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Obesity and reproductive disorders in women

Renato Pasquali[1], Carla Pelusi, Silvia Genghini, Mauro Cacciari and Alessandra Gambineri

Division of Endocrinology, Department of Internal Medicine and Gastroenterology, S. Orsola-Malpighi Hospital, University of Bologna, Italy

[1]To whom correspondence should be addressed at: U.O. di Endocrinologia, Ospedale S. Orsola-Malpighi, Via Massarenti 9, 40138 Bologna, Italy. E-mail: rpasqual@almadns.unibo.it

Obesity, particularly the abdominal phenotype, is associated with several reproductive disturbances. Whereas mechanisms by which obesity affect fertility are complex and still not completely understood, an important role appears to be played by the presence of a condition of functional hyperandrogenism and hyperinsulinaemia, which accompanies the insulin-resistant state. In women with the polycystic ovary syndrome, abdominal obesity may be co-responsible for the development of hyperandrogenism and associated chronic anovulation, through mechanisms primarily involving the insulin-mediated overstimulation of ovarian steroidogenesis and decreased sex hormone-binding globulin blood concentrations. By these mechanisms, obesity may also favour resistance to clomiphene and gonadotrophin-induced ovulation and reduce outcomes of IVF/ICSI procedures. Due to the beneficial effects of weight loss, lifestyle intervention programmes should represent the first-line approach in the treatment of infertile obese women. Insulin-sensitizing agents may add further benefits, particularly if administered in combination with hypocaloric dieting. Therefore, individualized pharmacological support aimed at favouring weight loss and improving insulin resistance should be widely extended in clinical practice in obese infertile patients. This may be beneficial even during pregnancy, thereby permitting favourable physiological delivery and healthy babies.

Key words: insulin sensitizers/obesity/polycystic ovary syndrome/reproduction/weight loss

Reproductive disorders in obese women: epidemiological aspects

Overweight and obesity represent a rapidly growing threat to the health of populations and an increasing number of countries worldwide (World Health Organization, 1997). Many dietary, lifestyle and possibly ethnic factors may prove to be important in determining the magnitude of the complications associated with obesity. These include non-insulin-dependent diabetes mellitus, cardiovascular diseases, cancers, gastrointestinal diseases and arthritis. In addition, significant associations are seen in reproductive endocrinology between excess body fat (particularly abdominal obesity) and irregular menstrual cycles, reduced spontaneous and induced fertility, increased risk of miscarriage and hormone-sensitive carcinomas (Kirschner, 1982; Pasquali and Casimirri, 1993). Distinct changes in circulating sex hormones appear to underline these abnormalities.

The association between alterations of the reproductive functions in women was recognized long ago. In an original description (Stein and Leventhal, 1934), obesity—together with hirsutism and infertility—represented one of the characteristics of the eponymous syndrome. Much later, others (Rogers and Mitchell, 1952) showed that 43% of women affected by various menstrual disorders, infertility and recurrent miscarriages were either overweight or obese. More recently, it was shown that the presence of anovulatory cycles, oligoamenorrhoea and hirsutism, either separately or in association, were significantly higher in obese than in normal-weight women (Hartz *et al*., 1979). In addition, the same authors found that the incidence of obesity during puberty and early adolescence was greater in adult married women without children than in those having had one or multiple pregnancies, thus confirming the existence of a correlation between obesity and infertility. Similar findings have been reported by others (for a review, see Norman and Clark, 1998).

The relationship between excess body fat and reproductive disturbances appears to be stronger for early-onset obesity, although this remains a controversial issue due largely to the heterogeneity of overweight or obese pre-adolescent or adolescent populations investigated (Azziz, 1989). There are several epidemiological studies which suggest that changes in body weight and/or body composition are critical factors regulating pubertal development in young women (Frisch and McArthur, 1974; Frisch, 1980; Fishman, 1985). The discovery of leptin provided a unique explanation in this complex circuit. Leptin is a main product of body fat (Considine *et al*, 1996) and, at the same time, regulates the gonadotrophin surge which initiates the development

of pubertal stages (Farooqi *et al*., 1999). Indirect confirmation of this derives from evidence that in the leptin-deficient *ob/ob* mouse the reproductive system remains pre-pubertal (O'Railly, 1998). Several studies have repeatedly reported that the age of menarche generally occurs at a younger age in obese girls than in normal-weight girls (Montemagno *et al*., 1979; Bruni *et al*., 1985). Just as the onset of menarche is earlier in obese women, so data also suggest that the onset of ovarian failure and increased production of FSH at menopause occurs several years earlier in obese than in normal-weight women (Bray, 1997; Norman and Clark, 1998). In adolescent and young women, the age of onset of obesity and that of menstrual irregularities are significantly correlated (Pasquali *et al*., 1985). In addition, data exist which indicate that the association with menstrual disorders may be more frequent in girls with onset of excess body weight during puberty than in those who were obese during infancy. These findings have been substantially confirmed in a large study which was performed in approximately 6000 women (Lake *et al*., 1997) and showed that obesity in childhood and in the early twenties increased the risk of menstrual problems. It is therefore likely that overweight and obesity do contribute to a significant proportion of menstrual disorders in young women.

Although many multiparous women are obese, evidence exists that obesity may also affect fertility rates in women within the fertile age. In the Nurses' Health Study (Rich-Edwards *et al*., 1994) it was reported that the risk of ovulatory infertility increased in women with increasing body mass index (BMI) values. Several other cross-sectional and prospective studies have produced similar findings (Bray, 1997; Norman and Clark, 1998). Similarly, there are consistent data indicating that obesity is also associated with an increased risk of miscarriage (Norman and Clark, 1998). In contrast, others (Zaadstra *et al*., 1993), while examining a large group of nulliparous healthy women who presented for artificial insemination due to infertility of their partners, found that body fat distribution other than fat amount was associated with a decreasing chance of conception. Therefore, due to the increasing world epidemic of obesity during the past decade, it is believed that much more updated investigations should be performed in order to evaluate whether this is associated with a parallel increase of related adverse effects on fertility in women (Table I).

