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The chromosomal normality of unfertilized oocytes from patients with polycystic ovarian syndrome

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The present study was designed to compare the cycle characteristics of in-vitro fertilization (IVF) and the chromosomal normality of oocytes in patients with polycystic ovarian syndrome (PCOS) with those of patients with tubal factor infertility. In all, 28 cycles of 24 PCOS patients and 55 cycles of 31 patients with tubal factor infertility (control) were investigated. Although a significantly greater number of oocytes were retrieved from PCOS patients (mean ± SD: 15.6 ± 6.4 versus 9.0 ± 4.0, PCOS versus control group, $P < 0.05$), the percentage of fertilized oocytes was significantly lower in the PCOS group compared with controls (40.1 versus 73.8%, $P < 0.01$). The pregnancy rate per embryo transfer did not differ between the two groups. Cytogenetic analysis was performed on 74 oocytes from PCOS patients and 73 oocytes from control patients. In the PCOS group, 10 of the 74 oocytes (13.5%) demonstrated aneuploidy, four (5.4%) oocytes were diploid and six (8.1%) oocytes were metaphase II with a prematurely condensed sperm chromosome (PCC). In the tubal infertility group, nine of the 73 (12.3%) oocytes showed aneuploidy, four (5.5%) oocytes were diploid and five (6.8%) oocytes were found to have PCC. There was no significant difference in the aneuploidy, diploidy and PCC rates between the two groups. These results suggest that the reduced fertilization observed in PCOS is not attributable to chromosomal aberrations or immaturity of oocytes recruited from patients with PCOS.

Key words: chromosome/oocytes/polycystic ovarian syndrome

Introduction

In-vitro fertilization (IVF) with embryo transfer has proven to be an effective therapy for polycystic ovarian syndrome (PCOS) patients. However, several reports have suggested reduced fertilization and cleavage rates in women with PCOS compared to those with other infertility diagnoses, although cumulative pregnancy rates per oocyte aspiration and per embryo transfer did not differ between the groups (Dor *et al.*, 1990; Urman *et al.*, 1992). It has also been demonstrated that significantly more oocytes are recovered from PCOS patients compared with those with other infertility factors (Homburg *et al.*, 1993; MacDougall *et al.*, 1993; Kodama *et al.*, 1995). These findings indicate that the additional number of oocytes recovered from PCOS patients may compensate for the potentially lower fertilization and cleavage rates. It has been postulated that the elevated tonic luteinizing hormone (LH) concentrations in the follicular phase observed in PCOS patients interfere with folliculogenesis, resulting in poor-quality oocytes and contributing to the reduced fertilization rates and increased rate of spontaneous abortion (Stanger *et al.*, 1985; Howels *et al.*, 1986; Regan *et al.*, 1990). Homburg *et al.* (1993) demonstrated that prevention of premature luteinization or reduction of tonic LH concentrations by using gonadotrophin-releasing hormone agonist (GnRHa) improved both the fertilization and pregnancy rates in PCOS patients compared with cycles receiving gonadotrophin only. However, Dor *et al.* (1992) demonstrated that administration of GnRHa in patients with PCOS did not affect the IVF results and suggested that development of poor-quality oocytes in PCOS patients may be attributable to reduced fertilization rates.

This evidence raises the possibility that chromosomal aberrations of the oocytes obtained from PCOS patients may result in poor fertilization rates. The present study was designed to investigate the chromosomal normality of unfertilized oocytes derived from patients with PCOS and, specifically, the differences in the incidence of chromosomal aberrations between the oocytes obtained from PCOS and those from tubal factor infertility patients.

Materials and methods

The study group was selected from patients who underwent IVF treatment between July 1993 and July 1995. Informed consent was obtained from all patients. In all, 28 cycles in 24 PCOS patients were included in this study. PCOS was defined as having typical ultrasound criteria (a hyperechogenic central stroma and at least 10 follicles of <9 mm diameter), LH:FSH ratio >1.5, and menstrual disturbances or anovulation. These patients had failed to conceive after at least three cycles of ovulation induction treatment with gonadotrophin. The control group consisted of 55 cycles of 31 patients with a pure tubal factor infertility. They were age-matched with patients in the PCOS group. Patients whose partner had abnormal semen quality were excluded from the study. Clinical characteristics of the patients in the PCOS and control groups are shown in Table I. The mean age and duration of infertility were similar for the two groups. Basal LH concentrations were significantly higher in the PCOS group than in the control group.

