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Do polymorphic variants of chromosomes affect the outcome of *in vitro* fertilization and embryo transfer treatment?

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BACKGROUND: The aim of this study was to investigate the effect of chromosomal polymorphic variations on the outcome of IVF and embryo transfer (IVF–embryo transfer) treatment for infertile couples.

METHODS: During the period from October 2006 to December 2009, 1978 infertile couples who had received their first IVF–embryo transfer treatment cycle in our hospital were selected for this retrospective study, and the frequency of chromosomal polymorphic variations was calculated. From these, 1671 couples were selected and divided into three groups: 1402 couples with normal chromosomes (Group 1/control group), 82 couples with chromosomal polymorphic variations in only females (Group 2) and 187 couples with chromosomal polymorphic variations in only males (Group 3). The clinical pregnancy rates (CPR), early miscarriage rates and ongoing pregnancy rates after IVF–embryo transfer treatment were compared.

RESULTS: There were no statistically significant differences among the three groups in implantation rates (29.37% in the control group, 29.70% in Group 2 and 31.41% in Group 3, $P > 0.05$) and CPR (45.86, 46.34 and 51.87%, respectively, $P > 0.05$). Although there was a trend toward higher first trimester pregnancy loss rates in Group 3 (male chromosomal polymorphic variations), but not in Group 2, compared with normal karyotype couples (10.31 versus 6.84%), the difference did not reach significance ($P > 0.05$).

CONCLUSIONS: Chromosomal polymorphic variations appear to have no adverse effects on the outcome of IVF–embryo transfer treatment.

Key words: chromosome polymorphism / IVF / pregnancy rate / early miscarriage

Introduction

Polymorphism variations mainly refer to the variants in the chromosomal heterochromatin region. Polymorphic variants on non-acrocentric chromosomes usually occur in the paracentric heterochromatin on the long arms of chromosomes 1, 9 and 16, the short-arm regions of D and G group chromosomes, and the distal heterochromatin of the Y chromosome. Increased lengths of the heterochromatic regions on the long arms of these chromosomes are designated as 1qh+, 9qh+, 16qh+ and Yqh+. Sometimes, the heterochromatin is reduced in these chromosomes, such as 1qh−, 9qh− and 16qh−. Increased lengths of the short-arm satellites and stalks of the acrocentric D and G group chromosomes (13, 14, 15, 21 and 22) are designated, for example, as 14ps+ and 13pstk+, while increased

lengths of the short arms themselves are designated as p+ (e.g. 15p+) (Madon *et al.*, 2005). For the heterochromatin that is formed by tandemly organized, highly repeated sequences of satellite DNA that do not encode proteins, the chromosomal polymorphism variations are considered normal karyotypes (Bhasin, 2005). However, more and more studies indicate that chromosome polymorphisms may cause certain clinical effects, such as infertility and spontaneous miscarriage (Madon *et al.*, 2005; Yuce *et al.*, 2007; Sahin *et al.*, 2008). Chromosome polymorphic variation is found to be higher in infertile patients, especially those receiving IVF and embryo transfer (IVF–embryo transfer) treatment, than in people with normal fertility (Madon *et al.*, 2005; Sahin *et al.*, 2008; Minocherhomji *et al.*, 2009). For example, in the study by Minocherhomji *et al.* (2009), the frequency of chromosomal polymorphism variation in 760

