

The impact of obesity and insulin resistance on the outcome of IVF or ICSI in women with polycystic ovarian syndrome

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The impact of insulin resistance on the outcome of IVF or intracytoplasmic sperm injection (ICSI) in women with polycystic ovarian syndrome (PCOS) was examined. Insulin sensitivity was measured by the continuous infusion of glucose with model assessment (CIGMA) test. Insulin-resistant ($n = 26$) and non-insulin-resistant women ($n = 30$) with PCOS underwent a total of 100 cycles of long-term down-regulation with busserelin acetate, stimulation with human recombinant FSH, and IVF or ICSI. Blood samples were taken throughout ovarian stimulation for hormone assays. Insulin-resistant and non-insulin-resistant women had similar concentrations of FSH, LH, testosterone and androstenedione throughout stimulation, but insulin-resistant women had hyperinsulinaemia and lower sex hormone binding globulin concentrations. Insulin-resistant women also had lower oestradiol concentrations during stimulation and required higher FSH doses, but these differences disappeared after controlling for the higher body weight in the group of insulin-resistant women. Groups had similar number of oocytes collected, similar implantation and pregnancy rates, and the incidence of ovarian hyperstimulation syndrome was also similar. Obesity, independent of hyperinsulinaemia, was related to a lower oocyte count and increased FSH requirement. It is concluded that in PCOS women receiving long-term down-regulation and stimulation with recombinant FSH, insulin resistance is neither related to hormone levels nor to IVF outcome. Obesity, independent of insulin resistance, is associated with relative gonadotrophin resistance.

Key words: insulin resistance/IVF/obesity/polycystic ovarian syndrome

Introduction

The features of polycystic ovarian syndrome (PCOS), a major cause of infertility, are hyperandrogenism and chronic anovulation (Franks, 1995). Many women afflicted with PCOS exhibit insulin resistance and hyperinsulinaemia. Hyperinsulinaemia in PCOS is attributed to obesity as well as to insulin resistance independent of body weight (Dale *et al.*, 1992; Holte *et al.*, 1994; Dunaif, 1997). Anovulatory infertility in PCOS often responds to clomiphene citrate treatment, ovulation induction with gonadotrophins, or ovarian surgery. In cases where these attempts fail or other fertility problems co-exist, IVF is the treatment of choice (Dale *et al.*, 1991; Buyalos and Lee, 1996; Homburg, 1996).

Obesity and insulin resistance, however, compromise the success of fertility treatment in PCOS. Indeed, obesity is more prevalent among PCOS women who remain anovulatory after ovarian electrocautery (Gjønnæss, 1994) and clomiphene citrate treatment (Polson *et al.*, 1989; Imani *et al.*, 1998). Ovulation induction with gonadotrophins in obese PCOS women requires higher doses than in lean PCOS women, the rate of ovulatory cycles is lower, and the rate of multifollicular development and incidence of miscarriage is higher in obesity

(Hamilton-Fairley *et al.*, 1992; Fridström *et al.*, 1997). Obesity may also jeopardize IVF results in PCOS: indeed, high intrafollicular concentrations of leptin—a hormone produced by adipose tissue—are related to relative gonadotrophin resistance during ovarian stimulation for IVF (Fedorcsák *et al.*, 2000a). Furthermore, android obesity—a common feature of PCOS—is associated with low pregnancy rate after IVF (Wass *et al.*, 1997), and obesity is also associated with an increased risk of miscarriage, partly due to the lower number of retrieved oocytes in obese women (Fedorcsák *et al.*, 2000c).

The independent effect of insulin resistance on infertility treatment in PCOS is less well defined. Regardless of body weight, insulin-resistant PCOS women need higher gonadotrophin doses during ovarian stimulation, and insulin resistance is also associated with a risk of multifollicular development and high cancellation rate (Fulghesu *et al.*, 1997; Dale *et al.*, 1998). Hyperinsulinaemic PCOS women are more likely to produce oocytes exhibiting low fertilization rates after IVF, and embryos that are unable to implant (Cano *et al.*, 1997). Furthermore, luteinized granulosa cells, derived from insulin-resistant PCOS women undergoing IVF, release less progesterone *in vitro* than cells from non-insulin-resistant women (Fedorcsák *et al.*, 2000b).