Table I. Potential adverse effects of obesity on fertility in women

Adverse effect
Precocious menarche
Irregular cycles, oligo/amenorrhoea
Chronic anovulation
Increased risk of miscarriage
Decreased conception rates after assisted reproductive technologies
Increased morbidity in pregnant women
Worsened outcomes of preterm deliveries
Increased androgenization
Pathophysiological implication in determining the polycystic ovary syndrome and associated metabolic syndrome

Obesity and pregnancy

Many women tend to become overweight or obese during pregnancy, and many are already obese when they become pregnant. Several environmental and dietary factors and genetic predisposition to diabetes may also favour an increased tendency to gain excess body fat throughout pregnancy. During the past few decades, a number of reports have been published on the potential risk of obesity on pregnancy and obstetric outcome (Calandra *et al*., 1981). In general terms, obesity increases morbidity for both the mother and fetus. In a large European study which included both overweight and normal-weight pregnant women (Galtier-Dereure *et al*., 1995), the former were found to have an increased risk in pregnancy of hypertension, toxaemia, gestational diabetes, urinary infections, Caesarean section and increased hospitalization. In that study, no perinatal mortality was observed, but in spite of a similar incidence of intrauterine growth retardation in both groups, many more infants of obese patients needed care in a paediatric unit. These findings were substantially confirmed by three studies (for a review, see Kalkhoff, 1991) which recorded perinatal mortality rates for infants born of normal-weight and obese mothers. It therefore appears that maternal obesity has no major effect on neonatal death rates. At variance, however, there are data which support the view that maternal obesity may have a greater deleterious effect on the outcome of pre-term deliveries than on full-term parturition (Lucas *et al*., 1988). Finally, there are data suggesting a greater risk for congenital malformation—particularly neurological—in fetuses of mothers with simple obesity, which appears to be independent of age (Waller *et al*., 1994; Prentice and Goldberg, 1996; Shaw *et al*., 1996; Watkins *et al*., 1996; Werler *et al*., 1996; Kallen, 1998). The negative impact of obesity on fertility may therefore be extended to the pregnancy state and newborns, who hopefully represent the final expected result of a physiological full reproductive process in each woman.

Mechanisms by which obesity may affect fertility in women

Fertility processes involve a complex of factors and mechanisms of both ovarian and extra-ovarian origin. Obesity may interfere with many neuroendocrine and ovarian functions, thereby reducing both ovulatory and fertility rates in otherwise healthy women. As previously reported, obesity affects reproductive function early in life, both before and during pubertal development. Moreover, it clearly appears that it is associated with an increased risk of hyperandrogenism and anovulation in women in reproductive age, as supported by the strong association between obesity and the polycystic ovary syndrome (PCOS), the most common hyperandrogenic disorder (Yen, 1980; Conway *et al*., 1989; Franks, 1989). The mechanisms for the relationship between hyperandrogenism and obesity are multifactorial, and this subject has recently been the subject of an extensive review (Gambineri *et al*., 2002a). In the following sections, available data will be summarized which support the concept that simple obesity—particularly the abdominal phenotype—may be associated with several alterations in the balance of sex hormones, particularly androgens, leading to a condition of 'functional hyperandrogenism'. The pathophysiological link between obesity and PCOS will then be reviewed. Finally, the available evidence will be summarized that weight

loss and correction of insulin resistance may offer a practical means of improving spontaneous and stimulated ovulation and fertility in obese women with PCOS, which further emphasizes the potential negative impact of obesity on fertility in selected individuals.

Obesity as a condition of sex hormone imbalance in women

It is well known that an increase in body weight and fat tissue is associated with several abnormalities of sex steroid balance, especially in fertile women. Such alterations involve both androgens and estrogens and overall their carrier protein, sex hormone-binding globulin (SHBG). Changes in SHBG concentrations lead to an alteration of androgen and estrogen delivery to target tissues. SHBG levels are regulated by a complex of factors, including estrogens, iodothyronines and growth hormone as stimulating agents, and androgens and insulin as inhibiting factors (Von Shoultz and Carlstrom, 1989). The net balance of this regulation is probably responsible for the decrement in SHBG concentration observed in obesity. Body fat distribution has also been shown substantially to affect SHBG concentrations (Pasquali *et al*., 1990). In fact, female subjects with central obesity usually have lower SHBG concentrations in comparison with their age- and weight-matched counterparts with peripheral obesity (Von Shoultz and Carlstrom, 1989). This seems to be dependent on higher circulating insulin in abdominally obese women and on the inhibiting capacity of insulin on SHBG liver synthesis. Reduction of circulating SHBG determines an increase in the metabolic clearance rate of circulating SHBG-bound steroids, specifically testosterone, dihydrotestosterone and androstenediol, which is the principal active metabolite of dihydrotestosterone (Samojlik *et al*., 1984). However, this effect is compensated by a consequent elevation of production rates. Obesity also affects the metabolism of the androgens not bound to SHBG. In fact, both production rates and metabolic clearance rates of dehydroepiandrostenedione and androstenedione are equally increased in obesity (Kirschner *et al*., 1990). The role of adipose tissue is crucial in controlling the balance of sex hormone availability in the target non-fat tissues; indeed, adipose tissue is able to store various lipid soluble steroids, including androgens. Most sex hormones appear to be preferentially concentrated within the adipose tissue rather than in the blood (Table II). As a consequence, since the amounts of fat in obesity are larger than their intravascular space, and the steroid tissue concentration is much higher than in plasma, the steroid pool in obese individuals is greater than that found in normal-weight individuals (Azziz, 1989). Fat also represents a site of intensive sex hormone metabolism and inter-conversion, due to the presence of several steroidogenic enzymes, such as 3β-dehydrogenase, 17β-hydroxydehydrogenase and the aromatase system. This topic has been extensively reviewed in previous studies (Azziz, 1989; Gambineri *et al*., 2002a; Pasquali *et al*., 2003). The pattern of body fat distribution can regulate androgen production and metabolism to a significant extent. In fact, women with central obesity have higher testosterone production rates than those with peripheral obesity (Kirschner *et al*., 1990). Accordingly, metabolic clearance rates of testosterone and dihydrotestosterone are significantly higher in women with central than peripheral obesity. The maintenance of normal circulating levels of these hormones in obesity may lead to predicting the presence of a sophisticated regulation which can adjust both the production rate and the metabolic clearance rate of these hormones to body size. Due to the greater reduction of SHBG concentrations, the percentage free testosterone fraction tends to be higher in women with central obesity than in those with peripheral obesity (Evans *et al*., 1990). An inverse correlation exists between waist-to-hip ratio (or other indices of body fat distribution) and testosterone or SHBG concentrations, regardless of BMI values (Evans *et al*., 1990). Therefore, a condition of 'relative functional hyperandrogenism' seems to be associated with the central obesity phenotype in women. Due to their action on the adipose tissue, it has been argued that this endocrine milieu may play a crucial role in favouring the development of visceral fat enlargement in women. For an extensive explanation on this important topic, the reader is referred to recent reviews (Wajchemberg, 2000; Pasquali *et al*., 2003)

Table II. Estimated sex hormone adipose tissue:blood ratio

Steroid	Adipose tissue:blood ratio[a]
Cortisol	0.4 ± 0.7
Dehydroepiandrosterone	13.2 ± 4.4
Androstenedione	7.7 ± 3.4
Testosterone	7.0 ± 3.0
Estrogens (estrone + estradiol)	2.2 ± 1.5
Progesterone	6.3 ± 7.0
17-Hydroxyprogesterone	4.0 ± 2.5

[a]Values are mean ± SEM.
Data from Feher and Bodrogi (1982) and Azziz (1989).