infertile patients was compared with that of 555 fertile ones. They found a higher frequency of chromosomal polymorphism variations in infertile couples with ICSI and IUI treatment (28.31 versus 15.16%, infertile women versus fertile controls, $P = 0.0007$; 58.68 versus 32.55%, infertile men versus fertile controls, $P = 0.0002$). In a controlled study by Madon *et al.* (2005), 842 patients who were ready for IVF−embryo transfer treatment for primary infertility or a history of multiple miscarriages were compared with the general population of the retrospective study by Bhasin (2005). They found that polymorphic variations such as 9qh+, Yqh+ and D/G group were more common in patients who were ready for IVF−embryo transfer treatment (7.6 versus 2.44%; 7.86 versus 2.85% and 8.91 versus 3.96%, respectively). According to these reports, chromosomal polymorphic variations cannot be ignored by clinicians. Therefore, whether polymorphic variants of chromosomes affect the outcome of assisted reproductive technique (ART) treatment has aroused general concern. Clinicians have speculated that infertile individuals with chromosomal polymorphic variations should use donor gametes or be treated with preimplantation genetic screening or undergo preimplantation genetic diagnoses. However, the literature regarding chromosome polymorphism in infertile couples has mainly focused on screening. Very few studies concerning the impact of chromosomal polymorphic variations on ART treatment have been reported (Silber *et al.*, 1998; van Golde *et al.*, 2001; Oates *et al.*, 2002; Choi *et al.*, 2004; Kihaile *et al.*, 2005; Yakin *et al.*, 2005). These studies concentrated on infertile men with severe oligozoospermia, azoospermia or Y chromosome variation, and the outcome of ICSI treatment. No report has studied whether chromosomal variation, other than Y chromosome variation, affects the outcome of IVF−embryo transfer treatment, including traditional IVF. More data are needed to resolve this question. Therefore, this retrospective study comprehensively analyzed the correlation between chromosome polymorphisms and the outcome of IVF−embryo transfer treatment for infertile couples who were treated in our department during the last 3 years.

Materials and Methods

Subjects and karyotype analysis

A retrospective analysis was conducted of all the IVF and ICSI treatments performed from October 2006 up to and including 12 December 2009 at the Department of Reproductive Medicine, Renji Hospital of Shanghai Jiaotong University. Only data from the first cycles were used. Excluding couples with abnormal chromosome karyotypes, the frequency of chromosomal polymorphic variations was detected in a total of 1978 infertile couples.

Chromosome karyotype analysis was carried out on peripheral blood lymphocytes for all infertile couples before ART. Peripheral blood lymphocytes were stimulated and cultured for 72 h, and then stained with the G-banding technique. At least 20 meta-phases were analyzed for each case, and five meta-phases were karyotyped using light microscopy. The banding resolution was 400−550 bands per haploid set (BPHS). C-banding and R-banding staining methods were adopted (when necessary) to assist karyotype analysis.

Heteromorphisms were reported according to International System for Chromosome Nomenclature 2009 after selective banding studies, such as C and nucleolar organizing region (NOR) banding, were conducted. Visualized polymorphic variations in the length of the centromeric heterochromatin on the long arms of chromosomes 1, 9 and 16 (1qh+/−, 9qh+/− and 16qh+/−), and the distal heterochromatic region of chromosome Y (Yqh+/−) were documented. Distinct polymorphic variants of the size of satellites (ps+) and lengths of stalks (pstk+) of the acrocentric (acro) chromosomes were also recorded.

To be classified as variants, heteromorphisms needed to be at least twice the size of the corresponding region on the other homolog. This served as an internal control to rule out cultural artifacts in a majority of meta-phases studied. Polymorphisms of the Y chromosome were evaluated such that Yqh+ occurred when it was larger than chromosome 18, and Yqh− occurred when the Y chromosome was smaller than the G-group chromosome (Hsu *et al.*, 1987). The pericentric inversion of chromosomes 9 and Y were also considered a heteromorphism. When heteromorphisms were detected, all karyotypes were examined by three independent laboratory technicians to avoid uncertainty and variable results.

Overall, 289 couples were eliminated from the study of the outcome of IVF−embryo transfer treatment for reasons including: female partner age >38 years, female basal FSH >10 IU/l, female partner with ovulation dysfunction (such as polycystic ovarian syndrome), female partner with anatomic defects of the reproductive system, female partner undergone reproductive system surgery and poor obstetric history (including spontaneous miscarriage, stillbirth and children with malformations or genetic abnormalities).

Thus 1689 infertile couples were grouped according to the karyotype analysis results: Group 1 (control group) included 1402 couples with normal chromosomes; Group 2 included 82 couples with polymorphic variants of chromosomes in only females; Group 3 included 187 couples with polymorphic variants of chromosomes in only males. There were 18 other infertile couples with polymorphic variants of chromosomes in both males and females. They were excluded from the study because of their small number.