Exercise, low-calorie diet and insulin-lowering drugs such as metformin, troglitazone and acarbose decrease insulin levels, correct the endocrine abnormalities induced by obesity and insulin resistance, and thus may improve the results of infertility treatment (Pasquali *et al.*, 1997; Clark *et al.*, 1998; Nestler *et al.*, 1998; Ehrmann, 1999). If insulin resistance impairs the outcome of IVF in PCOS women, it would warrant co-treatment with insulin-lowering drugs or weight reduction before and during down-regulation and ovarian stimulation. This led us to examine the impact of insulin resistance on the outcome of IVF or intracytoplasmic sperm injection (ICSI) in PCOS women.

Materials and methods

Patients

Fifty-six PCOS women receiving ovarian stimulation for IVF or ICSI, who agreed to undergo tests of insulin sensitivity during infertility work-up, were studied. The local ethics committee approved the study, and each patient gave informed consent. PCOS was defined by the presence of polycystic ovaries on vaginal ultrasound scan (at least 10 follicles between 2 and 8 mm in diameter) (Adams *et al.*, 1985) and at least two of the following criteria: oligo/amenorrhoea, hirsutism or hyperandrogenism. Attenuated 21-hydroxylase activity, Cushing's syndrome, androgen-secreting tumours and hyperprolactinaemia were excluded by appropriate tests. Before IVF or ICSI was offered, women had failed to ovulate or to conceive after repeated treatments with clomiphene citrate (up to four cycles with 150 mg for 5 days). Thirty-one women (55%) had also undergone ovarian wedge resection or electrocautery without conceiving.

CIGMA test

Insulin sensitivity was measured by the continuous infusion of glucose with model assessment (CIGMA) test (Hosker *et al.*, 1985; Dale *et al.*, 1992). After an overnight fast, the patients were infused with 5 mg glucose/kg ideal body weight per min over 60 min, and plasma glucose and insulin were measured at 50, 55 and 60 min. To assess insulin resistance and glucose tolerance, these concentrations were interpreted with a mathematical model of glucose and insulin homeostasis. The insulin resistance measured by CIGMA correlates well with data obtained with the euglycaemic clamp technique (Hosker *et al.*, 1985). No patient had glucosuria during the CIGMA test. A test value >4 indicated insulin resistance. Women were tested between days 4–7 of the menstrual cycle or at random in cases of amenorrhoea.

Ovarian stimulation and IVF

All patients received a similar stimulation regimen. Pituitary function was suppressed with a daily dose of 600 µg buserelin acetate (Suprefact; Hoechst, Frankfurt am Main, Germany). Down-regulation started 1 week before an expected menstrual bleed (or at random in case of amenorrhoea) and was given usually for 4 weeks until no follicles >10 mm were seen on vaginal ultrasound scan and serum concentrations of oestradiol were <0.2 nmol/l. Follicular development was then initiated with 75 or 150 IU human recombinant FSH (Gonal F; Serono, Switzerland/Puregon; Organon, The Netherlands) per day. The daily dose was increased with 75 IU FSH every 3–4 days according to the individual response. When the leading follicle was >18 mm and serum oestradiol concentrations were 1–15 nmol/l depending on the number of follicles, ovulation was induced with 10 000 IU human chorionic gonadotrophin (HCG) (Profasi; Serono). Buserelin acetate was given until the day of HCG injection. Cycles

were cancelled in case of insufficient follicular development (fewer than three follicles) or imminent ovarian hyperstimulation (enlarged multifollicular ovaries >10 cm in diameter and oestradiol concentrations >10 nmol/l).

