

Article

Chromosomal polymorphisms are associated with female infertility and adverse reproductive outcomes after infertility treatment: a 7-year retrospective study

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KEY MESSAGE

In this study, we explored the relationship between causes of chromosomal polymorphisms and female infertility by subgroup analysis, and probed the correlations in an adjusted model. The incidence of chromosomal polymorphisms was higher in infertile women compared with fertile controls. Chromosomal polymorphisms increased spontaneous miscarriage rates in fertile and infertile women.

ABSTRACT

Data from 19,950 women were retrospectively analysed to determine the effect of chromosomal polymorphisms on female infertility and pregnancy outcome; fertile women were used as controls. Frequency of chromosomal polymorphisms and adverse pregnancy outcomes were compared between groups. A significantly higher incidence of chromosomal polymorphisms was found in total infertile patients, and patients with tubal infertility, ovulatory dysfunction, cervical and uterine abnormalities, and unexplained infertility compared with controls [5.53% [$P < 0.001$], 4.86% [$P = 0.012$] 5.40% [$P < 0.001$], 5.75% [$P < 0.001$] and 8.51% [$P < 0.001$], versus 3.74%, respectively]. Infertile women had a higher incidence of 9qh+ and inv(9) compared with controls [$P < 0.001$ and $P = 0.027$]. Logistic regression analysis showed an effect of chromosomal polymorphisms on female infertility [adjusted

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OR 1.662, 95% CI 1.551 to 1.796, $P < 0.001$). All couples reported a phenotypically normal baby. In control and tubal infertility groups, miscarriage rates were higher in women with chromosomal polymorphisms than in women with normal chromosomes (4.95% versus 0.96%, $P = 0.001$ and 6.17% versus 1.08%, $P < 0.001$). Preterm birth rate showed a similar trend. Chromosomal polymorphisms adversely affected spontaneous miscarriage rates (adjusted OR 1.625, 95% CI 1.514 to 1.769, $P = 0.005$).

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Introduction

Chromosomal polymorphisms are considered to be a type of chromosomal variation, and are defined as the differences in size or staining of chromosome segments. These include heterochromatic segments, satellites and satellite stalks in the population (Hong et al., 2011). Polymorphic variations on non-acrocentric chromosomes usually occur in the paracentric heterochromatin on the long arms of chromosomes 1, 9, 16 and distal heterochromatin of the Y chromosome. For acrocentric chromosomes, such as those in D and G groups (chromosomes 13, 14, 15, 21 and 22), variations mostly occur on satellites, satellite stalks or short arms (Maden et al., 2005). Pericentric inversions on chromosomes 9[inv(9)] are also categorized as polymorphisms (Hong et al., 2011).

For some time, chromosomal polymorphisms, most of which take no effect on phenotypes, have been considered 'harmless', without clinical significance, therefore receiving less attention (Evans et al., 1974). Emerging evidence, however, suggests that variations of chromosomes may be associated with deleterious reproductive consequences, recurrent pregnancy loss and reproductive failure or infertility (Dong et al., 2013; Evans et al., 1974; Hong et al., 2011; Madon et al., 2005; Sahin et al., 2008).

Infertility is a significant condition worldwide, affecting up to 12% of women (Gurunath et al., 2011). Although the important role of genetic factors in pathogenesis of infertility is now increasingly recognized, the relationship between chromosome polymorphisms and infertility is still controversial, particularly the correlation between female infertility and chromosomal variations.

Studies have suggested that the incidence of inv(9) in women with reproductive disorders is not significantly higher, compared with the normal population (Mierla and Stoian, 2012a). By contrast, several investigators have suggested possible associations between inv(9) with female infertility (Ceylan et al., 2008; Collodel et al., 2006; Gekas et al., 2001). Previous studies have also confirmed no difference of chromosomal variants between genders. Among infertile patients, the incidence of chromosomal polymorphisms in men was found to be higher than that in women (Maden et al., 2005; Minocherhomji et al., 2009). Few studies, however, have focused on the genetic variations factors of female infertility and the effect of chromosomal polymorphisms on pregnancy results after infertility treatment.

The objective of this study was to determine the association between the polymorphic variants of chromosomes and female infertility, and to evaluate the effect of chromosomal polymorphisms on pregnancy outcomes after infertility treatment.