The stimulation protocol included the long and short down-regulation protocol. The long-term pituitary down-regulation started from the late luteal phase. After that, 150−225 IU of FSH were injected daily. The short-term pituitary down-regulation began on the cycle day 2 or day 3. When there were more than three follicles with diameter ≥18 mm in bilateral ovaries, hCG was injected intramuscularly at a dose of 5000−10 000 IU, and the oocytes were retrieved 34−36 h later. Traditional IVF or ICSI was performed 4−6 h after the oocyte retrieval procedure, and the fertilization of oocytes was checked the next day. The embryo-transfer procedure was performed on Day 2 or Day 3 according to embryo development and patient condition. Oocyte retrieval procedures without embryo transfer were excluded from the study.

Blood or urine β-hCG levels were tested 14 days after the embryo-transfer procedure to confirm biochemical pregnancy. The gestational sac seen on ultrasonography was used as evidence of clinical pregnancy 4 weeks after the embryo-transfer procedure. Pregnancy termination before 10 weeks after the oocyte retrieval procedure (corresponding to a gestational age of 12 weeks) was considered early miscarriage. Pregnancy over 10 weeks after the oocyte retrieval procedure was considered ongoing pregnancy.

The clinical pregnancy rates (CPR), embryo implantation rates (IRs), early miscarriage rates and ongoing pregnancy rates (OPRs) of fresh embryo transfer were calculated and compared between the three groups.

Statistical analysis

SPSS 16.0 software was used for statistical analysis. $P \leq 0.05$ was considered significant. One-way ANOVA was used to test numerical data. The exact χ^2 or Fisher's exact probability test was used to test for significant differences of categorical data.

A binary logistic regression model was used to compute the odds ratios (ORs) of the chromosomal polymorphic variations as variables predictive

of clinical and ongoing pregnancies and early miscarriage after fresh embryo transfer. Other independent variables included female age, IVF or ICSI, female basal FSH, protocol of ovarian stimulation, sperm parameters, dosage of gonadotrophin (Gn) used for controlled ovarian hyperstimulation, estradiol level on the day of HCG injected, thickness of endometrium on HCG day, number of oocytes obtained and number of high-quality embryos transferred. All of the above variables were categorical variables or were transformed into categorical variables. Chromosomal polymorphic variation was a multicategorical variable, with the different values having no real numerical relationship with each other; this was a code to a dummy variable. The control group was the category to which the other two categories were compared.

Results

The incidence of chromosomal polymorphic variations in infertile couples is shown in Table I. The most common variant observed

was Yqh+ in infertile men (145, 7.33%). Other chromosomal variants with a high incidence included 1qh+ (34 in women, 1.72%) and 16qh+ (19 in women, 0.96%). Inv(9) was the least common polymorphic variation in infertile couples (18, 0.91% in men; 13, 0.66% in women).

The basal data of the three groups were compared by two methods. Statistical analyses of not only numerical data, but also categorical data, showed no differences among the three groups for the methods of insemination, female ages, infertility years, etiology of infertility, female basal FSH, endometrial thickness, number of oocytes retrieved and the numbers of high-quality embryos transferred ($P > 0.05$; Table II).

Table III shows no differences between the polymorphic groups and the control group in the IR, CPR and OPR ($P > 0.05$). Compared with the control group, Group 2 (women with polymorphic chromosome variants) had a similar early miscarriage rate (7.89 versus 6.84%,

Table I Frequency of chromosomal polymorphism variation.

Karyotypes		No. of males with heteromorphism (n = 1978)	Frequency (%)	No. of females with heteromorphism (n = 1978)	Frequency (%)
Total		258	13.04	114	5.76
1, 9, 16qh+		39	1.97	65	3.29
	1qh+	22		34	
	9qh+	5		19	
	16qh+	10		9	
	1qh+16qh+	1		1	
	9qh+16qh+	0		2	
	1qh+9qh+	1		0	
Chromosome variation in D/G genomes		43	2.17	35	1.77
	13ps+	4		0	
	14p+	6		5	
	15p+	9		11	
	13cenh+	2		0	
	14cenh+	0		1	
	15cenh+	3		6	
	21p+	5		8	
	22ps+	12		4	
	22cenh+	1		0	
	15p12,21p12	1		0	
Inv(9)		18	0.91	13	0.66
Y chromosome variation		155	7.84		
	Yqh+	145			
	Yqh−	7			
	inv(Y)	3			
Multiple variation		3		1	
	1qh+,15ps+	1		0	
	9qh−	0		1	
	1qh+,inv(9)(p12q13)	1		0	
	Yqh+,16qh+	1		0	

Table II Basal characteristics of infertile couples.

