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ARTICLE

Low aneuploidy rate in early pregnancy loss abortuses from patients with polycystic ovary syndrome

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Abstract A prospective cohort study was conducted to determine whether chromosome aneuploidy increases the risk of early spontaneous abortions in patients with polycystic ovary syndrome (PCOS). A total of 1461 patients who conceived after IVF and embryo transfer were followed; 100 patients who had experienced clinical spontaneous abortion were recruited, 32 with PCOS and 68 without PCOS. Before 2013, genetic analysis comprised conventional cultured villus chromosome karyotyping and a multiplex ligation-dependent probe amplification subtelomere assay combined with fluorescence in-situ hybridization; since 2013, array-based comparative genomic hybridization technique combined with chromosome karyotyping has been used. Age, BMI, pregnancy history, gestational age and total gonadotrophin dosage did not differ significantly between the PCOS and non-PCOS groups. In the PCOS group, 28.1% of abortuses demonstrated aneuploidy, which was significantly lower ($P = 0.001$) than in the non-PCOS group (72.1%). Further statistical analyses controlling for maternal age demonstrated that abortuses of women with PCOS were significantly less ($P = 0.001$) likely to have chromosome aneuploidy. Embryonic aneuploidy does not play a vital role in early spontaneous abortion in women with PCOS. Maternal factors resulting in endometrial disorders are more likely to be responsible for the increased risk of early spontaneous abortion in patients with PCOS.

KEYWORDS: aneuploidy, chromosome, early miscarriage, embryo, IVF, polycystic ovary syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinology disorder that affects about 5–15% of women of reproductive age (Asunción et al., 2000; Azziz et al., 2004). Clinical features of PCOS include irregular menses and amenorrhoea, oligoovulation and anovulation, and elevated circulating concentrations of androgens, signs of hyperandrogenism, or both. A high proportion of women with PCOS are referred for assisted reproductive technique treatment to overcome infertility. Furthermore, it is well known that patients with PCOS have a risk of early pregnancy loss of up to 50% (Balen et al., 1993). Previous studies have shown that the infertility associated with PCOS could derive from chronic anovulation, poor oocyte quality and implantation failure (Cardozo et al., 2011; Ludwig et al., 1999). In recent years, oocyte quality has been a controversial topic in PCOS studies. A number of studies have suggested that increased oocyte abnormalities are caused by endocrine and paracrine abnormalities, metabolic dysfunction, and altered oocytes gene expression (Berker et al., 2009; Dumesic, 2008; Patel et al., 2008). A novel study on oocyte gene profiles reported that 374 out of 8123 mRNA abundances of genes significantly differed in oocytes from women with PCOS, and annotation of the data demonstrated that a subset of these genes were associated with chromosome alignment and segregation during mitosis, meiosis, or both (Wood et al., 2007). Nevertheless, some other studies have reported that the oocyte quality and spindle, and chromosome configurations, did not differ compared with the control groups (Nafiye et al., 2010; Vieira et al., 2011). Furthermore, a recent report has shown that the chromosomes of oocytes from women with PCOS were similar to those of controls following maturation *in vitro*, but some aspects of oocyte metabolism are perturbed by PCOS, and elevated pyruvate consumption was associated with abnormal oocyte karyotype (Harris et al., 2010).

Embryonic chromosomal anomalies are responsible for 50–70% of cases of early pregnancy loss after assisted reproductive techniques (Martínez et al., 2010). Little information, however, is available on karyotypes of embryos from women with PCOS. Despite many PCOS studies, few have focused on the causes of high spontaneous abortion rates in these patients. Some studies have speculated that maternal factors such as high levels of luteinizing hormone (Homburg et al., 1988; Regan et al., 1990) or testosterone (Cocksedge et al., 2008; Okon et al., 1998), anomalies in progesterone production (Doldi et al., 1998), insulin resistance (Eng et al., 2007; Irwin et al., 1993) and delayed endometrial development (Dosiou and Giudice, 2005) may contribute to early spontaneous abortion in women with PCOS. It remains unclear, however, whether the high spontaneous abortion rate in patients with PCOS is caused by embryonic or maternal factors, and whether abnormalities in the oocytes cause abnormalities in the embryos, which may contribute to spontaneous abortion. To date, no prospective studies have examined the extent to which embryonic chromosomal anomalies account for spontaneous abortions in women with PCOS or whether embryonic chromosomal anomalies are more common in women with PCOS than among controls. Consequently, we carried out a prospective observational cohort study to examine the rate of embryonic chromosomal anomalies in the abortuses of spontaneous abortions from women with PCOS.

Materials and methods

Case selection and sampling

The patients followed in this cohort study were treated in the Center of Reproductive Medicine at the First Affiliated Hospital of Sun Yat-sen University between January 2010 and February 2014. The study was approved by the Hospital Ethics Committee on 19 January 2009 (reference number: 2009; number 46), and written informed consent was obtained from all patients. A total of 1461 patients with tubal factor infertility who conceived after the first cycle of assisted reproduction were followed during the first trimester of pregnancy. The definition of PCOS was based on the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004); two of the following three criteria had to be fulfilled: oligoovulation, anovulation, or both; excess androgen activity, or polycystic ovaries, and no other endocrine secretion diseases, including thyroid disease, adrenal hyperplasia, and androgen-secreting tumour could be present. None of the patients in the control group showed evidence of PCOS. For both groups, patients with abnormal karyotypes, history of spontaneous abortion, endometriosis, uterine adenomyosis, or both, evident endometrial abnormalities, malformation of the reproductive system and any other endocrine secretion diseases were excluded. Conception was defined as a rising HCG level. Clinical pregnancy was defined as ultrasound evidence of a gestational sac. Ultimately, a total of 100 patients who had experienced spontaneous abortion of an intrauterine singleton clinical pregnancy were recruited, including 32 patients with PCOS (PCOS group) and 68 without PCOS (non-PCOS group) (**Figure 1**). All patients used recombinant follicle stimulating hormone for ovarian stimulation in the gonadotrophin-releasing hormone agonist stimulation protocols, including 39 long-acting protocols and 61 short-acting protocols. Luteal phase support was provided with a combination of a daily intramuscular injection of 40 mg of progesterone and a twice-daily application of 200 mg of vaginal progesterone (Utrogestan, Besins Manufacturing, Brussels). Repeat transvaginal ultrasound was carried out at 6–8 weeks' gestation to confirm early pregnancy loss. Chorionic villus samples were collected under sterile conditions with vacuum curettage. Long-term cultured preparations combined with molecular cytogenetic analysis were used to investigate chromosomal aberrations (Kennerknecht et al., 1992). Before 2013, multiplex ligation-dependent probe amplification (MLPA) subtelomere assay combined with fluorescence in-situ hybridization (FISH) was carried out in each case in addition to standard chromosome analyses ($n = 85$). Since January 2013, diagnoses have been made using array-based comparative genomic hybridization (arrayCGH) combined with conventional chromosome karyotyping ($n = 15$).

Conventional cultured villus chromosome karyotyping

Samples were examined and maternal tissue was carefully removed under a microscope (8–10x). About 50 mg of chorionic villus tissues were selected for incubation for each abortus. The remaining chorionic villus samples were