Materials and methods

Subjects

A retrospective, single-centre cohort study was conducted of all karyotype analyses carried out for reproductive problems or preconception

genetic tests between January 2008 and February 2015 at the Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu, Sichuan. After excluding infertile couples with male-factor infertility, and women aged 15–19 years and 45–49 years, when fewer couples are seeking a child, we investigated infertile women aged 20–44 years (Mascarenhas et al., 2012). The infertile women were subgrouped according to causes of infertility, which include tubal disease, ovulatory dysfunction, cervical or uterine abnormalities, unexplained infertility and endometriosis-related infertility. Women in the same age group seeking preconception genetic testing for recurrent spontaneous miscarriages or stillbirth, advanced maternal age (35 years or older), a history of malformed childbearing, a previous child with a genetic condition or concerns about birth defects were considered to be a sample of the fertile population and included as a control group. This group included 819 women who were nullipara at the time of the karyotype analysis and became pregnant spontaneously less than 12 months after the analysis. Women with abnormal chromosome karyotypes and their male partners with chromosomal aberration or polymorphic variants were excluded. A flow chart of the study design is presented in Figure 1. (Figure 2)

This study was approved by the Ethics Committee of West China Second University Hospital on 3 March 2014 (Reference number: 2014019).

Karyotype analysis

For cytogenetic analysis, chromosome karyotype analysis was carried out according to the standard laboratory protocol using Giemsa–Trypsin–Giemsa (GTG) banding (Hamerton, 1971). Peripheral blood from every included patient were cultured for 72 h in the presence of phytohemagglutinin (PHA) and then stained with the G-banding technique. At least 20 metaphases were analysed for each case, and five metaphases were karyotyped using light microscopy. High-resolution banding was carried out when necessary. All the results were confirmed by two independent readers.

Classification of chromosomal variations

The karyotype analysis results were presented on the basis of the existing version of the international cytogenetic nomenclature (Shaffer et al., 2013). We recorded visualized variants, including those with increased stalk lengths on the short arm of the acrocentric chromosomes(pstk+), those with changes of satellites on the short arm of chromosomes 13/14/15/21/22(pss/ps+), those with pericentric inversions of chromosomes 9/16[inv(9) and inv(16)] and those with increase in the length of the pericentric heterochromatin on the long arms of chromosomes(qh+).

Infertility treatment

All infertile women included in this study underwent treatment directed at correcting the pathology and restoring reproductive function.

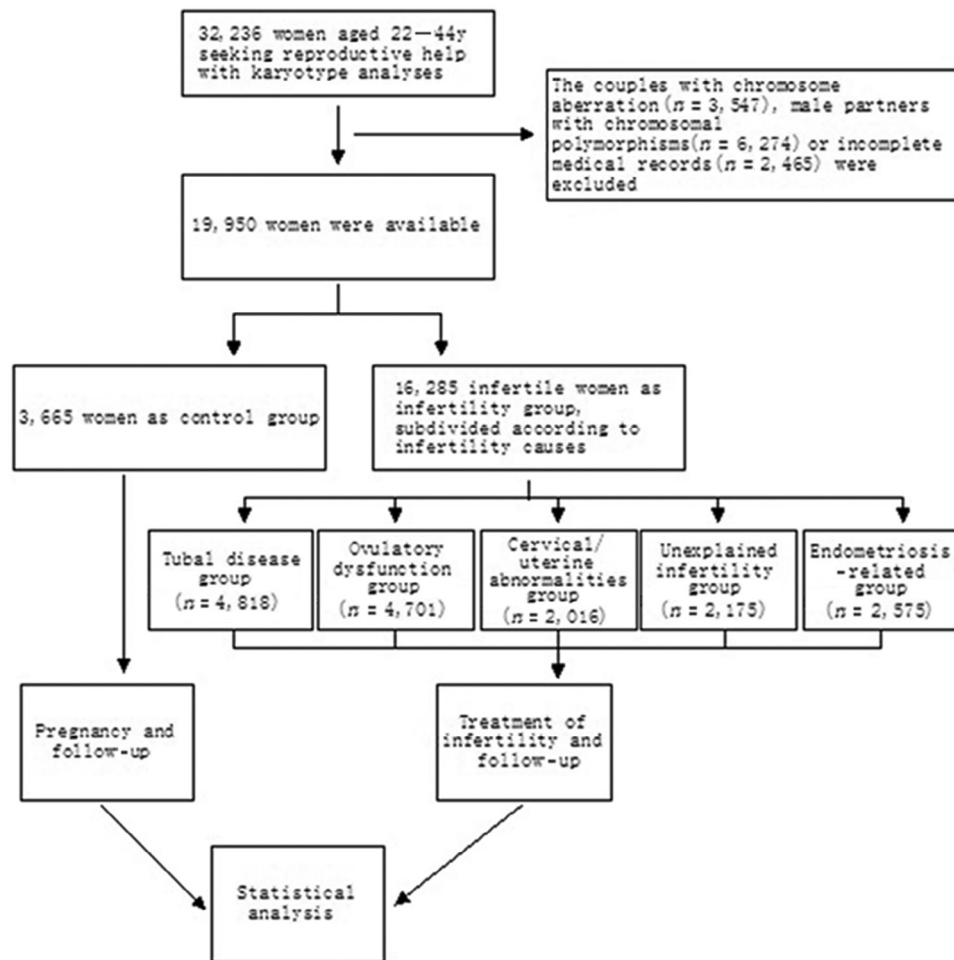


Figure 1 – Flow chart of the study design.