		Group 1 (*n* = 1402)	Group 2 (*n* = 82)	Group 3 (*n* = 187)	*P*[a]	*P*[b,c]
Method of insemination						
	IVF cycles	657	37	74		0.17[b]
	ICSI cycles	745	45	113		
Female age (years)	Mean ± SD	29.56 ± 3.54	29.78 ± 3.13	29.47 ± 3.57	0.80	
	<25	86	4	15		0.79[c]
	25–29	636	37	85		
	30–34	538	36	68		
	≥35	142	5	19		
Etiology of infertility						
	Tubal factor	410	24	51		0.52[c]
	Male factor	592	34	93		
	Multiple factor	368	21	40		
	Idiopathic	32	3	3		
Basal FSH (IU/l)	Mean ± SD	6.70 ± 1.50	6.85 ± 1.38	6.61 ± 1.48	0.48	
	<8	1121	65	151		0.95[b]
	8–10	281	17	26		
Protocol of ovarian stimulation						0.15[b]
	Long	1026	66	142		
	Short	376	14	45		
Sperm parameters						
	Normal	442	27	54		0.85[b]
	Oligozoospermia	285	19	42		
	Severe oligozoospermia and azoospermia	675	36	91		
Dosage of Gn (IU)	Mean ± SD	1619.33 ± 529.99	1543.15 ± 412.82	1556.35 ± 426.31	0.15	
	≤1125	201	9	24		0.36[c]
	1126–2999	1166	72	162		
	≥3000	35	1	1		
E2 level on HCG day (pg/ml)	Mean ± SD	3504.53 ± 1711.38	3370.82 ± 1653.96	3418.36 ± 1606.33	0.66	
	≤1000	39	3	5		0.53[c]
	1001–3499	971	58	142		
	3500	392	21	40		
Thickness of endometrium on HCG day (mm)	Mean ± SD	9.86 ± 1.85	9.94 ± 1.79	10.1 ± 2.13	0.23	
	≤7	118	6	20		0.92[b]
	>7	1284	76	157		
Numbers of oocytes obtained	Mean ± SD	12.72 ± 6.54	12.07 ± 5.46	12.71 ± 6.42	0.68	
	≤5	171	12	26		0.92[b]
	6–15	799	46	107		
	>15	432	24	54		
Numbers of high-quality embryos transferred	Mean ± SD	1.73 ± 0.67	1.67 ± 0.72	1.68 ± 0.68	0.55	
	0	111	8	16		0.90[c]
	1	227	15	34		
	2	1000	55	131		
	3	64	4	6		

[a]One-way ANOVA.
[b]χ^2.
[c]Fisher's exact test.

Table III Comparison of the outcomes of fresh IVF–embryo transfer cycles among the three groups.

	Group 1	Group 2	Group 3	P
Implantation rate	29.37% (843/2870)	29.70% (49/165)	31.41% (120/382)	0.71
Clinical pregnancy rate	45.86% (643/1402)	46.34% (38/82)	51.87% (97/187)	0.30
Early miscarriage rate	6.84% (44/643)	7.89% (3/38)	10.31% (10/97)	0.39
Ongoing pregnancy rate	41.16% (577/1402)	42.68% (35/82)	45.99% (86/187)	0.44

Table IV Comparison of outcomes of fresh IVF–embryo transfer cycles among the three groups with normal sperm parameters.

	Group 1	Group 2	Group 3	P
Implantation rate	28.68% (261/910)	30.91% (17/55)	27.93% (31/111)	0.92
Clinical pregnancy rate	45.92% (203/442)	44.44% (12/27)	46.30% (25/54)	0.99
Early miscarriage rate	5.42% (11/203)	8.33% (1/12)	12.00% (3/25)	0.27*
Ongoing pregnancy rate	41.40% (183/442)	40.74% (11/27)	38.89% (21/54)	0.94

*Fisher's exact test.

