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Sustained fertility from 22 to 41 years of age in women with polycystic ovarian syndrome

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BACKGROUND: Subfertility due to chronic anovulation is common in women with polycystic ovary syndrome (PCOS) and is often treated with IVF. Women with PCOS have an increased ovarian follicle and oocyte count, increased ovarian reserve and/or a slower rate of follicle atresia. If so, one would expect women with PCOS to display a delayed reduction in fertility with advancing age as compared with eumenorrheic women.

METHODS: To test this hypothesis, we compared oocyte count and live birth rates among two groups undergoing IVF, 500 women with PCOS and 500 eumenorrheic women with infertility due to tubal factor only.

RESULTS: Across the age range of 22–41 years, oocyte count and live birth rates remained stable in women with PCOS. In the eumenorrheic comparison group, these parameters decreased significantly with age.

CONCLUSIONS: Women with PCOS display sustained fertility with advancing age as compared with infertile eumenorrheic women.

Key words: PCOS / ageing / fertility / IVF

Introduction

Polycystic ovary syndrome (PCOS) is a condition characterized by increased ovarian androgen production, insulin resistance and infrequent ovulation (Dunaif, 1997). Despite ovarian dysfunction and attendant subfertility, women with PCOS display increased follicle and oocyte count both unstimulated and during ovarian stimulation and oocyte retrieval for IVF (Homburg et al., 1993). In general, female fertility declines with advancing age (Malizia et al., 2009). Increasing infertility reflects the quantity and quality of the remaining oocyte pool, indeed 50% of women are sterile at the age of 41 (te Velde and Pearson, 2002). The size of the initial follicle stock and the proportion undergoing atresia or commencing growth are mainly genetically determined, and these, together with environmental factors, such as diet, cigarette smoking and cancer treatment, influence the rate of ovarian aging (Alviggi et al., 2009).

Anti-Müllerian hormone (AMH) is secreted by proliferating granulosa cells in growing follicles that have started to mature and Franks and colleagues have demonstrated AMH secretion from the primordial stage onwards. They conclude that 'even squamous granulosa cells undergo a low rate of cell division' (Stubbs et al., 2005) and 'that a proportion of these follicles were growing at a very slow rate' as also shown in the macaque (Gougeon, 1996) and rat (Hirshfield, 1989).

AMH reaches its maximum secretion in small antral follicles up to 6 mm (Durlinger et al., 2002). Beyond this point, follicular growth becomes FSH dependent and AMH expression diminishes. AMH inhibits the recruitment of primordial follicles (Themmen, 2005) and in the mouse, FSH stimulated pre-antral follicle growth is attenuated in the presence of AMH (Durlinger et al., 2001). AMH expression is less prevalent in primordial and transitional follicles in PCOS women than in controls and it has been speculated whether this may permit increased recruitment of follicles in PCOS women (Stubbs et al., 2005). As they also display prolonged survival of pre-antral follicles, these two factors may contribute to the presence of an increased number of visible follicles in PCOS women (Webber et al., 2007). Both the excessive accumulation of antral follicles and their increased granulosa cell, AMH secretion (Catteau-Jonard et al., 2008) may

explain the increased circulating AMH level in PCOS women (Wang et al., 2007). Age-related female infertility is mainly based on changes in ovarian reserve, defined as the number and quality of the remaining follicles in the ovaries at a given age. As AMH correlates well with age and antral follicle count, it may constitute a sensitive marker for ovarian aging. Moreover, the size of the monthly growing follicle pool may reflect the unmeasureable number of primordial follicles in the ovaries and thus indicate the ovarian reserve (Ledger, 2010).

Women with PCOS achieve similar pregnancy and live birth rates per treatment cycle as normo-ovulatory women (Heijnen et al., 2006). Since women with PCOS have both higher follicle counts (Webber et al., 2007) and serum AMH concentrations (Tehrani et al., 2010), we hypothesized that they could also have sustained fertility compared with controls. To test this assumption, we compared oocyte number and live birth rates to chronological age in women with PCOS and eumenorrheic controls undergoing IVF.