For anovulatory patients, controlled ovarian stimulation is given using ovulation-inducing agents, such as clomiphene, letrozole, HCG and gonadotrophins. Assisted reproductive techniques, such as IVF and embryo transfer, intrauterine insemination, frozen embryo transfer and intracytoplasmic sperm injection, are carried out according to indications and standard protocols. For tubal infertility, IVF treatment or surgical intervention to reconstruct tubal is carried out according to individualized characteristics. Endometriosis-related infertility is treated using medical intervention, IVF or surgical ablation of the

endometrial implants and lysis of adhesions. Comprehensive treatment approach is used for unexplained infertility.

Data collecting and outcomes

After participants were enrolled, medical records were reviewed, demographic and potential confounding variables abstracted, and karyotype analysis results recorded. Pregnancy and neonatal outcomes were carried out by phone or outpatient visit. The parameters

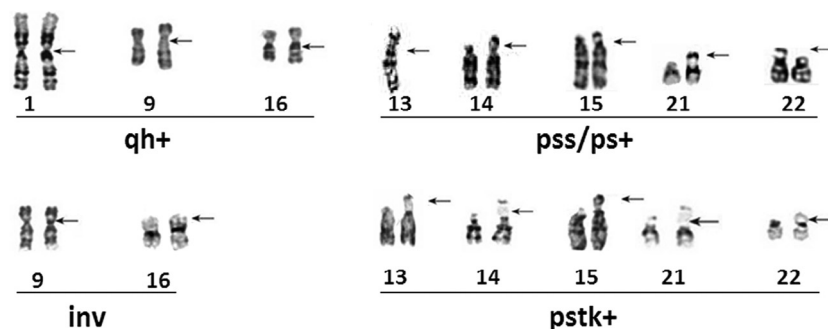


Figure 2 – Examples of chromosome polymorphisms.

compared included the frequency of chromosomal variations, spontaneous miscarriage rates, preterm birth rates and congenital birth defects rates. Standard definitions for outcome measurements were used. Clinical pregnancy was confirmed by ultrasonographic visualization of one or more gestational sacs or fetal heartbeat. A miscarriage is the spontaneous loss of a fetus before the 20th week of pregnancy. Clinical pregnancy loss before gestational age of 12 weeks or 10 weeks after the oocyte retrieval procedure was considered early miscarriage. Preterm birth was defined as delivery at less than 37 weeks gestational age.

Our primary outcomes are the difference of chromosomal variations rates between infertile women and control women. As secondary outcomes, spontaneous miscarriage rates, preterm birth rates and congenital birth defects rates were included for analysis to investigate the effect of chromosomal variations on pregnancy outcomes. The infertile group was stratified in accordance with infertility causes to further analyse the relationship between infertility causes and chromosomal variations.

Statistical analysis

SPSS software version 21.0 (IBM Corp., USA) was used for all statistical analyses. $P < 0.05$ was considered statistically significant. Descriptive statistics was used to present demographic data. Chi-square or Fisher's exact test was used for categorical variables, such as the frequencies of variations, demographic variables and pregnancies outcomes. Logistic regression analysis was used to evaluate the association between the female infertility and age, ethnicity, levels of education, history of surgery and previous pregnancies and previous history. To evaluate the associations between the polymorphic variations and adverse pregnancy results, odds ratio and 95% confidence interval were calculated using unconditional logistic regression analysis with adjustments for potential confounding variables.

Results

A total of 19,950 women composed of 3665 fertile women as the control group and 16,285 infertile women as the infertile group were included and analysed in this retrospective study. The subgroups of infertility were tubal disease group ($n = 4818$), ovulatory dysfunction group ($n = 4701$), cervical/uterine abnormalities group ($n = 2016$), unexplained infertility ($n = 2175$) and endometriosis-related infertility group ($n = 2575$). The basic characteristics of the groups are shown in **Table 1**.