Obesity can also be considered a condition of increased estrogen production, the rate of which correlates significantly with body weight and the amount of body fat (Kirschner *et al*., 1990). Reduced SHBG concentrations may in turn lead to an increased exposure of target tissues to free estrogens. In addition, obesity is associated with a decreased formation of inactive 17β-estradiol metabolites (e.g. 2-hydroxyestrogens) which are virtually devoid of peripheral estrogen activity, and a higher production of estrone sulphate, an important reservoir of active estrogens. Altogether, these alterations lead to an increased ratio of active to inactive estrogens as a final result. However, in spite of these changes, blood estrogen levels are usually normal or only slightly elevated in both premenopausal and post-menopausal obese women (Pasquali and Casimirri, 1993). This finding may be attributed to the ability of enlarged body fat to act as a storage for excess formed estrogen, contributing in this way to maintain normal levels of circulating hormone. In addition, there are no systematic differences in the estrogen blood levels in women with different obesity phenotypes, although estrogen production rates have been found to be particularly increased in women with peripheral obesity (Pasquali and Casimirri, 1993). Increased estrogenization in simple obese women may be important in protecting against the development of the abdominal obesity phenotype which may, as previously reported, be a sign of tissue-specific androgenization in both fertile and post-menopausal women. The dichotomy of fat distribution according to sex is under the control (among other

hormones) of both androgens and estrogens (Wajchemberg, 2000). In the adipose tissue of women, the androgen receptors seem to have the same characteristics as those found in male adipose tissue. However, estrogens down-regulate the density of these receptors (whereas testosterone up-regulates them), and this may be a mechanism whereby estrogens protect adipose tissue from androgen effects. In addition, estrogen receptors are expressed in human adipose tissue and show a regional variation of density; although the quantity of these receptors appears to be of physiological importance, this has not been clearly established (Wajchemberg, 2000)

The obesity-PCOS link: pathophysiological aspects

As cited above, PCOS—the most common cause of anovulatory infertility in young women—is frequently associated with obesity. In fact, approximately 50% of PCOS women are overweight to some degree (Yen, 1980; Conway *et al*., 1989; Franks, 1989) and the history of weight gain frequently precedes the onset of clinical manifestations of the syndrome, suggesting a pathogenetic role of obesity in the development of PCOS and related infertility. Moreover, there are numerous studies indicating that obese women with PCOS (notably those with an abdominal body fat distribution phenotype) have more severe insulin resistance (Conway *et al*., 1992; Grulet *et al*., 1993; Holte *et al*., 1994; Dunaif and Finegood, 1996; Morales *et al*., 1996; Ciaraldi *et al*., 1997; Dunaif, 1997; Morin-Papunen *et al*., 2000a), dyslipidaemia (Hartz *et al*., 1979; Mahabeer *et al*., 1990; Conway *et al*., 1992; Holte *et al*., 1994) and hyperandrogenic state (Pasquali *et al*., 1993; Holte *et al*., 1994; Morales *et al*., 1996) along with more frequent menses abnormalities (Pasquali *et al*., 1993; White *et al*., 1996) and lower incidences of spontaneous and stimulated ovulations and pregnancy rates (Lobo *et al*., 1982; Galtier-Dereure *et al*., 1997; Fedorcsák *et al*., 2000b; 2001) than their normal-weight counterparts. Mechanisms by which obesity interferes with the pathophysiology and clinical expression of PCOS are complex and not completely understood. This topic was recently reviewed extensively (Gambineri *et al*., 2002a), and consequently only some of the relevant aspects will be summarized in this text. As reported in the previous section, obesity *per se* represents a condition of functional hyperandrogenism. In women with PCOS, however, obesity is believed to play a distinct pathophysiological role in the development of hyperandrogenism. In an obese PCOS woman, the former presence of obesity in her mother during pregnancy appears to influence the susceptibility to develop hyperandrogenism and the PCOS phenotype later in time, although pathophysiological mechanisms for this have not been defined (Creswell *et al*., 1997). By contrast, it has been hypothesized recently that in-utero androgen excess may be an important factor programming subsequent PCOS development during puberty (Abbot *et al*., 2002). This theory appears to be substantiated by studies performed in non-human primates (Abbot *et al*., 1998; Eisner *et al*., 2000) and sheep (Padmanabham *et al*., 1998; Robinson *et al*., 1999). By these mechanisms, it has been suggested that a primary ovarian disorder may even occur early in the woman's life, leading to the development of a hyperandrogenized ovary later in life (Abbott *et al*., 2002). Along with this line of thinking, it is also possible that the early onset of overweight or obesity—that is, during peripubertal age—may play a role in the development of hyperandrogenism by the intervention of multiple interrelated mechanisms which primarily involve inappropriate signals from different hormones and/or alterations of specific hormone regulatory pathways. These include insulin, the insulin-growth-factor system, the opioid system, estrogens and several newly discovered cytokines.

It is well known that obesity—particularly the abdominal phenotype—is a condition of insulin resistance and compensatory hyperinsulinaemia. Contrary to what occurs in the classical target tissues (muscle, liver, adipose tissue) of insulin action that have become resistant to insulin, the ovaries remain responsive to insulin throughout the interaction with its own receptor. During the past two decades a large number of in-vitro studies have shown that, in the ovaries of PCOS women, excess insulin is capable of stimulating steroidogenesis and excessive androgen production from the theca cell system (Poretsky *et al*., 1999). In addition, by inhibiting SHBG synthesis by the liver proportionally to its blood levels, excess insulin may further increase the delivery of free androgens to target tissues (Poretsky *et al*., 1999). *In vivo*, numerous studies have subsequently shown that both acute and chronic hyperinsulinaemia can stimulate testosterone production and that suppression of insulin levels can conversely decrease blood androgen concentrations (for reviews, see Poretsky *et al*., 1999; Gambineri *et al*., 2002a). The excess in local ovarian androgen production induced by excess circulating insulin may also cause premature follicular atresia and then favour anovulation (Poretsky *et al*., 1999). It can therefore be speculated that insulin resistance and hyperinsulinaemia, which develop together with the obese state, may play a dominant role in favouring hyperandrogenism in women susceptible to the development of PCOS, particularly during pubertal age. The same dangerous insulin-dependent process on both ovarian steroidogenesis and androgen metabolism may obviously occur also in women who had PCOS before becoming obese, and this worsens both hyperandrogenism and associated clinical features, including anovulation.