Follicles were aspirated within 34–38 h after ovulation induction with HCG, and collected oocytes were fertilized *in vitro* by IVF or ICSI (Åbyholm *et al.*, 1991; Tanbo *et al.*, 1998). One or two embryos were transferred on day 2, 3 or 4 after follicle puncture. Transfer of three embryos was only allowed in selected cases. Progesterone (25 mg/day) was injected for luteal phase support. Pregnancies were defined by >10 IU/l plasma β -HCG concentration on day 14 after follicle puncture. Vaginal ultrasound scans at 6 and 12 weeks gestation confirmed fetal viability or miscarriage. Implantation rate was the ratio of the number of gestational sacs at 6 weeks ultrasound scan over the total number of transferred embryos.

Ovarian hyperstimulation syndrome was defined by enlarged ovarian diameter >10 cm, abdominal discomfort [corresponding to World Health Organization (WHO) stages I and II], and eventual ascites, hydrothorax or coagulation abnormalities (corresponding to WHO stage III).

Hormone assays

For baseline hormone assays, fasting blood samples were collected in the early follicular phase of cycling women, or at random in amenorrhoea. Blood samples were also taken on the day when FSH stimulation was started, between days 4 and 6 of ovarian stimulation, and on the day of ovulation induction. The serum concentrations of FSH, LH, oestradiol, androstenedione, testosterone, sex hormone-binding globulin (SHBG), glucose, insulin and C-peptide were determined using routine laboratory methods (Dale *et al.*, 1998). Briefly, FSH, LH and oestradiol were measured with dissociation-enhanced lanthanide fluoroimmunoassay (DELFLIA; Wallac Oy, Turku, Finland); testosterone, androstenedione and SHBG were assayed by radioimmunoassay (Dale *et al.*, 1998); and insulin was measured with radioimmunoassay using mono-iodinated insulin and anti-insulin antiserum (Linco Research, St Louis, MO, USA) (Dale *et al.*, 1998). Glucose concentrations were determined using glucose-oxidase method with the Elite™ glucometer (Bayer Diagnostics, Paris, France), while C-peptide was assayed with radioimmunoassay (Diagnostic Systems Laboratories, Webster, Texas, USA). The free testosterone index was calculated using the formula: testosterone $\times 100$ /SHBG.

Statistical analysis

Data of the groups were compared with the Mann–Whitney test or χ^2 test for proportions. Correlations between variables were assessed in a way to take account of the unequal number of treatment cycles in patients. Thus, Pearson's correlation coefficient between subject means were calculated, while means were weighted with the number of cycles; *P* values were based on the number of patients and not on the total number of cycles (Bland and Altman, 1995). Cumulative pregnancy rates were calculated with the Kaplan–Meier method, and compared with the log-rank test. A *P*-value < 0.05 was considered statistically significant.

Results

Patient characteristics

The median CIGMA score (the measure of insulin resistance) was 4 (range 1–20). Based on the CIGMA test, 26 women were insulin-resistant (CIGMA score >4) and 30 women had normal insulin sensitivity. The age of insulin-resistant and

Table I. Baseline hormone concentrations in non-insulin-resistant and insulin-resistant women with polycystic ovarian syndrome (PCOS)

	Non-insulin-resistant (n = 30)	Insulin-resistant (n = 26)
FSH (IU/l)	4.6 (2.7–7.0)	5.65 (3.0–8.6) ^a
LH (IU/l)	13.0 (2.3–32.4)	12.1 (5.1–18.7)
LH/FSH ratio	2.7 (0.6–7.0)	2.0 (0.9–5.2)
Testosterone (nmol/l)	1.55 (0.8–3.7)	2.1 (0.7–24.0)
Androstenedione (nmol/l)	6.0 (2.4–13.5)	6.7 (2.7–128.0)
SHBG (nmol/l)	30.5 (8.0–60.0)	23.0 (6.0–60.0) ^a
Free testosterone index (%)	5.2 (2.1–27.3)	11.4 (3.0–50.0) ^b
Fasting insulin (pmol/l)	102 (59–196)	150 (71–491) ^c
Fasting C-peptide (pmol/l)	721 (356–1504)	1149 (429–2120) ^b

Values are median (range).

^aP < 0.05; ^bP < 0.01; ^cP < 0.001 by Mann–Whitney test.