The incidence of chromosomal polymorphic variations in participants included is shown in **Table 2**. Of the 19,950 specimens karyotyped, the chromosomal polymorphisms were identified in 1038 (5.20%) women. The incidence of total variants in control individuals was 3.74% (137/3665), whereas the incidence of variants in total infertile patients was 5.53% (901/16285), with the highest incidence in unexplained infertility group (8.51% [185/2175]), followed by 5.75% (116/2016) in cervical/uterine abnormalities group, 5.40% (254/4701) in ovulatory dysfunction group, 4.86% (234/4818) in tubal infertility group and 4.35% (112/2575) in endometriosis-related infertility group. Compared with control group, the incidence of chromosomal polymorphisms in total infertile patients, and patients with tubal infertility, ovulatory dysfunction, cervical and uterine abnormalities, and unexplained infertility, were significantly higher [5.53% versus 3.74%, P

Table 1 – Characteristics of control women and infertile women sub-grouped according to cause of infertility.

Characteristic	Subgroups of infertility						
	Control group (n = 3665)	Group of infertility (n = 16,285)	Tubal disease (n = 4818)	Ovulatory dysfunction (n = 4701)	Cervical/uterine abnormalities (2016)	Unexplained infertility (n = 2175)	Endometriosis (n = 2575)
Age (years)	Mean ± SD	30.75 ± 5.02	30.73 ± 5.03	30.70 ± 5.00	30.60 ± 4.97	31.08 ± 5.09	30.71 ± 4.99
	<25	29.84 ± 4.88 492 (13.4)	30.75 ± 5.02 1638 (10.1)	30.73 ± 5.03 504 (10.5)	30.70 ± 5.00 481 (10.2)	31.08 ± 5.09 191 (8.8)	30.71 ± 4.99 265 (10.3)
	25–34	2510 (68.5)	10860 (66.7)	3213 (66.7)	3119 (66.3)	1446 (66.5)	1706 (66.3)
	≥35	663 (18.1)	3787 (23.3)	1101 (22.9)	1101 (23.4)	538 (24.7)	604 (23.5)
Ethnicity	Han	3615 (98.6)	15672 (96.2)	4613 (95.7)	4532 (96.4)	2092 (96.2)	2493 (96.8)
	Tibetan	50 (1.4)	613 (3.8)	205 (4.3)	169 (3.6)	83 (3.8)	82 (3.2)
Levels of education	Primary education or lower	1232 (33.6)	5872 (36.1)	1725 (35.8)	1635 (34.8)	863 (39.7)	874 (33.9)
	Secondary education	1224 (33.4)	5883 (36.1)	1694 (35.2)	1724 (36.7)	770 (35.4)	941 (36.5)
	Bachelor	1199 (32.7)	4097 (25.2)	1257 (26.1)	1208 (25.7)	470 (21.6)	697 (27.1)
	Master or upper	10 (0.3)	433 (2.7)	142 (2.9)	134 (2.9)	72 (3.3)	63 (2.4)
Number of previous pregnancies	0	819 (22.3)	7968 (48.9)	2273 (47.2)	2214 (47.1)	1047 (48.1)	1205 (46.8)
	≥1	2846 (77.7)	8317 (51.1)	2545 (52.8)	2487 (52.9)	1128 (51.9)	1370 (53.2)
Infertility diagnosis	Primary infertility	–	7962 (48.9)	2272 (47.2)	2213 (47.1)	1045 (48.0)	1205 (46.8)
	Secondary infertility	–	8323 (51.1)	2546 (52.8)	2488 (52.9)	1130 (52.0)	1370 (53.2)
Data are shown as mean ± SD for continuous variables and number (percentage) for dichotomous variables.							

Data are shown as mean \pm SD for continuous variables and number (percentage) for dichotomous variables.

Table 2 – Frequency of chromosomal polymorphisms in fertile women and infertile women.