Follicular stimulation was achieved with a desensitizing protocol, using a combination of GnRHa (buserelin acetate, Suprecur; Hoechst Japan, Tokyo, Japan) and human menopausal gonadotrophin (HMG, Pergonal; Teikokuzouki, Tokyo, Japan). Suprecur was administered on day 2 after menstrual or withdrawal bleeding followed by injection of HMG after ovarian down-regulation was ascertained. The dose of

Table I. Clinical characteristics of the patient groups with polycystic ovarian syndrome (PCOS) and tubal factor infertility. Values are means ± SD

	PCOS	Tubal factor
No. of patients	24	31
No. of cycles	28	55
Age (years)	32.5 ± 2.4	32.0 ± 3.0
(range)	(28–39)	(26–37)
Duration of infertility (years)	5.6 ± 3.1	5.8 ± 2.9
Basal FSH (mIU/ml)	7.1 ± 2.6	7.3 ± 2.8
Basal LH (mIU/ml)	13.5 ± 5.2	6.1 ± 2.7[a]

FSH = follicle stimulating hormone; LH = luteinizing hormone.
[a]$P < 0.01$ for significant difference between basal LH values for the PCOS and tubal factor groups.

Table II. Cycle characteristics of in-vitro fertilization and embryo transfer in patients with polycystic ovarian syndrome (PCOS) or tubal factor infertility. Values are means ± SD

	PCOS	Tubal factor
No. of cycles	28	55
No. (range) of oocytes retrieved per cycle	15.6 ± 6.4 (5–28)	9.0 ±4.0[a] (2–17)
No. of fertilized oocytes per cycle	6.1 ± 4.7	6.6 ± 3.3
Fertilization rate (%)	40.1	73.8[b]
No. of embryos transferred per cycle	4.1 ± 2.1	4.2 ± 2.5
No. (%) of clinical pregnancies per embryo transfer	4 (14.3)	6 (17.1)
No. (%) of miscarriages per embryo transfer	0	1 (16.7)

[a]$P < 0.05$ for difference between the PCOS and tubal factor groups.
[b]$P < 0.01$ for difference between the PCOS and tubal factor groups.

HMG was individually adjusted according to ovarian response. When the dominant follicle was at least 16 mm in diameter and serum oestradiol was >400 pg/ml, 10 000 IU human chorionic gonadotrophin (HCG) was administered. Oocyte recovery was performed 34–36 h after the HCG injection using transvaginal ultrasound-guided aspiration. All follicles >14 mm in diameter were aspirated. Oocyte cultures were inseminated with a sperm suspension containing 105 motile spermatozoa per oocyte. Oocyte culture and sperm preparation were performed in human tubal fluid culture medium (Irvine Scientific, Santa Ana, CA, USA) supplemented with 10% maternal serum. The oocytes were examined for fertilization 16–18 h after insemination. Oocytes lacking two pronuclei and a second polar body were cultured for an additional 24 h.

Oocytes with no signs of fertilization and apparently normal morphology were prepared for chromosomal analysis by a gradual fixation–air-drying method, as described by Kamiguchi *et al.* (1993). Unfertilized oocytes were incubated for 5 min in 0.1% (w/v) pronase (type XIV; Sigma Chemical Co., St Louis, MO, USA) in phosphate-buffered saline to soften the zona pellucida; 0.9% sodium citrate with 7% fetal calf serum was used for 15 min as the hypotonic agent. Oocytes were fixed with fixative I (methanol:acetic acid:H_2O, 5:1:4 v/v/v) for 5 min, mounted on a grease-free slide, and covered with fixative II (methanol:acetic acid, 3:1 v/v). Thereafter, the slide was dipped into fixative II for 30 min. Finally, it was fixed with fixative III (methanol:acetic acid:H_2O, 3:3:1) for 1 min, and dried by blowing with moist air. Fixed preparations were stained with 2% Giemsa stain for 10 min.

Serum oestradiol was assayed using commercially available radioimmunoassay kits (Diagnostic Product Co., Los Angeles, CA, USA). LH and FSH were determined by an enzyme-linked fluorescent immunoassay (BioMérieux, Marcy l'Etoile, France). The intra- and inter-assay coefficients of variation were <10% for all assays.

Statistical significance of the data was determined by the χ^2 test, Fisher's exact test and Student's *t*-test as appropriate. The differences were considered significant at a level of $P < 0.05$.

Results

The IVF/embryo transfer cycle characteristics of the PCOS and tubal factor control groups are shown in Table II. Although a significantly greater number of oocytes was retrieved in the PCOS group ($P < 0.05$), the percentage of fertilized oocytes was significantly lower in the PCOS group compared with the controls ($P < 0.01$). The pregnancy rate per embryo transfer or per patient did not differ between the two groups. Similarly, no significant difference was observed in the miscarriage and the live birth rates between the two groups.

