced.

With respect to type of preparation, experience so far has failed to demonstrate improved treatment outcome when recombinant FSH is used rather than hMG.

With respect to co-interventions, patients pretreated with metformin then stimulated with FSH had significantly fewer follicles >15 mm in diameter on the day of hCG administration, significantly lower estradiol plasma levels and fewer cycle cancellations (DeLeo *et al*., 1999; Stadtmauer *et al*., 2001). The authors suggested that the decrease of estradiol levels in plasma and the reduction of multifollicular growth with the metformin administration can possibly reduce the incidence of OHSS and multiple pregnancies.

Patient-related factors

The most likely barrier to mono-ovulation in patients with normogonadotrophic anovulation and polycystic ovaries using conventional methods of ovulation induction is related to features intrinsic to the polycystic ovary itself. Thus, ultrasound evidence of polycystic ovaries predicts a multifollicular response to ovarian stimulation, irrespective of the ambient pituitary and ovarian hormone levels (Shoham *et al*., 1991). The multifollicular response is in part due to the large number of follicles present that are immediately sensitive to gonadotrophin stimulation. It also seems that the intra-ovarian autoregulatory process, by which one follicle normally becomes dominant and cohort follicles become inhibited, is readily overcome by stimulation with exogenous or antiestrogen-stimulated endogenous gonadotrophin.

Nevertheless, little is known about the clinical factors which predict ovarian sensitivity for stimulation by FSH, and whether these factors are also involved in ovulation induction outcome. Prospective follow-up studies on ovulation induction outcome in ~200 normogonadotrophic oligoamenorrhoeic infertile women established that patient age, the presence of polycystic ovaries and elevated testosterone levels are predictive of treatment outcome (Fauser *et al*., 1999; Imani *et al*., 2002). Further studies should focus on whether initial screening characteristics can predict the gonadotrophin doses needed, the likelihood of success and the probability of complications due to multiple follicle development.

Cycle management factors

Other factors that help to determine the outcome of gonadotrophin ovulation induction include careful monitoring of ovarian response (by transvaginal pelvic ultrasound, and possibly serum estradiol levels) and the conscientious application of criteria to cancel the cycle in case of multiple follicle development. Additional techniques to 'rescue' the cycle under these circumstances include 'coasting' or puncture of additional follicles.

Table IV. Background pregnancy rates in an infertile population (adapted from Gleicher et al., 1996)

Infertile couples waiting for treatment	1016
Untreated cycles	5541
Clinical pregnancies	112
Pregnancies per year (%)	20

Table V. Spontaneous pregnancy rate per year in couples with primary unexplained infertility (adapted from Collins and Rowe, 1989)

Duration of infertility (years)	Age of woman (years)	
	26–30	⩾35
3–5	31.8	0
⩾5	28.2	0

Table VI. Spontaneous pregnancy rate per year in couples with male infertility (adapted from Hargreave and Elton, 1986)

Duration of infertility (years)	Sperm concentration ($\times 10^6$)	
	<2	2–10
2–4	27	37
⩾4	0	20

Toward mono-ovulation in assisted reproductive treatment cycles

Limits of conventional treatment in subfertile couples

The prognosis for infertile couples in the absence of specific defects (anovulation, tubal disease, azoospermia) is not well known. Observations of subfertile couples conducted in specialized clinics indicate that 14–20% may have successful conceptions within 1 year but the overall success rate is not >50% (Table IV, Table V and Table VI). With duration of infertility <3 years, secondary infertility or a female partner <30 years old, the prognosis is above average. Although the prognosis is lower with longer duration and severe factors, there is continuing residual background fertility. In a 5 year follow-up study on 200 couples after they discontinued ICSI treatment, the cumulative rate of spontaneous conceptions leading to delivery was 10% after 36 months of follow-up (Osmanagaoglu *et al*., 2002).

In treating couples in whom conventional treatment has not been successful, clinicians rely on strategies designed aspecifically improve fertility by increasing the number of follicles and bringing gametes into closer proximity. These strategies may involve simple [CC and intrauterine insemination (IUI)] or advanced treatment protocols (ovulation induction and IVF with or without ICSI). Since ovarian hyperstimulation increases the risk of multiple pregnancy; every attempt should be made to achieve increased fertility in patients with unexplained or persistent female infertility without excessive numbers of follicles.

Derom *et al*. (1993) reported that 77% of twins and 72% of triplets born in Belgium were associated with isolated ovarian stimulation. Assisted reproductive treatment and ovulation induction each account for 40% of the triplet and higher-order births in the USA (Division of Reproductive Health, 2000). In two multicentre studies in the USA, the multiple birth rates were particularly high (29 and 33%) in ovulation induction cycles with gonadotrophins, and up to 9% of these were high-order multiple pregnancies (Guzick *et al*., 1999; Gleicher *et al*., 2000).

Milder ovarian stimulation as isolated treatment or used in combination with assisted reproductive technology cycles

Lack of power characterizes most studies of CC administered in ovulatory patients for the treatment of unexplained infertility. CC treatment has been evaluated in four trials involving 1588 women (Harrison and O'Moore, 1983; Fisch *et al*., 1989; Glazener *et al*., 1990; Fujii *et al*., 1997). The pregnancy rate with CC treatment was significantly higher in the combined results from three trials and significantly lower in the fourth (Fujii *et al*., 1997). In the fourth trial, the fecundity in the control group was >20% per cycle, that is, similar to non-infertile couples. The overall effect of CC treatment is small, however, even in the three positive trials: the number needed to treat is 40 cycles: one additional pregnancy would occur in 40 CC cycles (95% CI 22, 201) treated with CC compared with untreated control cycles. Although significant, the effect of CC treatment seems clinically unimportant. In a further trial, the effect of CC with IUI was somewhat better: one additional pregnancy in 16 CC/IUI cycles (95% CI 9, 165) compared with untreated control cycles (Deaton *et al*., 1990). The authors did not comment on multiple births. A retrospective analysis of 1713 cycles involving CC treatment and IUI reported 176 pregnancies, including 14 (8%) twins and three (2%) triplets or higher order (Dickey *et al*., 2002). These resulted in 127 births, including 12 (9%) twins, but no triplet births.