The influence of obesity on hyperandrogenism can also be mediated by other factors and mechanisms. As in simple obesity, a hyperestrogenic state is also present in obese PCOS women. Excess estrogens may exert a positive feed-back regulation on gonadotrophin release, triggering in turn a rise in ovarian androgen production, according to a still valid theory proposed many years ago (Yen, 1980).

An additional factor involved in the dysregulation of this complex circuit may be an increased tone of the opioid system, which has been demonstrated in the presence of obesity, as well as in women having PCOS (Aleem and McIntosh, 1984; Giuliano, 1984; Jewelovicz, 1984; Genazzani *et al*., 1986; Giuliano *et al*., 1987). Several studies have shown that β-endorphin is able to stimulate insulin secretion (Feldman *et al*., 1983; Giuliano *et al*., 1989). The possibility that increased opioid activity may favour the development of hyperinsulinaemia and, in turn, of hyperandrogenaemia, is further supported by the finding that both acute and chronic administration of opioid antagonists (e.g. naloxone, naltrexone) suppress basal and glucose-stimulated insulin blood concentrations (Pasquali *et al*., 1992). Whether alterations of the opioidergic system play a role in determining infertility in women is still undefined, however several studies have shown that opioid antagonists given to obese PCOS women may improve menses.

Finally, several peptides—particularly leptin and ghrelin—are currently emerging as potential candidates involved in the pathogenesis of hyperandrogenism and related infertility in PCOS women. Leptin is considered to be one of the main peripheral signals that affect food intake and energy balance, and obesity is a classic condition of circulating leptin excess (Considine *et al*., 1996). Leptin has been found to decrease appetite and food intake and reduce the massive obesity in leptin-deficient *ob/ob* mice, and the concentrations of leptin in the blood, during the feeding state, vary with the amount of adipose tissue in the body (Campfield *et al*., 1996). Leptin production and secretion by adipocytes is under complex regulation by many stimulating hormones, particularly insulin, glucocorticoids and cytokines (including tumour necrosis factor α and interleukin-1) and inhibiting hormones and factors, such as catecholamines, testosterone and peroxisome proliferator-activated receptor (PPAR)γ-agonists (Friedman and Halaas, 1998; Ahima and Flier, 2000; Harris, 2000). Under physiological conditions, the amount of leptin produced by fat tissue is directly correlated with both adipose tissue mass and local mRNA expression. In humans, leptin blood levels are two-fold higher in females than in males, and are also affected by growth and energy consumption. The discrepancy between high leptin blood levels and its central effects represents the basis to support the concept that most forms of obesity may represent a condition of leptin resistance. On the other hand, many tissues besides fat mass have been shown to express leptin and its receptors. For the purpose of this review, the focus will be on the interaction between leptin and the ovaries. Several lines of evidence indicate that leptin acts directly on the ovary. In particular, functional leptin receptors have been detected on the surface of ovarian follicular cells, including granulosa, theca and interstitial cells (Wiesner *et al*., 1999), and recent in-vivo data indicate that leptin may exert a direct inhibitory effect on ovarian function, by inhibiting both granulosa and thecal cell steroidogenesis, probably through the antagonism of stimulatory factors such as insulin-like growth factor-1, transforming growth factor-β, insulin and LH (Spicer and Francisco, 1997; 1998; Agarwal *et al*., 1999). Moreover, high leptin concentrations in the ovary may interfere with the development of dominant follicles and oocyte maturation, as demonstrated by both in-vitro and in-vivo studies (Duggal *et al*., 2000). Finally, exogenous leptin infusion has been shown to significantly decrease the ovulation rate in female rats (Duggal *et al*., 2000). Taking into account the involvement of leptin in the control of ovulation and reproduction, interest focused on leptin levels in PCOS women, who are generally obese. To date, contradictory results have been reported on leptin levels in PCOS women, and either higher levels than would be expected for their BMI (Brzechffa *et al*., 1996) or normal concentrations have been reported (Vicennati *et al*., 1998). Whether high leptin levels in the peripheral circulation and/or in the ovarian tissues may play a role in determining anovulation in obese PCOS women is presently unknown, although it cannot be excluded that this may somehow be involved in the processes leading to the development of infertile ovaries.

Another newly discovered peptide, ghrelin, may be involved in the pathophysiology of hyperandrogenism and infertility in obese PCOS women. This acylated 28-amino acid peptide, a natural ligand of the GH-secretagogue receptor (a G protein-coupled receptor primarily expressed in the pituitary and the hypothalamus; Howard *et al*., 1996), has been shown to be a potent stimulant of GH secretion (Arvat *et al*., 2000). Ghrelin is mainly produced in the stomach (Kojima *et al*., 1999), although several other tissues may express ghrelin and its receptors. Ghrelin has also attracted attention for its involvement in the control of food intake and energy balance. When administered centrally or peripherally to rodents and humans, ghrelin has been shown to enhance appetite, reduce fat utilization, and cause adiposity (Tschop *et al*., 2000; Wren *et al*., 2001). Moreover, a putative regulation of ghrelin secretion by nutrients or related factors such as insulin has also been hypothesized, since circulating levels of ghrelin increase on fasting and decrease following food intake (Ariyashu *et al*., 2001; Cummings *et al*., 2001). Plasma ghrelin concentrations have been shown to be lower in obese patients than in normal subjects (Shiiya *et al*., 2002; Tschop *et al*., 2002), though mechanisms responsible for this situation have not yet been defined. Interestingly, however, data are available which suggest that obese women with PCOS may have lower plasma ghrelin levels, compared with age- and weight-matched control subjects (Pagotto *et al*., 2002). A highly significant negative correlation has also been demonstrated between ghrelin and androgen levels, which is in line with recent in-vitro data indicating that gonads may be an important target of ghrelin action. In fact, ghrelin's binding sites have been detected in both human ovary and testis (Papotti *et al*., 2000). Ghrelin has also been shown to exert a strong inhibitory action on several steroidogenic enzymes implicated in androgen production under stimulated conditions, such as chorionic gonadotrophin and cAMP (Tena-Sempere *et al*., 2002). On the other hand, it also appears that androgens may be involved in the regulation of ghrelin secretion and/or metabolism. It has been shown recently that when androgen levels in obese PCOS women were suppressed by flutamide (a pure anti-androgenic compound which acts at the androgen receptor level and also inhibits androgen secretion from the gonads), a significant increase occurred in plasma ghrelin concentrations to values within the normal range. In addition, it has been shown that ghrelin levels in hypogonadal men were significantly lower with respect to weight-matched obese and normal-weight individuals, and significantly increased to expected values based on their BMI values after long-term testosterone replacement therapy (unpublished data). Taken together, these data point to an important role of androgens on ghrelin levels in humans. Since ghrelin concentrations are negatively correlated with insulin resistance (Tschop *et al*., 2000; Pagotto *et al*., 2002), it might be speculated that this peptide in some way represents a link between hyperandrogenaemia and the insulin system in conditions such as obesity and PCOS. It therefore appears that ghrelin, like leptin, may represent a further endocrine factor that is related not only to energy balance and metabolism, but also to the gonadal function. Whether alterations in ghrelin secretion and action are involved in the infertility associated with obesity and PCOS remain to be elucidated, however.