Patients and Methods

Selection of patients and study design

Patients in this cohort study were retrospectively identified in our clinical database with detailed records on cycles of assisted reproduction treatments with IVF or ICSI. The complete cohort of women with PCOS (n = 708) was retrieved from the database. The diagnosis of PCOS was based on the 2003 (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), that is, the presence of at least two of the following conditions: oligo/anovulation, hyperandrogenism and polycystic ovaries. Attenuated 21-hydroxylase activity, Cushing syndrome, androgen-secreting tumors and hyperprolactinemia were excluded by appropriate tests. The retrieved records were manually checked to identify and exclude women with co-existing diseases, such as tubal disease, male factor infertility and endometriosis. The final case cohort contained 500 women with PCOS.

The control group consisted of eumenorrheic women who underwent IVF or ICSI for tubal factor infertility only (n = 856). We excluded women with other co-existing conditions. These initial control cohorts differed with respect to mean age (PCOS, 31.3 years; tubal factor, 33.4 years; P < 0.001) and the shape of age distributions. To reduce bias in subsequent regression analyses and to obtain a similar age distribution in the case and control groups, one woman from the tubal factor cohort was matched with each woman in the PCOS cohort according to age across the age range of 20–44 years. Matching was performed with the nearest month of birth. Finally, each group consisted of 500 patients after matching.

Ovarian stimulation and IVF

Ovarian stimulation and IVF were performed as previously described (Bjercke et al., 2005). Briefly, pituitary down-regulation was achieved with GnRH agonist (Suprecur, Sanofi-Aventis, Germany or Synarela, Pfizer, Belgium) administered intranasally from the mid-luteal phase of the preceding cycle in patients with regular menses. Patients with PCOS who were oligomenorrheic were given a progestine to induce endometrial sloughing. Recombinant FSH (Gonal-F, Merck Serono, Geneva, Switzerland or Puregon, Organon, Oss, The Netherlands) or human menopausal gonadotrophin (Menopur, Ferring, Denmark) was given for ovarian stimulation. The standard starting dose was 75 IU daily in the PCOS group and 150 IU daily in the control group. The dose was adjusted after 5–9 days according to the ovarian response. In some cases (n = 7 in each group), down-regulation with GnRH agonist was not performed and spontaneous

ovulation was prevented by administration of GnRH antagonist (Cetrotide, Serono, Germany or Orgalutran, Organon, The Netherlands 0.25 mg daily), beginning on the sixth day of stimulation with FSH or the day when the largest follicle reached the diameter of 14 mm. Transvaginal oocyte retrieval was performed 34–36 h after administration of 6500 or 10 000 IU human chorionic gonadotrophin (hCG, Ovitrelle, Serono or Pregnyl, Organon). One or two embryos were transferred on Day 2 or Day 3 after follicle aspiration. Luteal phase support was provided as intravaginal progesterone capsules (Progestan, Organon, 600 mg daily) or gel (Crinone, Serono, 180 mg daily).

To avoid selection bias, only the first treatment was included in the analysis. Pregnancy was defined by serum hCG concentration >20 IU/l on Day 12 after embryo transfer. Ovarian hyperstimulation syndrome was diagnosed by a combination of clinical symptoms (abdominal distension, nausea, excessive follicle development of >25 follicles per ovary, or ascites) independent of hospitalization or outpatient management. Body mass index (BMI, kg/m^2) was assessed at the first visit.

Data analysis

Data are shown as mean and standard deviation or median and range. Between-group comparisons were performed with chi-square test, t-test or Mann–Whitney test as appropriate. P < 0.05 was considered statistically significant. To assess age-related ovarian capacity to mature follicles in response to FSH stimulation, multiple regression analyses were performed in the case and control group using age, mean daily FSH dose and BMI as independent variables (Fig. 1). In the PCOS group, a history of ovarian surgery was also considered as a covariate. The analysis was performed for the groups separately, and regression coefficients (slopes) for age were compared between the groups with t-test.