SHBG = sex hormone-binding globulin.

non-insulin-resistant women was similar [median 30 years (range 23–38) versus 31 years (range 25–38); P = 0.19]. Insulin-resistant women had a higher body mass index (BMI) [median 28.9 kg/m² (range 18.8–36.3)] than non-insulin-resistant women [median 24.7 kg/m² (range 19.7–33.3); P = 0.006]; indeed, more insulin-resistant women were obese [18/26 (69%) versus 13/30 (43%); P = 0.05]. Baseline hormone concentrations reflected the effects of insulin resistance in PCOS: insulin-resistant women had higher fasting insulin and C-peptide concentrations, lower SHBG, but higher testosterone concentrations, and thus, higher free testosterone index; insulin-resistant women also had higher FSH concentrations and lower LH/FSH ratio (Table I). These differences in baseline hormone concentrations between insulin-resistant and non-insulin-resistant groups remained similar after accounting for BMI by co-variance analysis (data not shown).

Ovarian stimulation and IVF

Patients received one to four cycles of pituitary suppression and ovarian stimulation, the average number of cycles being slightly higher in non-insulin-resistant women (Table II). In the insulin-resistant group, 15 women had one cycle, nine women had two cycles, and two women had three cycles. In the non-insulin-resistant group, 12 women had one cycle, nine women had two cycles, five women had three cycles, and four women had four cycles. The duration of gonadotrophin-releasing hormone (GnRH) agonist down-regulation before the start of stimulation was similar (Table II). Insulin-resistant women required higher FSH doses for ovarian stimulation than women with normal insulin sensitivity (Table II). The total FSH dose correlated with BMI (r = 0.65; P < 0.001) and CIGMA score (r = 0.36; P < 0.01). By stepwise multiple linear regression (multiple R² = 0.44; P < 0.0001), only BMI (P < 0.0001) and the number of days on GnRH agonist before stimulation (P < 0.05) predicted independently the total FSH dose; the effect of CIGMA score was not significant (P = 0.75). The effect of previous ovarian wedge resection or electrocautery was also not significant (P = 0.15).

On the scheduled day of ovulation induction, HCG was withheld in two cycles in insulin-resistant and in six cycles in non-insulin-resistant women (proportions are similar by

Table II. Ovarian stimulation and IVF in PCOS women with or without insulin resistance

	Non-insulin resistant (n = 30)	Insulin-resistant (n = 26)
<i>Ovarian stimulation, IVF and embryo transfer</i>		
No. of started cycles	61	39
No. of cycles per patient	2 (1–4)	1 (1–3)
No. of ICSI cycles (%)	7/61 (11.5)	3/39 (7.7)
No. of days on GnRH _a before start of stimulation	21.5 (8–52)	22 (6–47)
Total FSH dose (IU)	1800 (650–6750)	2450 (975–8005) ^a
No. of collected oocytes	9 (1–35)	10 (3–37)
No. of normal fertilized oocytes	5 (0–20)	5 (1–23)
No. of cycles proceeding to embryo transfer (%)	47/61 (77)	33/39 (84.6)
Day of embryo transfer	3 (2–4)	3 (2–4)
No. of transferred embryos	2 (1–3)	2 (1–3)
<i>Cycle outcome</i>		
Pregnancy rate per started cycle (%)	22/61 (36.1)	14/38 (36.8)
Pregnancy rate per embryo transfer	22/47 (46.8)	14/32 (43.8)
Implantation rate (%)	25/89 (28.1)	17/65 (26.2)
Incidence of OHSS (%)	6/61 (9.8)	4/39 (10.3)
<i>Pregnancy outcome</i>		
Delivery (%)	18/22 (82)	9/14 (64)
Abortion before week 6 (%)	3 (14)	2 (14)
Abortion between week 6–12 (%)	–	3 (21)
Abortion after week 12 (%)	1 (4)	–

Data are median (range) or proportion (%).

^aP < 0.05 by Mann–Whitney test.

GnRH_a = gonadotrophin-releasing hormone agonist; ICSI = intracytoplasmic sperm injection; OHSS = ovarian hyperstimulation syndrome.