Strategies to improve ovulation and fertility in obese PCOS women

Several therapeutic strategies have been investigated with an aim to reducing body weight, hyperandrogenism and hyperinsulinaemia, and thereby improve spontaneous ovulation and fertility rates in obese women with PCOS.

Lifestyle intervention and insulin sensitizers

Therapies aimed at favouring weight loss should represent the primary interventional strategy in obese women with PCOS. The effects of weight loss on the clinical course of women with obesity and PCOS have been partly neglected in the past, whereas impressive clinical efforts have been made in the pharmacological management of the syndrome. Nevertheless, available studies indicate that weight loss represents a simple and effective way to improve clinical, metabolic and endocrinological features of obese women presenting PCOS (Pasquali and Gambineri, 2002). Most importantly, a beneficial impact of weight loss on menses abnormalities and on both ovulation and fertility rate has been described (Pasquali *et al*., 2000; Pasquali and Gambineri, 2002). This does not appear to be related to the amount of weight lost, since it can be achieved after only mild to moderate weight loss. This has been shown in several studies examining major fertility outcomes in women who have lost ~5% of their baseline body weight compared with those who lost ⩾10% (Pasquali and Gambineri, 2002). Moreover, this has been confirmed by studies investigating the effects of lifestyle modification programmes that did not primarily involve significant caloric restriction, and resulting in a moderate weight loss during which the appearance of ovulatory cycles in anovulatory obese PCOS women has been documented (Huber-Buchholz *et al*., 1999). These results underline that even a modest reduction in body weight may lead to a restoration of ovulatory cycles, suggesting that the effects of caloric restriction may be as important as weight loss itself. Reduction of hyperinsulinaemia appears to be the key factor responsible for the beneficial effect of body fat reduction on menses alterations and fertility (Abbott *et al*., 2002). However, this does not occur universally in all obese PCOS women; in fact, some women do not improve menses alterations and ovulation rates in spite of weight reduction (Pasquali *et al*., 1989; Holte *et al*., 1995; Huber-Buchholz *et al*., 1999). The only significant differences between women who had an improvement in menstrual cyclicity or conceived after energy restriction and those who did not, were found in a greater reduction of fasting insulin values and insulin resistance levels in the responders than in non-responders (Moran *et al*., 2003). It appears therefore that more accurate large and long-term studies should be performed to obtain reliable predictors of the response to both short- and long-term weight loss programmes in obese women with PCOS.

Given the importance of hyperinsulinaemia in the development of hyperandrogenism and disrupted folliculogenesis, the use of insulin-sensitizing drugs seems reasonable in order to facilitate spontaneous ovulation and restore fertility. Such studies in PCOS have used either metformin (which belongs to the class of biguanides; Bailey and Turner, 1996), thiazolidinediones (which are selective ligands for PPARγ, a member of the nuclear receptor superfamily of ligand-activated transcription factors; Lehmann *et al*., 1995), or D-chiro-inositol (a component of the signal transduction system of insulin; Larner, 1994; Nestler *et al*., 1998b).

Metformin is the drug most commonly used during the past decade, and apart from two negative reports (Acbay and Gundogdu, 1996; Ehrmann *et al*., 1997), all the other open-label and/or placebo-controlled studies have documented its effectiveness in reducing hyperinsulinaemia and improving hyperandrogenism and the related clinical features of PCOS women (Velazquez *et al*., 1994; 1997b; Nestler and Jakubowicz, 1996; 1997; Diamanti-Kandarakis *et al*., 1998a; Morin-Papunen *et al*., 1998; Glüeck *et al*., 1999a; Pirwany *et al*., 1999; Unluhizuci *et al*., 1999; Kolodziejczyk *et al*., 2000; Moghetti *et al*., 2000; Ibánez *et al*., 2001; Vrbikova *et al*., 2001) including menstrual cyclicity and ovulation rates (Velazquez *et al*., 1994; 1997b; Nestler and Jakubowicz, 1996; Diamanti-Kandarakis *et al*., 1998b; Morin-Papunen *et al*., 1998; Nestler *et al*., 1998b; Glüeck *et al*., 1999a; 2001b; La Marca *et al*., 1999; Pirwany *et al*., 1999; Unluhizuci *et al*., 1999; Ibánez *et al*., 2000a; 2001; Kolodziejczyk *et al*., 2000; Moghetti *et al*., 2000; Baysal *et al*., 2001; Vrbikova *et al*., 2001; 2002; Fleming *et al*., 2002). Although in general only obese PCOS women have been investigated, there are nonetheless studies which indicate that insulin sensitizers may prove also to be effective in non-obese, insulin-resistant PCOS women. In an uncontrolled study (Ibánez *et al*., 2001), it was shown that 14 of 18 (78%) normal-weight adolescents ovulated after taking metformin for 6 months. However, others (Nestler *et al*., 1998b) found that eight of 19 (42%) normal-weight PCOS women ovulated after only 4–6 weeks on metformin therapy, although this ovulation rate was higher (but not statistically different) with respect to that seen in women taking placebo. Further studies are therefore required to establish whether metformin therapy is effective only in obese PCOS women or whether it may also improve ovulation in PCOS patients who are not obese. One open question is also whether the benefits produced by metformin on obese PCOS women can be attributable to the direct action of the drug or to metformin-induced weight loss. As has been discussed in a recent review on this topic (Pasquali and Gambineri, 2002), the balance of opinion seems to favour the concept that the beneficial effects of metformin are over and above those due to dietary modifications and associated weight loss and weight changes, since endocrine and metabolic abnormalities have been reported to be improved also by metformin in obese women who did not lose weight and independently of changes in body weight. Whichever approach is used, however, it is believed that the best way to improve fertility other than metabolic alterations and hyperandrogenism in obese women with PCOS should be a combination of hypocaloric dieting and an insulin sensitizer, such as metformin. In a recent controlled randomized study performed in obese women with PCOS, it was found that metformin (850 mg, twice daily) had significant and synergistic additive effects to hypocaloric diet on metabolic and endocrine alterations and, particularly, on menses abnormalities (Pasquali *et al*., 2000).