Age-related changes in live birth rate were compared between the groups by calculating age-specific regression coefficients with logistic regression analysis. The age of the woman and the number of replaced embryos were used as covariates in these analyses (Fig. 2).

The R program package (available at www.r-project.org) version 2.5.0 was used for statistical analysis and plotting the data.

Results

The age was similar in the two groups but women with PCOS had higher BMI than women with tubal factor infertility and received lower FSH dosage than controls, with a lower daily dose but a more prolonged stimulation (Table I). The number of collected oocytes was statistically comparable between the groups, whereas the number of diploid fertilized oocytes was lower in the PCOS compared with the tubal factor infertility group. The number of cycles in which no embryos were transferred was higher in the PCOS than in the control group. No statistical differences were revealed among the groups in terms of total pregnancy rate and pregnancy outcome (Table I).

In the PCOS group, no significant association between age and number of collected oocytes was observed [regression coefficient (slope) +0.11, SE: 0.09, P = 0.23]. In the tubal factor infertility group, advanced age was associated with a reduced number of retrieved oocytes (slope −0.12, SE: 0.06, P = 0.05). The slopes for regression lines (Fig. 1) differed significantly between the PCOS group and tubal factor infertility (P = 0.01).

In the PCOS group, a significant association between age and live birth rate was not observed (coefficient: −0.001, P = 0.96). In the tubal factor infertility group, advanced age was associated with a
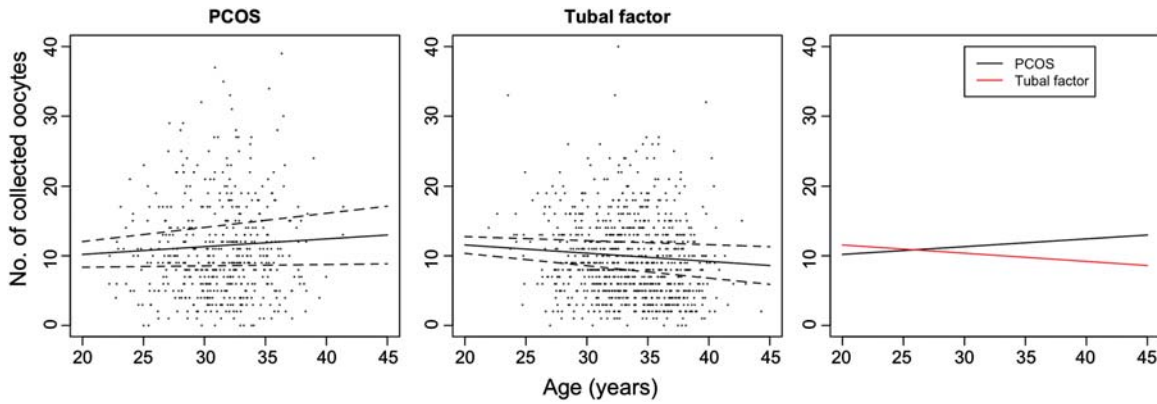
Figure 1 Number of collected oocytes during first treatment with IVF in women with PCOS or tubal factor infertility. Dotted lines indicate SE.
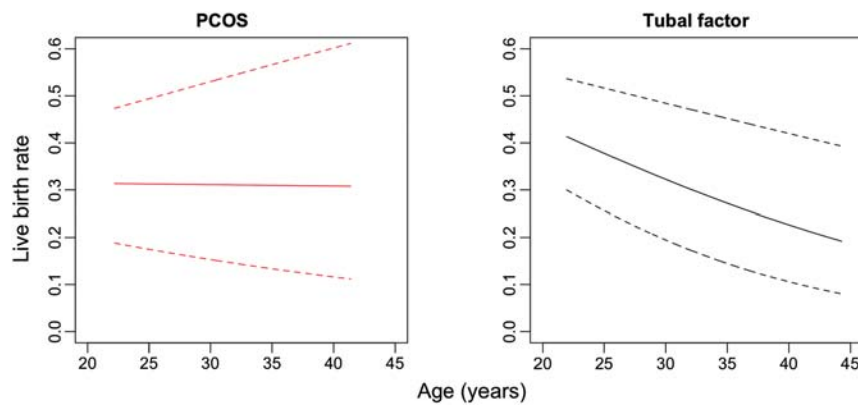