Characteristic		Subgroups of infertility						
		Control group (n = 3665)	Group of infertility (n = 16,285)	Tubal disease (n = 4818)	Ovulatory dysfunction (n = 4701)	Cervical/uterine abnormalities (n = 2016)	Unexplained infertility (n = 2175)	Endometriosis (n = 2575)
qh+	1qh+	17 [12.4]	87 [9.7]	24 [10.3]	22 [8.7]	14 [12.1]	15 [8.1]	12 [10.7]
	9qh+	31 [22.6]	231 [25.6] ^a	54 [23.1]	71 [28.0] ^a	29 [25.0]	55 [29.7] ^a	22 [19.6]
	16qh+	12 [8.8]	28 [3.1]	7 [3.0]	7 [2.8]	4 [3.4]	5 [2.7]	5 [4.5]
Inv	inv(9)	29 [21.2]	196 [21.8] ^a	53 [22.6]	51 [20.1]	24 [20.7]	45 [24.3] ^a	23 [20.5]
	inv(16)	4 [2.9]	21 [2.3]	6 [2.6]	6 [2.4]	2 [1.7]	3 [1.6]	4 [3.6]
pss/ps+	13pss/ps+	3 [2.2]	29 [3.2]	8 [3.4]	9 [3.5]	4 [3.4]	5 [2.7]	3 [2.7]
	14pss/ps+	4 [2.9]	40 [4.4]	11 [4.7]	12 [4.7]	5 [4.3]	7 [3.8]	5 [4.5]
	15pss/ps+	3 [2.2]	35 [3.9]	10 [4.3]	11 [4.3]	4 [3.4]	6 [3.2]	4 [3.6]
	21pss/ps+	6 [4.4]	71 [7.9] ^a	19 [8.1]	17 [6.7]	9 [7.7] ^a	16 [8.6] ^a	10 [8.9]
	22pss/ps+	5 [3.6]	44 [4.9]	13 [5.6]	12 [4.7]	6 [5.2]	7 [3.8]	6 [5.4]
pstk+	13pstk+	1 [0.7] ^b	13 [1.4]	3 [1.3]	4 [1.6]	2 [1.7]	2 [1.1]	2 [1.8]
	14pstk+	1 [0.7] ^b	6 [0.7]	1 [0.4] ^b	2 [0.8] ^b	0 [0.0] ^b	2 [1.1] ^b	1 [0.9] ^b
	15pstk+	5 [3.6]	34 [3.8]	8 [3.4]	10 [3.9]	5 [4.3]	6 [3.2]	5 [4.5]
	21pstk+	9 [6.6]	31 [3.4]	8 [3.4]	9 [3.5]	4 [3.4]	5 [2.7]	5 [4.5]
	22pstk+	7 [5.1]	35 [3.9]	9 [3.8]	11 [4.3]	4 [3.4]	6 [3.2]	5 [4.5]
Total		137	901 ^a	234 ^a	254 ^a	116 ^a	185 ^a	112

Data are shown as number (incidence).

^a Compared with control group ($P < 0.05$).^b Fisher's exact test.

Table 3 – Logistic regression for factors associated with female infertility.

Potential factors	Female infertility			
	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age	1.041 (1.033 to 1.049)	<0.001	1.038 (1.030 to 1.046)	<0.001
Ethnicity	2.674 (1.982 to 3.607)	NS	2.828 (2.115 to 3.781)	NS
Levels of education	0.934 (0.893 to 0.978)	NS	0.929 (0.891 to 0.969)	NS
History of surgery	4.015 (3.919 to 5.276)	<0.001	4.027 (3.975 to 5.384)	0.007
Number of previous pregnancies	0.842 (0.818 to 0.866)	NS	0.846 (0.825 to 0.866)	NS
Number of previous gravida	0.740 (0.661 to 0.829)	NS	0.530 (0.482 to 0.583)	NS
Chromosomal polymorphisms	1.477 (1.388 to 1.586)	<0.001	1.662 (1.551 to 1.796)	<0.001
NS, not statistically significant.				

<0.001, 4.86% versus 3.74%, $P = 0.012$, 5.40% versus 3.74%, $P < 0.001$, 5.75% versus 3.74%, $P < 0.001$ and 8.51% versus 3.74%, $P < 0.001$, respectively). The variant showing the highest frequency was 9qh+ followed by inv(9) in the control group and almost all infertile subgroups, but not in the endometriosis-related group, in which inv(9) showed the highest frequency.

Significant differences were found in the incidence of 9qh+ in the total infertile patients, tubal infertility group, ovulatory dysfunction group and unexplained infertility group compared with the fertile control group (1.42% versus 0.85%, $P < 0.001$, 1.51% versus 0.85%, $P < 0.001$, 2.53% versus 0.85%, $P < 0.001$, respectively). Compared with the fertile control group, the incidence of inv(9) in the total infertile patients and unexplained infertility group were significantly high (1.20% versus 0.79%, $P = 0.027$ and 2.07% versus 0.79%, $P < 0.001$). The incidence of 21pss/ps+ in the total infertile patients, cervical/uterine abnormalities group and unexplained infertility group were significantly higher than that in the fertile control individuals (0.44% versus 0.16%, $P = 0.015$, 0.45% versus 0.16%, $P = 0.043$, and 0.74% versus 0.16%, $P < 0.001$). In contrast, no differences were presented between the infertility groups and the control group in the incidence of 1qh+, 16qh+, inv(16), 13/14/15/22pss/ps+ and 13/14/15/21/22pstk+. It also did not show significant difference by further subgroup analysis according to causes of infertility.