Only one study has been performed to investigate the effectiveness of metformin on pregnancy rate in unselected PCOS patients, although pregnancy was not a primary end

point (Fleming *et al*., 2002). This large randomized, double-blind, placebo-controlled study on predominantly obese PCOS patients showed that 17% of women on metformin (16 weeks) conceived compared with 5% on placebo, though the result was not significantly different. Other studies have shown that metformin may reduce the rate of first-trimester spontaneous abortion (Glüeck *et al*., 2001a; Jakubowicz *et al*., 2002), which is three-fold higher in PCOS women than in the normal population. This association is not surprising, given the apparent association of hypofibrinolytic plasminogen activator inhibitor (PAI-Fx) and hypofibrinolytic 4G4G polymorphism of the *PAI-1* gene with spontaneous abortion and with major complications of pregnancy respectively (Glüeck *et al*., 2002b). Hypofibrinolytic PAI-Fx appears to promote placental insufficiency by reducing the lysis of thrombi in the spiral arteries of the uterus (Velazquez *et al*., 1997a; Glüeck *et al*., 1999b). PAI-Fx is higher and hypofibrinolytic 4G4G homozygosity for the *PAI-1* gene is more common in PCOS women than in non-affected controls (Glüeck *et al*., 2002a). Even though the full mechanisms have not yet been elucidated, it has been suggested that insulin may play a role in the regulation of the system. The decrease in insulin levels induced by metformin may favour reduced PAI-Fx, thus providing one mechanism for the reduction of early abortion rates in PCOS women (Glüeck *et al*., 2002a). Moreover, it has been shown that insulin reduction with metformin may increase luteal phase serum glycodelin concentrations and enhance uterine vascularity and blood flow in PCOS, both of which changes will stabilize pregnancy (Jakubowicz *et al*., 2001). It is important to note that these results may be consistent with the use of metformin during pregnancy in PCOS women, particularly if obese. Notably, metformin has been extensively proved to be safe, effective and absolutely free of potential teratogenic properties (Denno and Sadler, 1994; Pasquali *et al*., 2000; Glüeck *et al*., 2002a; Heard *et al*., 2002; Jakubowicz *et al*., 2002). At the present time, however, no randomized controlled studies on metformin and pregnancy exist in women with PCOS. Nonetheless, there are studies indicating that, as well as reducing abortion rates, metformin treatment may also be effective in controlling glucose metabolism and gestational diabetes (Glüeck *et al*., 2002b). Whether metformin added to a controlled diet may favour better metabolic control during pregnancy and more safe deliveries requires further investigation, however.

Like metformin, the PPARγ-binding agent troglitazone has been found to improve insulin sensitivity, decrease testosterone and increase spontaneous ovulation and conception in PCOS women (Dunaif *et al*., 1996; Azziz *et al*., 2001). The principal mechanism responsible for the overall benefit of troglitazone on hyperandrogenism and fertility relates to its ability to decrease insulin levels. Unfortunately, this compound has been withdrawn because of its potential liver toxicity. To date, no data have been published on the use of the newer PPARγ-binding agents, rosiglitazone and pioglitazone, in women with PCOS. Available studies performed in patients with type 2 diabetes indicate that, unlike metformin, rosiglitazone and pioglitazone are either weight-neutral or may even cause a modest weight gain (Dunaif *et al*., 1996). Moreover, studies conducted in animals have shown retarded fetal development during pregnancy administration of rosiglitazone and pioglitazone (Iuorno and Nestler, 2001); this effect may be related to their activation of the PPAR system, which is important in embryonic development. Thus, the use of rosiglitazone and pioglitazone as inducers of ovulation—particularly during pregnancy—cannot presently be recommended until data on their safety and efficacy in humans are available.

Among other insulin-sensitizing agents, the potential use of D-chiro-inositol in PCOS treatment is currently under investigation. D-chiro-inositol is a phosphoglycan that mediates insulin action through mechanisms involving rate-limiting enzymes of non-oxidative and oxidative glucose disposal (Nestler *et al*., 1998b). A deficiency in specific D-chiro-inositol-containing inositolphosphoglycan may contribute to the development of insulin resistance. The use of D-chiro-inositol in syndromes of insulin resistance as the obesity-PCOS condition may thus offer another opportunity to improve insulin-mediated effects on the ovaries, particularly excess androgen production and altered ovulatory pathways. In a recent placebo-controlled study (Nestler *et al*., 1999), it was shown that this drug could decrease fasting and glucose-stimulated insulin values, increase plasma SHBG, reduce free testosterone levels and, finally, restore spontaneous ovulation.

In summary, it is clear that weight loss and insulin sensitizers, through their capacity to improve insulin resistance and reduce insulin blood levels, may represent valid therapeutic procedures to improve spontaneous fertility in obese PCOS women, and may offer good metabolic control during pregnancy, thereby permitting favourable physiological delivery and healthy babies. It is also believed that insulin-sensitizing agents should always be administered in association with lifestyle rules (nutritional, etc.) which have been proved safely to resolve infertility in many patients with obesity and PCOS.

A role for anti-androgens ?

Anti-androgens such as flutamide, finasteride, spironolactone, cyproterone acetate and GnRH agonist have been used extensively in the treatment of PCOS in order to reduce androgen levels and related clinical manifestations, such as hirsutism and menses abnormalities (Codsland *et al*., 1992; Diamanti-Kandarakis *et al*., 1995; 1998b; Moghetti *et al*., 1996; Dahlgren *et al*., 1998; De Leo *et al*., 1998; Paoletti *et al*., 1999; Ibánez *et al*., 2000a; Morin-Papunen *et al*., 2000b; Armstrong *et al*., 2001; Falsetti *et al*., 2001; Carmina, 2002; Mastorakos *et al*., 2002; Odling *et al*., 2002). Androgens are important mediators of intermediate metabolism, and their excess in women may be associated with multiple metabolic abnormalities, including insulin resistance and lipid disorders. In addition, as has been stated previously, excess androgens appear to play an important role in determining the development of the abdominal obesity phenotype. Treatment with anti-androgens is therefore expected to be effective in PCOS.