Table III. Chromosomal analysis of oocytes from patients with polycystic ovarian syndrome (PCOS) or tubal factor infertility

	PCOS	Tubal factor
No. of oocytes fixed	142	125
No. of metaphase II oocytes	112	101
No. of oocytes analysed	74	73
No. (%) of normal oocytes	59 (79.7)	60 (82.2)
No. (%) aneuploid oocytes	10 (13.5)	9 (12.3)
No. (%) hyperhaploid oocytes	4 (5.4)	4 (5.5)
No. (%) hypohaploid oocytes	6 (8.1)	5 (6.8)
No. (%) diploid oocytes	4 (5.4)	4 (5.5)
No. (%) oocytes at MII + sperm PCC[a]	6 (8.1)[b]	5 (6.8)

[a]MII + sperm PCC = oocytes that were metaphase II and had an additional set of prematurely condensed sperm chromosomes at the G_1 phase.
[b]One oocyte was diploid metaphase II and was also included in the diploid group.

A total of 142 oocytes recruited from the PCOS group and 125 oocytes from the tubal factor group were processed for chromosomal slide preparation. Oocytes with apparently normal morphology were randomly selected. Out of the 142 oocytes derived from PCOS patients that were fixed for chromosomal preparation, seven (4.9%) were lost accidentally, 12 (8.5%) contained no chromosomes, seven (4.9%) contained metaphase I chromosomes, and four (2.8%) were fertilized but had failed to divide. Similarly, of the 125 oocytes in the control group used for preparation, seven (5.6%) were accidentally lost, nine (7.2%) showed no chromosomes, five (4.0%) contained metaphase I chromosomes, and three (2.4%) were fertilized. Of the 112 (78.9%) and 101 (80.8%) oocytes in the respective groups which were in the second meiotic metaphase (MII), 74 (66.1%) and 73 (72.3%) oocytes respectively produced analysable preparations. The results of chromosomal analysis for each group are shown in Table III. In the PCOS group, 10 of 74 (13.5%) analysable preparations demonstrated aneuploidy, including four (5.4%) hyperploid and six (8.1%) hypoploid oocytes. Four (5.4%) oocytes were diploid and six (8.1%) oocytes were metaphase II with an additional set of prematurely condensed sperm chromosomes at the G_1 phase (PCC). In one case, PCC was accompanied by diploidy. In the tubal infertility group, nine of 73 (12.3%) oocytes were aneuploid, including

four (5.5%) hyperploid and five (6.8%) hypoploid, and four (5.5%) oocytes were found to be diploid. There were five (6.8%) oocytes with PCC. There was no significant difference in the incidence of aneuploidy, diploidy or PCC between oocytes from patients with PCOS or tubal factor infertility.

Discussion

Several reports have suggested reduced fertilization rates and an increased incidence of spontaneous abortion in women with PCOS when compared with patients with tubal factor infertility, although these reports have been conflicting (Dor *et al.*, 1990; Urman *et al.*, 1992; Homburg *et al.*, 1993; Hardy *et al.*, 1995). The mean number of embryos transferred and the pregnancy rates do not differ between PCOS and other infertility patients, because the significantly greater number of oocytes recovered from PCOS patients compensates for the lower fertilization rate (MacDougall *et al.*, 1993; Kodama *et al.*, 1995; Buyalos and Lee, 1996). Elevated concentrations of LH in the follicular phase have been reputed to have a crucial role in reduced fertilization rates as well as pregnancy wastage in PCOS, since the reduction in LH induced by GnRHa administration is associated with improved fertilization rates (Homburg *et al.*, 1993). In addition, Ashkenazi *et al.* (1995) demonstrated that the oocytes of patients with PCOS who were exposed to GnRHa had a significantly higher implantation rate than those from patients treated with FSH and HMG alone in oocyte retrieval cycles. In our present study, we confirmed that a significantly greater number of oocytes, with lower fertilization rates, were recruited in the PCOS group compared with the tubal factor infertility group, even though all patients were treated with a GnRHa long protocol.