Fisher’s exact test; P = 0.48). These cycles were cancelled because of imminent ovarian hyperstimulation (n = 4), insufficient follicular development (n = 2), or for personal reasons (n = 2). In the 92 cycles that proceeded to ovulation induction and oocyte retrieval, the number of collected oocytes was similar in insulin-resistant and in non-insulin-resistant women (Table II), and there was no association between CIGMA score and the number of oocytes (r = –0.02). The number of oocytes, however, was lower in women requiring a higher dose of FSH (r = –0.44; P < 0.001). A higher BMI was also related to a lower number of retrieved oocytes (r = –0.28; P < 0.05), even after accounting for the history of ovarian wedge resection or electrocautery (P < 0.04).

Hormone concentrations during ovarian stimulation were available in 42 cycles of non-insulin-resistant women and 31 cycles of insulin-resistant women (Table III). The proportion of cycles for this analysis was as follows. In the insulin-resistant group 13 women had one cycle, and nine women had two cycles, while in the non-insulin-resistant group 16 women had one cycle, three women had two cycles, four women had three cycles, and two women had four cycles. Insulin-resistant women tended to have lower SHBG and higher fasting insulin concentrations throughout ovarian stimulation, although these differences were statistically significant (P < 0.05) only at the start of FSH stimulation (higher insulin) and at days 4–6

Table III. Hormone concentrations in non-insulin-resistant (NIR) and insulin-resistant (IR) women with PCOS on the day when FSH stimulation was started, between days 4 and 6 of ovarian stimulation, and on the day of ovulation induction

	Start of stimulation	Stimulation days 4–6	Day of HCG administration
FSH (IU/l)			
NIR	3.0 (1.2–7.6)	5.3 (3.6–10.1)	7.0 (4.0–11.9)
IR	3.2 (1.6–7.4)	5.2 (2.3–10.7)	6.2 (3.0–9.3)
LH (IU/l)			
NIR	2.0 (0.6–7.0)	1.4 (0.5–5.6)	0.8 (0.4–3.3)
IR	2.4 (0.6–7.2)	1.4 (0.5–5.1)	0.7 (0.6–1.5)
Oestradiol (nmol/l)			
NIR	0.1 (0.0–0.32)	0.26 (0.08–9.98)	7.1 (2.19–24.0)
IR	0.13 (0.04–0.31)	0.16 (0.06–0.41) ^a	2.93 (0.57–15.0) ^a
Testosterone (nmol/l)			
NIR	1.1 (0.5–2.4)	1.5 (0.8–4.0)	1.75 (1.2–3.6)
IR	1.4 (0.5–2.9)	1.1 (0.5–6.7)	2.1 (0.5–3.5)
Androstenedione (nmol/l)			
NIR	2.7 (1.7–7.0)	3.85 (1.7–10.9)	7.2 (2.6–117.0)
IR	3.6 (1.2–8.1)	3.1 (1.8–7.7)	6.7 (5.1–13.6)
SHBG (nmol/l)			
NIR	33.5 (12–84)	39 (12–82)	59 (21–152)
IR	26 (6–87)	25.5 (7–76) ^a	46.5 (24–153)
Fasting insulin (pmol/l)			
NIR	122.5 (62–296)	124.5 (67–220)	134.5 (74–609)
IR	185.5 (152–495) ^a	245.5 (102–1312)	163 (100–827)

Data are median (range).

^a $P < 0.05$.

SHBG = sex hormone-binding globulin.

of stimulation (lower SHBG). Concentrations of FSH, LH, testosterone and androstenedione were similar between groups. Oestradiol concentrations were also similar at the start of FSH stimulation, but thereafter were significantly lower in insulin-resistant women. Oestradiol concentrations on days 4–6 of stimulation and on the day of HCG administration, however, did not differ significantly between groups after controlling for differences in BMI by co-variance analysis [adjusted geometric means (95% confidence interval, CI)]: days 4–6 of stimulation, non-insulin-resistant group 0.28 (0.20–0.40) nmol/l versus insulin-resistant group 0.18 (0.11–0.30) nmol/l; day of HCG administration, non-insulin-resistant group 6.00 (4.16–8.67) nmol/l versus insulin-resistant group 3.62 (2.30–5.70) nmol/l.