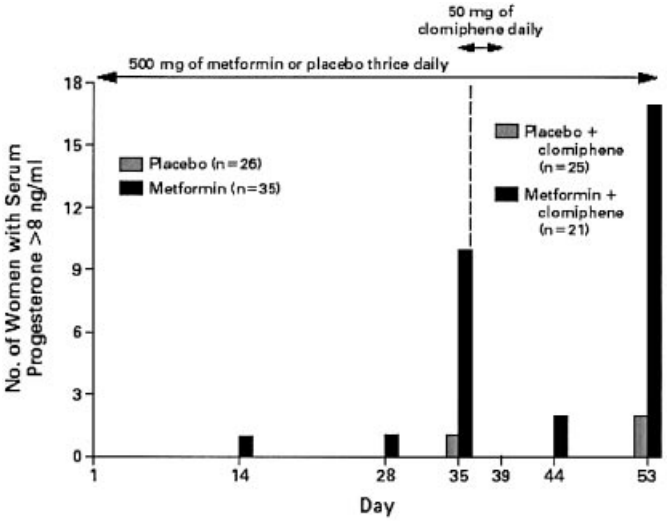

Figure 1. Numbers of women with serum progesterone level >8 ng/ml (indicative of ovulation) on days 14, 28 and 35 in the metformin and placebo groups (from Nestler *et al.*, 1998b, with permission from the editor). Note that no women had progesterone levels >8 ng/ml at days 14, 28 and 44 in the placebo/placebo + clomiphene groups. Reproduced from Nestler *et al.* (1998b). © 1998 Massachusetts Medical Society. All rights reserved.

Notably, they markedly reduce androgens (Dahlgren *et al.*, 1998; Morin-Papunen *et al.*, 2000b; Armstrong *et al.*, 2001; Falsetti *et al.*, 2001) and improve gonadotrophin secretion (Mastorakos *et al.*, 2002) and hirsutism (Dahlgren *et al.*, 1998; Morin-Papunen *et al.*, 2000b; Armstrong *et al.*, 2001; Falsetti *et al.*, 2001). Several studies have shown that anti-androgens may also improve lipid profile (Dahlgren *et al.*, 1998; Odling *et al.*, 2002), whereas it is still unclear whether this treatment may reduce insulin resistance (Dahlgren *et al.*, 1998; Armstrong *et al.*, 2001; Carmina, 2002; Odling *et al.*, 2002). Moreover, it is still unclear whether anti-androgens may have an important impact on menstrual abnormalities, probably because of the heterogeneity of the cohorts of PCOS investigated in the available studies, with particular reference to the degree of excess androgen blood levels, their glandular source (ovarian and/or adrenal) and the amount of body fat (Gambineri *et al.*, 2002b; Ibánez *et al.*, 2002). In addition, no clear significant effects on ovulation and fertility have been consistently described. Therefore, even considering the potential (although uncommon) toxicity of some of these compounds on fetal development (Imperato-McGinley *et al.*, 1992; Miyata *et al.*, 2002), their utilization as pro-fertility drugs should be discouraged.

On the other hand, it is speculated that a combination of insulin sensitizers and anti-androgens added to lifestyle interventions promises to have potential complementary effects in the treatment of obese PCOS due to their different spectra of action. In a preliminary study in young normal-weight women with PCOS (Ibánez *et al.*, 2002), it was found that the combined treatment of metformin and flutamide had additive benefits with respect to the monotherapies on insulin sensitivity and hyperandrogenaemia. However, flutamide did not have any effect on menstrual abnormalities. Similar results have been recently confirmed and extended in a cohort of obese insulin-resistant anovulatory PCOS women treated for 7 months with a low-calorie diet regimen added to placebo, metformin, flutamide or their combination, given in random order (Gambineri *et al.*, 2002b). The combined treatment was found to have additive or synergistic effects with respect to the monotherapies in reducing androgens and in improving lipid profile, particularly high-density lipid (HDL)-cholesterol concentrations. Metformin appeared to be the drug primarily involved in the improvement of menses abnormalities, whereas flutamide proved to be primarily responsible for reducing the hirsutism score and visceral fat depots, as measured by computed tomography at the mid-abdomen level. These data further emphasize that anti-androgens do not represent target treatment, even added to dietary-induced weight loss, when the objective is to improve menses abnormalities, spontaneous ovulation and fertility rates.

Obesity and pharmacological strategies to induce ovulation and fertility

The administration of clomiphene citrate, or gonadotrophins and pulsatile GnRH still represents standard pharmacological strategy to achieve ovulation in women with chronic anovulation. Clomiphene citrate has a great index of responsiveness in infertile women in terms of ovulatory cycles and conception (Hammond *et al.*, 1983; Opsahl *et al.*, 1996; Kousta *et al.*, 1997). Obesity negatively influences the responsiveness to clomiphene (Lobo *et al.*, 1982; Polson *et al.*, 1989), and obese women require multiple courses and high doses of clomiphene to achieve ovulation (Lobo *et al.*, 1982; Kousta *et al.*, 1997). Since obesity is associated with increased insulin resistance and hyperinsulinaemia, high levels of insulin rather than obesity itself have been considered the cause of poor responsiveness to clomiphene administration in PCOS women. Several studies have reported the effectiveness of metformin in combination with clomiphene citrate on restoration of ovulation and/or pregnancy in unselected PCOS women. One group (Nestler *et al.*, 1998b) examined the ovulation rate of 61 obese PCOS women randomly treated with metformin or placebo for 34 days, and subsequently with one cycle of clomiphene if ovulation was not achieved by day 34. These authors found that administration of metformin before and during low-dose clomiphene administration was more effective in achieving ovulation than placebo (Figure 1). Similar findings have also been described by others (El-Biely and Habba, 2001), who compared the effect of metformin plus clomiphene with clomiphene alone in 90 obese PCOS patients. A higher ovulation rate and a higher pregnancy rate were found in patients treated with metformin plus clomiphene than in those given clomiphene alone.

Moreover, an improvement in both ovulation and pregnancy rate in a large cohort of clomiphene-resistant PCOS women has been demonstrated after metformin administration (Kocak *et al.*, 2002). Others (Parsanezhad *et al.*, 2001) described that among 41 obese, clomiphene-resistant PCOS patients, 39% ovulated and 24% conceived when metformin was added to clomiphene. The same results have also been reported in previous smaller studies (Ng *et al.*, 2001; Vandermolen *et al.*, 2001).