CC/IUI treatment is cheaper than gonadotrophin treatment for persistent infertility, the multiple birth rate is lower and the difference in effectiveness between CC and gonadotrophin is not large (Ecochard *et al*., 2000).

In a recent randomized study comparing CC (51 patients) and FSH (49 patients) ovarian stimulation in a donor insemination programme, CC treatment was associated with a lower pregnancy rate (6.1 versus 14.4%), a reduced twin rate (12.5 versus 20.0%) and (more important) with the absence of triplet pregnancy (6% in the FSH group) (Matorras *et al*., 2002).

In the past it was impossible to reduce the risk of multiple births without reducing the conception rate (Collins, 1994). With the combined use of low-dose FSH and a GnRH antagonist, promising results have been recently reported. In 27 women undergoing IUI, the pregnancy rate was surprisingly high, and all gestations were singleton pregnancies (Alagna *et al*., 2002).

Table VII. Reported success in unstimulated IVF cycles

Authors	Pregnancy rate per cycle (%)	Comment
Claman *et al.* (1993)	3	
Macdougal *et al.* (1994)	0	14 attempts
Daya *et al.* (1995)	5	
Ng *et al.* (2001a)	13	
Omland *et al.* (2001)	10	Endometriosis
	11	Tubal defect
	3	Unexplained infertility
Nargund *et al.* (2001)	13	Indomethacin

One of the key points of controlled ovarian stimulation (COS) for IVF is the achievement of adequate synchronization of follicular growth so that ovulation can be triggered when the follicles have reached a similar level of maturity. However, the premature, gradual exposure of follicles to FSH during the late luteal phase of a previous cycle (Hillier *et al*., 1980; Roseff *et al*., 1989), may accelerate the development of more sensitive follicles and accentuate follicular size discrepancies observed during the first days of the subsequent cycle. Follicular heterogeneity during the early follicular phase provides a possible explanation for the better results reported with COS protocols preceded by GnRH agonist or oral contraceptive administration as compared to those without some form of luteal FSH control (Gonen *et al*., 1990; Cramer *et al*., 1999).

In a recent study (R.Fanchin personal communication) the administration of estradiol during the luteal phase effectively reduced: the discrepancy between the sizes of the follicles, the mean diameter of early antral follicles, and the mean ovarian volume. In addition the study suggests that the subsequent administration of a GnRH antagonist may further improve the coordination in the set of growing follicles.

Administering gonadotrophin preparations during the mid- and late-follicular phase may also be a realistic means of subtle interference with single dominant follicle selection for IVF (de Jong *et al*., 2001; Hohmann *et al*., 2003).

IVF in the untreated cycle

The first attempts at IVF took place in the untreated cycle. Edwards *et al*. (1980) reported the outcome of the first 68 patients in whom an LH surge could be identified. Pre-ovulatory oocytes were aspirated from most of the patients. Fertilization and cleavage occurred in 34 instances, and 32 embryos were transferred. Four patients became pregnant.

In an attempt to increase pregnancy chances, ovarian stimulation was introduced in order to replace more than one embryo (Fishel *et al*., 1985).

More recently, in response to the improved laboratory and clinical IVF procedures, the mean number of embryos transferred was reduced, especially in Europe, in an attempt to reduce the frustratingly high multiple pregnancy rates and this new strategy coincided with the resurgence of interest in IVF in the untreated cycle.

Success in unstimulated IVF cycles has been somewhat lower than in stimulated cycles, although there was a rising trend in publications from 1993 through 2001 (Table VII). The pregnancy rate was 13% per treatment cycle when indomethacin was used in some of the patients to delay follicle rupture and prevent weekend retrievals (Nargund *et al*., 2001). Patients undergoing untreated cycle IVF are more likely to have abandoned cycles, produce fewer follicles and oocytes, and less likely to reach embryo transfer than stimulated cycle patients, but of course there is less complexity and cost in untreated cycles. Daya *et al*. (1995) suggested that despite the high failure rate at each step in the process, untreated cycles are more cost-effective than stimulated cycles because the incremental cost per live birth in stimulated cycles is higher due to the obstetrical and neonatal complications of the associated multiple pregnancies.

Conclusions

Serious attempts to achieve mono-ovulation in treatments involving ovarian stimulation are needed to prevent the burden of illness associated with multiple births. Mono-ovulation is more likely when the initial steps in any treatment plan address changes in diet and exercise, and when pharmacological treatment begins with drugs that restore or influence ovulation at the hypothalamic or pituitary level. When gonadotrophins are used, their direct effect on the ovary should be evaluated by careful monitoring, and over-stimulation should be minimized by the use of low-dose protocols. More trials are needed to evaluate the safety and efficacy of insulin lowering agents and ovarian diathermy, compared with gonadotrophin treatment. Given the current limits of IVF treatment outcomes, it may be appropriate to explore minimal stimulation protocols that would reduce multiple births, complexity and cost while improving access to this important treatment.

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