The number of normal fertilized oocytes, proportion of cycles proceeding to embryo transfer, day of embryo transfer, number of transferred embryos and implantation rate were similar in insulin-resistant and non-insulin-resistant women (Table II). The pregnancy rate was also similar between groups (Table II), even after accounting for the unequal number of cycles by Kaplan–Meier analysis [cumulative pregnancy rate, insulin-resistant group: 64% (95% CI 24–100%) versus non-insulin-resistant group: 81% (95% CI 63–100%)]. Ovarian hyperstimulation syndrome developed in six cycles after ovulation induction, and its overall incidence was similar in the two groups (Table II).

Of the 36 pregnancies, 27 (75%) were carried to term. Five

(14%) abortions occurred before 6 weeks pregnancy, three (8%) between 6–12 weeks, and one (3%) after 12 weeks pregnancy. The proportion of deliveries was somewhat lower in the insulin-resistant group, although this difference was not significant [9/14 (64%) versus 18/22 (82%); $P = 0.24$].

Discussion

In this study, infertile women with PCOS underwent long-term down-regulation with buserelin acetate, ovarian stimulation with recombinant FSH, and IVF or ICSI. The impact of insulin resistance on infertility treatment was examined, and it was found that the presence of insulin resistance did not affect the number of collected oocytes, the number of normal fertilized oocytes, implantation and pregnancy rates, the incidence of ovarian hyperstimulation syndrome, and pregnancy outcome. Moreover, insulin resistance had no effect on hormone levels during ovarian stimulation, as insulin-resistant and non-insulin-resistant women had similar concentrations of FSH, LH, testosterone and androstenedione throughout ovarian stimulation. Insulin-resistant women, however, tended to have lower SHBG and higher insulin concentrations during stimulation. Furthermore, insulin-resistant women needed more FSH for ovarian stimulation and had lower oestradiol concentrations during ovarian stimulation than women with normal insulin sensitivity. These differences, however, were not significant after accounting for BMI, suggesting that they were mostly due to obesity, and hyperinsulinaemia had minimal impact on both FSH dose and oestradiol concentrations.

In studies where PCOS women received low-dose purified FSH without prior GnRH agonist down-regulation, it was shown that insulin resistance alters ovarian response to stimulation. Indeed, hyperinsulinaemia independent of body weight was associated with increased FSH requirement, higher oestradiol concentrations, risk of multifollicular development and high cancellation rate (Fulghesu *et al.*, 1997; Dale *et al.*, 1998). In contrast to these reports (amongst which one is from our centre; Dale *et al.*, 1998), it was not possible to find such effects of insulin resistance on ovarian stimulation in the present study. This conflict with earlier studies may be related to the different stimulation regimens, as down-regulation with buserelin acetate and stimulation with recombinant FSH may neutralize the impact of hyperinsulinaemia on ovarian response in PCOS.

In response to stimulation with LH and FSH, growing follicles secrete androgens and oestrogen: LH induces androgen synthesis by theca cells, while granulosa cells aromatize androgens in response to FSH; granulosa cells, however, also become sensitive to LH when the follicle matures (Richards, 1994). Studies on cultured granulosa cells show that insulin stimulates both androgen synthesis and aromatase activity by enhancing the effects of FSH and LH *in vitro* (Franks *et al.*, 1999). In the current study, however, LH concentrations may have been too low for insulin to stimulate androgen secretion, since GnRH agonist down-regulation suppressed LH secretion and women received recombinant FSH without LH activity. Indeed, similar androgen concentrations were found in insulin-resistant and non-insulin-resistant women throughout the stimu-

lation, despite the marked hyperinsulinaemia in insulin-resistant women. Lower intrafollicular androgen concentrations may have resulted in a healthier intrafollicular environment and in restored gonadotrophin sensitivity. Furthermore, lower androgen production meant less substrate for aromatization, and in this way the excessive oestradiol release that was seen during low-dose FSH stimulation (Fulghesu *et al.*, 1997) was avoided.