Figure 2 Predicted age-specific live birth rates for women with PCOS or tubal factor infertility undergoing first treatment with IVF. Dotted lines indicate SE.

significantly reduced probability of live birth (coefficient: -0.049, $P = 0.03$) (Fig. 2).

Discussion

The results indicate that women with PCOS maintain a stable oocyte count and live birth rate across the age range of 22–41 years during IVF treatment, whereas normo-ovulatory women with tubal factor infertility experience a significant decline with increasing age during this period. Although the finding support the hypothesis that women with PCOS have a sustained fertility compared with controls, conclusions about the pace of ovarian aging cannot be drawn, since the age range of 22–41 years does not cover subsequent events during reproductive aging, such as the age at cessation of fertility or menopause.

Sustained fertility in PCOS women is supported by many observations. First, women who had been infertile due to anovulation experienced spontaneous pregnancies late in reproductive age (Lunde et al., 2001; Vulpoi et al., 2007) and entered menopause later than ovulatory women (Dahlgren et al., 1992). Second, increased ovarian volume and follicle number was observed in

PCOS compared with controls across the age range of 20 years to menopause (Asamarai et al., 2009), which is in accordance with the observation that the serum AMH level is increased and shows a slower rate of decline with aging in PCOS women (Mulders et al., 2004; Tehrani et al., 2010). Finally, when treated with in vitro maturation, which does not entail FSH stimulation, women with PCOS have an increased number of oocytes collected and higher live birth rate compared with controls (Child et al., 2001). A sustained number of growing follicles, however, fails to gain support by some other studies. Inhibin B is a marker of ovarian antral follicle cohort in PCOS (Bili et al., 2001) and eumenorrheic women (Danforth et al., 1998). Bili et al. (2001) found an age-related reduction in the number of antral follicles and a decrease in inhibin B in 472 PCOS patients and Elting, in 27 women found an age-related reduction in inhibin B increment during FSH-stimulated ovarian reserve testing (Elting et al., 2001). However, Piltonen et al. (2004) found unchanged age-related inhibin B levels in 42 women with PCOS. Inhibin B inhibits FSH release, and a lower inhibin B level may contribute to a higher FSH level and thus favor follicle growth with more regular and more frequent ovulatory cycles in older women with PCOS.

Table I Clinical characteristics, ovarian stimulation and treatment outcome among age-matched women with PCOS and tubal factor infertility undergoing IVF.

	PCOS (*n* = 500)	Tubal factor infertility (*n* = 500)	*P*-value
Age (years)	31.3 (3.6)	31.6 (3.2)	
BMI (kg/m^2)	26.5 (5.2)	23.5 (3.9)	<0.001
Total FSH dose (IU)	1650 (525–9825)	1725 (525–2500)	0.04
FSH treatment (days)	12 (6–39)	11 (6–28)	<0.001
Number of collected oocytes	9 (0–39)	9 (0–33)	0.20
Number of diploid fertilized oocytes	5 (0–25)	6 (0–23)	<0.001
Number of cycles with transfer of embryos			<0.001
0 embryo	65 (13%)	35 (7%)	
1 embryo	102 (20%)	79 (16%)	
2 embryos	333 (67%)	386 (77%)	
No. of pregnancies	169 (33.8%)	169 (33.8%)	0.99
No. of spontaneous abortions	47 (9.4%)	36 (7.2%)	0.25
No. of ectopic pregnancies	0	6	
No. of stillbirths	1	0	
No. of live births	121 (24.2%)	124 (24.8%)	0.82
Ovarian hyperstimulation syndrome	11 (2.2%)	3 (0.6%)	0.03