Crude and adjusted odds ratios for female infertility are presented in **Table 3**. Data showed an effect of chromosomal polymorphisms on female infertility [OR 1.477, 95% CI 1.388 to

1.586, $P < 0.001$; adjusted OR 1.662, 95% CI 1.551 to 1.796, $P < 0.001$]. Some potential variables, such as age and history of surgery, also showed an effect on female infertility [OR 1.041, 95% CI 1.033 to 1.049, $P < 0.001$; adjusted OR 1.038, 95% CI 1.030 to 1.046, $P < 0.001$ and OR 4.015, 95% CI 3.919 to 5.276, $P < 0.001$; adjusted OR 4.027, 95% CI 3.975 to 5.384, $P = 0.007$]. Other factors, including ethnicity, levels of education, number of previous pregnancies and number of previous gravida, showed no statistical effect on reproductive ability in females.

To further identify the effects of chromosomal polymorphisms on pregnancy outcomes, the adverse pregnancy rates between groups were compared (**Table 4**). The pregnancy and birth outcomes of 18,963 women were available, with data of 987 women missing. In total, there were 10,214 gestations during our study period, of which 510 were spontaneous miscarriages and 190 preterm birth. All couples reported a phenotypically normal baby, and no congenital birth defects were recorded. In the control group and tubal infertility group, the rates of spontaneous miscarriages in women with chromosomal polymorphisms were significantly higher than that in women with normal chromosomes (4.95% versus 0.96%, $P = 0.001$ and 6.17% versus 1.08%, $P < 0.001$). In the control group, tubal infertility group, ovulatory dysfunction group, and cervical and uterine abnormalities group, the rates of preterm birth in women with chromosomal polymorphisms were significantly higher than that in women with normal chromosomes (2.97% versus 0.92%, $P = 0.046$; 7.41% versus 1.36%, $P < 0.001$; 6.13% versus 1.28%, $P < 0.001$; and 8.89% versus 2.50%, $P = 0.016$).

Table 4 – Pregnancy outcomes of fertile and infertile women sub-grouped according to infertility causes.

		Women without chromosomal polymorphisms	Women with chromosomal polymorphisms	Total
Control group	Miscarriage rate % (n)	1.0 (26/2704)	5.0 (5/101) ^a	1.1 (31/2805)
	Preterm birth rate % (n)	0.9 (25/2704)	3.0 (3/101) ^a	1.0 (28/2805)
Tubal disease group	Miscarriage rate % (n)	1.1 (23/2135)	6.2 (5/81) ^a	1.3 (28/2216)
	Preterm birth rate % (n)	1.4 (29/2135)	7.4 (6/81) ^a	1.6 (35/2216)
Ovulatory dysfunction group	Miscarriage rate % (n)	7.7 (168/2188)	10.4 (17/163)	7.8 (184/2351)
	Preterm birth rate % (n)	1.3 (28/2188)	6.1 (10/163) ^a	1.6 (38/2351)
Cervical/uterine abnormalities group	Miscarriage rate % (n)	6.6 (61/921)	6.7 (3/45)	6.6 (64/966)
	Preterm birth rate % (n)	2.5 (23/921)	8.9 (4/45) ^a	2.8 (27/966)
Unexplained infertility group	Miscarriage rate % (n)	10.1 (75/742)	15.4 (16/104)	10.8 (91/846)
	Preterm birth rate % (n)	3.2 (24/742)	4.8 (5/104)	3.4 (29/846)
Endometriosis group	Miscarriage rate % (n)	10.7 (107/997)	12.1 (4/33)	10.8 (111/1030)
	Preterm birth rate % (n)	2.7 (27/997)	18.1 (6/33) ^a	3.2 (33/1030)

Data are shown as incidence.

^a Compared with women without chromosomal polymorphisms ($P < 0.05$).

The odds ratios of potential variables were calculated to evaluate the effect on spontaneous miscarriages rate and preterm birth rate (Table 5). The result indicated a positive effect of chromosomal polymorphisms on spontaneous miscarriages rate [OR1.711, 95% CI 1.488 to 1.967, $P = 0.003$; adjusted OR 1.625, 95% CI 1.514 to 1.769, $P = 0.005$]. Other factors, including age and number of previous pregnancies, also showed a positive association with spontaneous miscarriage rate [OR 1.012, 95% CI 1.051 to 1.041, $P = 0.043$; adjusted O: 1.046, 95% CI 1.029 to 1.051, $P = 0.035$; and OR1.042, 95% CI 1.008 to 1.265, $P = 0.001$; adjusted OR 1.346, 95% CI 1.258 to 1.465, $P = 0.028$]. The data, however, failed to suggest the female chromosomal polymorphisms affect preterm birth rate [OR1.175, 95% CI 0.988 to 1.367, $P = 0.006$; adjusted OR 1.126, 95% CI 0.955 to 1.209, $P = 0.009$].