It has been demonstrated that decreased fertilization and implantation rates are observed in patients who are high responders to gonadotrophins (Pellicer *et al.*, 1989). Tarin *et al.* (1990) reported that a significantly greater incidence of diploidy and of PCC was found in women where >11 oocytes were obtained, compared with women with a moderate response to gonadotrophins, although the total incidence of chromosomal abnormalities was not different between the groups. This finding suggests a higher degree of cytoplasmic immaturity as the number of oocytes recruited increases, since PCC and diploidy are considered to be characteristic of oocytes with immature cytoplasm (Badrenas *et al.*, 1989; Calafell *et al.*, 1991). Tarin *et al.* (1991) also demonstrated a lower rate of diploid oocytes from fertile control women compared with inseminated unfertilized oocytes from infertile women, although the incidence of aneuploidy was not different between the two groups. This suggested that a lower fertilization rate could be the consequence of oocyte immaturity. Thus, some selection against immature oocytes may occur at fertilization. Any selection against aneuploid oocytes seems to be very weak.

Taken together, it can be speculated that the observed low fertilization rates of oocytes from PCOS patients may be attributable to the high incidence of immature oocytes recruited. However, the present findings demonstrated that there were no differences in the incidence of metaphase II, diploidy, PCC or aneuploidy between the PCOS and control groups. In cases with a high ovarian response to gonadotrophin, the cohort of multiple follicles recruited represents follicles in the early stages of development (Tarin *et al.*, 1990). In contrast, the cohort of follicles recruited in PCOS patients seems to comprise follicles at more mature stages of development. The multiple follicles associated with PCOS are simply arrested at the small antral stage, being neither atretic nor apoptotic and following normal development after stimulation by gonadotrophin (Fauser, 1994). Hardy *et al.* (1995) also reported that embryos from PCOS patients under an optimized ovulation induction regimen were of good quality and developmental potential. These concepts may explain the difference in the incidence of immature oocytes between PCOS and high responder groups. Another reason for the absence of any difference in the incidence of diploid oocytes in PCOS and control patients may be the similarity of the ages of the patients in the two groups in our study. Roberts and O'Neill (1995) have demonstrated that the proportion of diploid oocytes increases significantly with advancing maternal age, being particularly prevalent in women aged >35 years.

It has been suggested that diploid oocytes originate following disruption of the endocrine control of meiosis, resulting in impaired extrusion of the first polar body (Hansmann *et al.*, 1983). Although the pathogenesis of PCOS is still unclear, elevated concentrations of LH, insulin resistance and over-production of androgens are associated with this syndrome (Kazer *et al.*, 1989; Buyalos *et al.*, 1992; Homburg *et al.*, 1996). Elevated free insulin-like growth factor (IGF) and decreased IGF-binding protein-1 (IGFBP-1) concentrations in combination with LH may stimulate androgen production in PCOS patients through dysregulation of P450c 17α activity (Rosenfield *et al.*, 1990). Based on the present results, this endocrine disruption, including possible effects on the growth hormone (GH)/IGF-1 system, may be mediated either via a modified accumulation of RNA or through the response of granulosa cells to FSH during oocyte growth (Hardy *et al.*, 1995). It is unlikely, however, to influence either the meiotic maturity or the chromosomal normality of oocytes. This finding is, in part, supported by recent observations that a high incidence of miscarriage in PCOS patients is not correlated with fetal chromosomal abnormality (Hasegawa *et al.*, 1996) and that, with an optimized ovulation induction, oocytes from PCOS patients are of good quality (Hardy *et al.*, 1995). The reason for lower fertilization in oocytes from PCOS patients was not determined in the present study but may be related to our ovulation induction protocol. A suboptimal ovulation regimen may lead to the development of a hyperandrogenic intrafollicular milieu or to elevated concentrations of LH in PCOS patients, as suggested by Hardy *et al.* (1995), who demonstrated that suboptimal ovulation induction may have a detrimental effect on oocyte quality. The mechanism by which endocrine disruption causes impairment of oocyte quality is not well understood. It has been postulated that LH induces premature meiotic maturation of oocytes (Abdulwahid *et al.*, 1985) and may cause detrimental prolongation of the interval between completion of first meiosis and fertilization. In addition, Anderiesz and Trounson (1995) reported that androgen

could adversely affect murine oocyte maturation, fertilization and development *in vitro*.

In conclusion, our results demonstrate that a significantly greater number of oocytes were retrieved but a smaller proportion fertilized in patients with PCOS compared with those with tubal factor infertility. This study also suggests that reduced fertilization rates are not attributable either to an increased rate of chromosomal aberrations or to immaturity of oocytes recruited from patients with PCOS. Unfavourable endocrine environments in the follicles, including hyperandrogenism, elevated concentrations of LH and disruption of the GH/IGF system, as have been reported in PCOS, may adversely affect both the quality of oocytes and fertilization.

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