An alternative, though insufficiently corroborated, explanation for sustained fertility in PCOS is a reduced pace of ovarian aging, possibly involving pathways implicated in ovarian aging and aging in general. In experimental models with worms (Tissenbaum and Ruvkun, 1998; Mukhopadhyay and Tissenbaum, 2007; Angelo and Van Gilst, 2009) and mice (Baba *et al.*, 2005), conditions that slow metabolism and confer metabolic efficiency also slow gamete aging, especially when the metabolic efficiency is not the direct result of undernutrition (Martin *et al.*, 2007). Women with PCOS have been shown to be metabolically efficient proportional to the extent of insulin resistance (Robinson *et al.*, 1992) which may be a manifestation of underlying metabolic efficiency (Georgopoulos *et al.*, 2009). However, our study did not permit us to investigate whether this finding might be associated with or causally related to metabolic efficiency or insulin resistance.

We wish to highlight some limitations of our study. First, it is uncertain whether oocyte count in IVF is a suitable marker for sustained fertility or ovarian aging in PCOS patients. Indeed, there is a positive correlation between basal AMH levels and the number of retrieved oocytes in women undergoing ovarian stimulation (La Marca *et al.*, 2010), but this is not the case for PCOS patients (Wang *et al.*, 2007). Moreover, the longer lifeline of follicles in PCOS may make the correlation between the number of resting primordial follicles and growing follicles, i.e. AMH, less direct compared with controls. Second, the differences in endocrine and metabolic dysfunctions and thereby phenotype in PCOS women constitute a wide continuum. Minor fertility derangements may initially be successfully treated by simple treatment such as hormonal ovulation induction with clomiphene citrate, ovarian surgery or low-dose FSH stimulation therapies. The PCOS group in this study required more complex therapy like IVF and the results of this study may therefore constitute a subgroup of PCOS women. Third, collection of an increased number of oocytes in older women with PCOS may not imply a sustained oocyte quality.

It is uncertain how oocyte quality would be affected in aging polycystic ovaries. An increased density of follicles (Hughesdon, 1982; Webber *et al.*, 2003; Maciel *et al.*, 2004), lower apoptotic rate of granulosa cells (Das *et al.*, 2008) and decreased follicle loss through atresia (Webber *et al.*, 2007) may result in a larger resting follicle cohort, which may be associated with an improved oocyte quality. Indeed, in young women and also cattle, the antral follicle number and AMH levels correlate with the number of morphologically healthy follicles and oocytes, response to ovulation induction, number of high-quality transferable oocytes and blastocyst development (Scheffer *et al.*, 2003; Ireland *et al.*, 2008). Furthermore, production of androstenedione and testosterone by theca cells may be inherently related to the number of healthy growing follicles and low-circulation androgen levels reflect a low number of follicles (Mossa *et al.*, 2010) and a poor ovarian response (Qin *et al.*, 2011). As androgen levels are higher in older PCOS women than in controls, they may experience a better hormonal follicular function contributing to a relative better oocyte quality. In addition, circulating FSH concentration is inversely related to morphologically healthy follicles and oocytes (Ireland *et al.*, 2007). FSH is a positive regulator of aromatase, eastadiol receptors and estradiol production by granulosa cells (Silva and Price, 2000). In normal women, FSH begins to increase at the age of 35 with a marked increase beyond 40 years, whereas women with PCOS at this age may achieve a normal menstrual cycle reflecting a sound hormonal constitution (Elting *et al.*, 2000). Nonetheless, the ovarian microenvironment may still undergo age-related deterioration increasing the proportion of oocytes with meiotic abnormalities. Assessment of these defects, however, is challenging since routine morphological scoring of oocytes fails to reflect age-related biological changes (Stensen *et al.*, 2010).

We conclude that our data indicate a better fertility with advanced age in PCOS women compared with controls and that future research may reveal the exact mechanisms for this phenomenon.

Authors' roles

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