Discussion

Chromosomal polymorphism, usually categorized as a balanced rearrangement of chromosome, has been shown to be clinically relevant in many studies, most of which have presented an increased frequency of polymorphic variations in infertile patients [Dong et al., 2013; Madon et al., 2005; Mierla and Stoian, 2012a; Sahin et al., 2008]. It is becoming increasingly difficult to ignore the potential relationship between chromosomal polymorphism and reproductive capacity. Only a few studies, however, have paid special attention to the effect of chromosomal polymorphism on female fertility and pregnancy outcomes after infertility treatments. Furthermore, few studies have stratified female infertility according to infertility causes [Düzcan et al., 2003], and most of previous reports simply presented a difference frequency of chromosomal polymorphism [Caglayan et al., 2010a; Mierla and Stoian, 2012a; Šípek et al., 2015]. In this study, we subdivided the female infertility group in line with causes of infertility to explore the association between chromosomal polymorphisms and female infertility.

Our data showed a total incidence of chromosomal polymorphisms of 5.20%, with 3.74% in the control group and 5.53% in total infertile patients, which is similar to previous cytogenetic studies [Madon et al., 2005]. The highest incidence observed was in the unexplained infertility (8.51%). The incidence of chromosome variants in the current study, however, is high, compared with a previous population survey in the general population (3.96%) [Bhasin, 2005]. The laboratory incidence from analysis of cohorts of individuals with certain clinical indications could not reflect the incidence in the general population. In the current total cohort, the variant showing the highest frequency was 9qh+ (1.31%) followed by inv(9) (1.13%). For 9qh+, the incidence in our study is less than that of previous surveys (2.44%) [Bhasin, 2005]; most earlier reports, however, presented a higher incidence (7.60%) [Hong et al., 2011; Madon et al., 2005]. The incidence of inv(9) in the study is similar to that in the large epidemiological studies (1.58% and 1.01%) [Demirhan et al., 2000; Šípek et al., 2015]. The incidence of chromosomal polymorphisms, however, could vary within a broad range, fluctuating with the heterogeneity of subjects included.

Although it is widely debatable, increasing evidence has confirmed that female reproductive disorders are closely related to chromosomal polymorphisms. Yet, most studies have reported an increasing incidence of chromosomal polymorphism variation in infertile men or couples, ignoring the same trend in infertile women [Caglayan et al., 2010b; Yakin et al., 2005]. A study in the Romanian population

Table 5 – Crude and adjusted odds ratios for pregnancy outcomes and preterm birth in fertile and infertile women.

	Spontaneous miscarriages rate			Preterm birth rate		
	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)
Age	1.012 (1.051 to 1.041)	0.043	1.046 (1.029 to 1.051)	1.012 (1.051 to 1.041)	0.043	1.046 (1.029 to 1.051)
Ethnicity	0.64 (0.463 to 1.431)	NS	0.817 (0.526 to 1.393)	1.067 (0.991 to 1.570)	NS	0.854 (0.516 to 1.071)
Levels of education	0.541 (0.559 to 0.984)	NS	0.693 (0.589 to 0.895)	0.917 (0.879 to 0.991)	0.008	0.893 (0.691 to 0.977)
History of surgery	1.146 (0.923 to 1.237)	NS	0.961 (0.875 to 1.143)	0.515 (0.391 to 0.867)	NS	0.672 (0.456 to 0.843)
Number of previous pregnancies	1.042 (1.008 to 1.265)	0.001	1.346 (1.258 to 1.465)	0.814 (0.783 to 0.867)	0.046	0.963 (0.873 to 1.065)
Number of previous gravida	0.824 (0.617 to 0.852)	NS	0.830 (0.625 to 0.931)	1.040 (1.006 to 1.593)	NS	1.531 (1.428 to 1.637)
Chromosomal polymorphic variations	1.711 (1.488 to 1.967)	0.003	1.625 (1.514 to 1.769)	1.175 (0.988 to 1.367)	0.006	1.126 (0.955 to 1.209)
NS, not statistically significant.						