In search of predictors of responsiveness to clomiphene, one group (Imani *et al*., 1998) investigated many clinical, endocrine and sonographic characteristics during an initial screening of anovulatory infertile women. It was found that hyperandrogenaemia, obesity, higher mean ovarian volume and oligoamenorrhoea could be identified as screening parameters for unsuccessful clomiphene treatment. In a subsequent study from the same group (Imani *et al*., 2000), it was found that insulin levels, insulin:glucose ratio and leptin levels were significantly higher while, in contrast, insulin-like growth factor (IGF) binding protein-1 (IGFBP-1) levels were lower in those women remaining anovulatory after clomiphene administration. Overall, these studies indicate that insulin resistance and obesity are unfavourable conditions to ovarian response during induction of ovulation by clomiphene.

Obese PCOS women also present a reduced responsiveness to gonadotrophin injections and require higher doses of these drugs to induce ovulation than their lean counterparts (McClure *et al*., 1992; Hamilton-Fairley and Franks, 1994; White *et al*., 1996; Fridstom *et al*., 1997). Unfortunately, high doses of gonadotrophins can induce ovarian hyperstimulation and, consequently, multiple pregnancies. The different responsiveness to gonadotrophins between overweight and normal-weight PCOS women and the high-dose gonadotrophin regimens needed to achieve ovulation in overweight PCOS cannot be attributed to a decreased drug bioavailability, as blood levels of FSH during hMG treatment are usually higher in obese than non-obese patients (Fridstom *et al*., 1997). Among putative factors, insulin resistance has been indicated as a potential cause explaining reduced fertility rates after gonadotrophin administration. One group (Homburg *et al*., 1996) found that the amount of hMG required to achieve ovulation in a group of anovulatory PCOS women correlated positively with BMI and insulin concentrations, and negatively with IGFBP-1. However, a multiple regression analysis revealed that insulin concentration was the most significant determinant of the total dose of hMG required to induce ovulation. Moreover, by comparing insulin-resistant and normal insulin-sensitive PCOS women, the former were found to require more time and larger FSH doses to achieve follicular maturation; these women also presented a reduced conception rate, regardless of their body weight (Dale *et al*., 1998).

Two discrepant studies evaluating the effect of pre-treatment with metformin on the responsiveness to gonadotrophin administration in infertile PCOS women resistant to clomiphene have also been reported. The first (De Leo *et al*., 1999) showed that the PCOS women receiving FSH plus metformin had, in comparison with those treated with FSH alone, fewer dominant follicles, a lower peak plasma estradiol level and a lower percentage of cycles withheld because of excessive follicular development. The second study (Yarali *et al*., 2002) showed that the administration of metformin did not improve ovulation and pregnancy rates.

Moreover, one interesting study (Farhi *et al*., 1995) which evaluated the effect of ovarian electrocautery on the response to gonadotrophic stimulation in clomiphene citrate-resistant women with PCOS, indicated an increased ovarian sensitivity to gonadotrophins after laparoscopic ovarian electrocautery, together with an increase in ovulation and pregnancy rate. Further studies are, however, warranted to better delineate the effect of metformin and ovarian electrocautery on exogenous gonadotrophin treatment in infertile PCOS women.

Only a few studies have investigated the impact of pulsatile GnRH analogue regimens in anovulatory PCOS women. One group (Bringer *et al*., 1985) showed that in PCOS women resistant to clomiphene, obesity reduced the effectiveness of pulsatile GnRH to achieve follicular growth. Others (Filicori *et al*., 1991) compared the clinical outcome of pulsatile GnRH cycles in women with different ovulatory disorders and found that obesity reduced the ovulatory rate in PCOS women, but did not affect treatment outcome in a group of hypogonadotrophic patients. Therefore, endocrine disorders such as hyperinsulinaemia and hyperandrogenism appear to be related to a poorer efficiency of pulsatile GnRH treatment observed in obese patients (Eshel *et al*., 1988; Filicori *et al*., 1994).

In conclusion, although there is a lack of systematic data and controlled studies, the available evidence indicates that obesity and insulin resistance adversely affect fertility rate after gonadotrophin or GnRH analogue therapeutic protocols. This further emphasizes the opportunity that programmes aimed at obtaining weight loss should precede each pharmacological intervention in obese PCOS women, in order to further improve fertility and pregnancy outcome.

Obesity and assisted reproductive technologies outcome

IVF and ICSI represent the treatment of choice in the case of failure of pharmacologically induced ovulation (Dale *et al*., 1991). Here again, obesity—particularly the abdominal phenotype—appears adversely to affect the outcome of IVF or ICSI, impairing fecundity and reducing the pregnancy rate (Crosignani *et al*., 1994; Wass *et al*., 1997). This appears to be mediated by several factors, including androgens (Smith, 1996), insulin (Porestky and Piper, 1994) and leptin (Karlsson *et al*., 1997; Agarwal *et al*., 1999). The importance of insulin resistance and compensatory hyperinsulinaemia in affecting ovarian responsiveness to IVF or ICSI was recently highlighted (Fedorcsák *et al*., 2001), it being shown that insulin-resistant infertile PCOS women needed a higher FSH dose and had lower estradiol levels during ovarian stimulation compared to women with normal insulin sensitivity. Another study (Stadtmauer *et al*., 2001) involved the retrospective investigation of 46 PCOS women resistant to clomiphene, unselected for obesity, who underwent 60 cycles of IVF with ICSI treatment. These authors found that cycles with co-administered metformin had an equivalent number of dominant follicles, but a significantly lower total number of follicles, more mature oocytes, higher fertilization rate and higher pregnancy rate. High intrafollicular concentrations of leptin have also been demonstrated in pre-ovulatory follicles of PCOS patients resistant to gonadotrophins administered during ovarian stimulation by IVF (Fedorcsák *et al*., 2000a). A higher number of spontaneous abortions in patients undergoing IVF or ICSI has also been reported in the presence of obesity (Fedorcsák *et al*., 2000b). Obese women, in fact, present a low oocyte count and reduced embryo quality at transfer, both of which are responsible for an increased risk of miscarriage (Millis, 1992) and early pregnancy loss (Mitwally, 1999; Wang *et al*., 2001).

Many more controlled studies should be carried out in this area to evaluate whether reduction of body fat or correction of insulin

resistance by the administration of insulin-sensitizers may improve the responsiveness of obese subjects to assisted reproduction technology and decrease the risk of the procedures.

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