Besides stimulating androgen synthesis, insulin was also shown to increase aromatase activity in isolated granulosa cells *in vitro* (Poretsky *et al.*, 1988; Erickson *et al.*, 1990; Pierro *et al.*, 1997). Nonetheless, in the current study similar oestradiol concentrations were found in insulin-resistant and non-insulin-resistant women after controlling for differences in body weight, and the number of collected oocytes was also alike—suggesting that oestradiol synthesis per growing ovarian follicle was similar between groups. These findings do not support the fact that stimulation of aromatase by insulin *in vitro* results in an increased oestradiol release in hyperinsulinaemic PCOS women *in vivo*. Several causes may account for this disparity: the intricate mechanism that regulates aromatase activity *in vivo*, including gonadotrophins, GnRH, androgens and growth factors (Richards, 1994), may have a more pronounced effect on ovarian steroid secretion than does insulin, particularly when women receive long-term GnRH agonist treatment. Furthermore, long-standing hyperinsulinaemia may down-regulate insulin receptors in the ovary, and as a result reduce insulin's effect on granulosa cells (Samoto *et al.*, 1993; Fedorcsák *et al.*, 2000b).

Insulin resistance in PCOS is also associated with an impaired progesterone synthesis by cultured granulosa-lutein cells *in vitro* (Fedorcsák *et al.*, 2000b). Such a defect of progesterone release during the luteal phase may impair outcome of low-dose FSH stimulation in insulin-resistant PCOS women (Fulghesu *et al.*, 1997; Dale *et al.*, 1998), since luteal phase support is usually not given with ovulation induction protocols. During long-term down-regulation and ovarian stimulation for IVF or ICSI, women receive luteal support (in the current study, progesterone), which may hence overcome impaired corpus luteal function in hyperinsulinaemia. Taken together, the results of the present study show that the effects that hyperinsulinaemia has on ovarian steroid synthesis *in vitro* or during low-dose FSH stimulation *in vivo* are minor when PCOS women receive long-term down-regulation and stimulation with recombinant FSH.

In contrast to insulin resistance, obesity had a marked impact on infertility treatment in PCOS women. Indeed, it was also found that obesity is associated with higher gonadotrophin requirement during stimulation, and fewer collected oocytes. These effects were independent of the insulin resistance index, suggesting that factors other than hyperinsulinaemia contribute to relative ovarian gonadotrophin resistance in obesity. One such factor could be the altered pharmacokinetics of gonadotrophins in obese women, resulting in lower effective concentrations of exogenous FSH (Fridström *et al.*, 1997). Another possible factor inducing gonadotrophin resistance is the adipocyte-derived hormone, leptin. Indeed, leptin inhibits the stimulatory effect of FSH on steroid synthesis by granulosa cells *in vitro* (Zachow and Magoffin, 1997; Agarwal *et al.*, 1999),

and high intrafollicular leptin concentrations are associated with relative gonadotrophin resistance in obese PCOS women (Fedorcsák *et al.*, 2000a). In either way, increased gonadotrophin doses to compensate for relative gonadotrophin resistance induced by obesity might result in impaired oocyte or embryo quality, implantation failure and pregnancy complications. Indeed, superovulation in mice induces various defects, such as abnormal embryonic development and decreased invasional capacity of blastocysts *in vitro*, lower implantation rate, delayed implantation, increased length of gestation, lower birth weight and developmental retardation *in vivo* (Ertzeid and Storeng, 1992; Ertzeid *et al.*, 1993). Although these defects were shown in mice, in the current study no significant differences were found in conception rate and pregnancy outcome between insulin-resistant and non-insulin-resistant women, even though hyperinsulinaemic women received higher FSH doses.

In summary, serum hormone concentrations of insulin-resistant and non-insulin-resistant PCOS women were similar after controlling for body weight, and insulin resistance did not affect the outcome of IVF treatment in this study. Obesity, however, independently of insulin resistance, was associated with a relative gonadotrophin resistance, as shown by higher gonadotrophin requirement, a lower number of collected oocytes and lower peak oestradiol concentrations.

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