has found a rising prevalence of inversion of chromosome 9 in infertile women, without further investigations and explanations [Mierla and Stoian, 2012b]. In this study, we found a higher incidence of chromosomal polymorphisms in total infertile patients, and the tubal infertility group, ovulatory dysfunction group, cervical and uterine abnormalities group and unexplained infertility group compared with the control group. Interestingly, the highest incidence observed was in the unexplained infertility group. Our result also indicated that the incidence of 9qh+ in total infertile patients, individuals with tubal infertility, ovulatory dysfunction and unexplained infertility, were higher than that in the control group. The incidence of inv(9) in total infertile patients and those with explained infertility, and the incidence of 21pss/ps+ in total infertile patients, cervical and uterine abnormalities, and unexplained infertility shared a similar trend. Logistic regression analysis showed a negative effect of chromosomal polymorphisms on female reproductive ability. Consequently, a statistical association between chromosomal polymorphisms, such as 9qh+, inv(9) and 21pss/ps+ and female infertility was confirmed, suggesting chromosomal polymorphisms are causal factors leading to fertility problems in women, which is consistent with previous studies [Šípek et al., 2014].

It is still unclear how chromosomal polymorphisms affect fertility. Some reports have proposed a potential explanation for some types of pericentric inversions: the inversion itself could interfere with the pairing of homologous chromosomes during meiosis [Coop and Przeworski, 2007]. Verification of this theory is not possible in the present study, as our data did not include the cytogenetic analyses of their offspring. Interchromosomal effect is another mechanism suggested, which is that the pairing of other chromosomes can be affected by inv(9), because the unpaired segments of homologous chromatids can also interfere with other bivalents [Anton et al., 2005]. Unfortunately, the interchromosomal effect in inv(9), leading to reproductive problems, has only been reported in a few studies of men [Amiel et al., 2001]. Recent studies using fluorescence in-situ hybridization, have revealed the diversity of the molecular-cytogenetic characteristics of pericentric inversions in specific patients [Kosyakova et al., 2013; Ramos et al., 2015]. Some clinical findings, however, have not been explained well; for instance, couples with inv(9) commonly suffered from secondary infertility [Kumar et al., 2012]. Nevertheless, the mechanism of 9qh+ and 21pss/ps+ affecting reproductive ability has still been inadequately researched.

Concerns over whether polymorphic variants of chromosomes affect the outcome of infertility treatment have been raised. The effect of chromosomal variants on the outcomes of infertility treatments remains unknown, although numerous studies have focused on the topic. Most studies concentrated on the outcome of assisted reproduction technique treatment in fertile men with chromosomal variations [Van Golde et al., 2001; Yakin et al., 2005]. The change of DNA repeats at specific regions of Y chromosome was reported to affect the pairing and synapsis of X and Y chromosomes during meiosis [Antonelli et al., 2000]. The increase long arm of the Y chromosome may inhibit the expression of genes of spermatogenesis by position-effect variegation and decrease reproductive capacity [Minocherhomji et al., 2009]. Treatments of infertility in our study, however, were more comprehensive than assisted reproduction techniques. The present data showed that the rates of spontaneous miscarriages and preterm birth in women with chromosomal polymorphisms were higher than that in women without chromosomal polymorphisms, and a positive effect of chromosomal polymorphisms on spontaneous miscarriages rate was also confirmed. This is inconsistent with a previous

study by Hong et al. [2011], in which only the early miscarriage rate of male carriers tended to be higher. In another study, Liang et al. [2014] concluded that only male chromosomal polymorphisms adversely influence fertilization rates of IVF cycles. The reason for the inconsistency may be the difference of treatment approaches that enrolled individuals received, and that our study only included women, ruling out the confounding of male chromosomal polymorphisms which has been closely correlated to reproductive conditions [Ferlin et al., 2007].

The results show a close association between chromosomal polymorphisms and reproductive disorders in the unexplained infertility group. The highest incidence of chromosomal polymorphisms was in the unexplained infertility; this finding could contribute to our understanding of the mechanism of unexplained infertility in women. The explanation of this finding remains unknown, although few studies have confirmed a relationship between chromosomal aberrations and unexplained infertility, but have failed to establish a connection between chromosomal polymorphisms and unexplained infertility [Düzcan et al., 2003].

Our results are limited by the observational nature of the study design. Furthermore, chromosomal analyses should be carried out on the pregnancies and babies to ascertain whether or not the chromosomal polymorphisms are inherited after treatments. Part of the birth defects and congenital malformations data of babies were obtained from their parents, without a confirmation from experts.

In conclusion, based on our results, it is suggested that chromosomal polymorphisms play a significant role in female infertility and adversely affect pregnancy outcomes after infertility treatment, increasing the spontaneous miscarriage rate. Future studies, however, should evaluate the clear mechanism of chromosomal polymorphisms on female infertility and adverse affect on pregnancy outcomes after infertility treatment.

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