Table V Comparison of outcome of fresh IVF–embryo transfer cycles among the three groups with oligozoospermia and azoospermia.

	Group 1	Group 2	Group 3	P
Implantation rate	29.69% (582/1960)	29.09% (32/110)	32.84% (89/271)	0.56
Clinical pregnancy rate	45.83% (440/960)	47.27% (26/55)	54.14% (72/133)	0.20
Early miscarriage rate	7.50% (33/440)	7.69% (2/26)	9.72% (7/72)	0.75*
Ongoing pregnancy rate	41.04% (394/960)	43.64% (24/55)	48.87% (65/133)	0.22

*Fisher's exact test.

$P > 0.05$), while Group 3 (men with polymorphic chromosome variants) had a higher miscarriage rate (10.31 versus 6.84%), though the difference was not significant ($P > 0.05$). Further subgroup analysis according to the male sperm parameters also did not demonstrate significant difference neither in couples with normal sperm parameters (Table IV) or in those with oligozoospermia and azoospermia (Table V).

Results of the binary logistic regression model are shown in Table VI. The model resulting from the analysis yielded ORs for CPR, the early miscarriage rate and OPR. Some potential confounders showed an effect on the CPR and OPR; for example, the long-term down-regulation protocol showed a much better treatment outcome than the short-term protocol (OR for CPR 0.428, $P < 0.001$; OR for OPR 0.423, $P < 0.001$); the dosage of Gns displayed a negative correlation with the CPR (OR for CPR 0.739, $P = .0030$), and similar trend with OPR (OR for OPR 0.758, $P = 0.051$) and the more high-quality embryos that were transferred, the higher was the pregnancy rate obtained (OR for CPR 1.574, $P < 0.001$; OR for OPR 1.490, $P < 0.001$). Furthermore, other factors such as sperm parameters and female age had no effect on the outcome of IVF/ICSI treatment. After adjusting for these factors, Group 2 (female polymorphism variation couples) displayed similar CPR, OPR and early miscarriage rates to Group 1 (ORs 0.993, 1.034 and 1.210, respectively, $P > 0.05$). Group 3 (male polymorphism variation couples) seemed to have a higher CPR (1.310-fold higher than Group 1) and higher early miscarriage rate (1.620-fold higher than Group 1); however, neither difference was significant ($P = 0.095$ and $P = 0.140$, respectively).

Discussion

Chromosomal polymorphism variation, a synonym of chromosomal heteromorphism, was considered normal for a long period of time. In recent years, more and more studies have shown an increased incidence of chromosomal polymorphism variation in infertile couples. There seems to be some relationship between chromosomal polymorphism variation and decreased fertility; however, the impact of chromosomal heteromorphism on infertility treatments such as IVF–embryo transfer remains unknown. In 2005, Yakin investigated 210 infertile males who were involved in ICSI treatment; they found that the CPRs and IRs were significantly lower for men with heterochromatin polymorphism than those of normal karyotype (CPR 21.7 versus 40.6%; IR 12.9 versus 19.9%). However, since this study investigated only infertile couples in which the male partner presented with severe oligoasthenoteratozoospermia (OAT) and non-obstructive azoospermia (NOA), the results do not fully reflect the impact of chromosomal polymorphisms on the outcome of IVF and embryo-transfer techniques.

In the present study, more infertile couples, including male partners presenting with normal sperm parameters, mild to moderate OAT and obstructive azoospermia, as well as infertile males presenting with NOA or OAT were enrolled. Traditional IVF transfer cycles were also included. The effect of chromosomal heteromorphism expression on the outcome of IVF/ICSI treatment in infertile women was also evaluated. The outcomes of IVF/ICSI treatment were also compared in subgroups of normal and abnormal sperm parameters. Furthermore to eliminate the effect of sperm parameters and insemination methods, the ratios of these variables among the three groups were evaluated and found to be no different, even when using a logistic regression model to analyze the effects of these variables with respect to IVF/ICSI treatment.

The IR, CPR and OPR were first compared among the three groups by the χ^2 method, and no difference was found, even in subgroups according to sperm parameters. The male carrier group with oligozoospermia and azoospermia displayed a higher IR, CPR and OPR,

Table VI Estimated OR for CPR, early miscarriage rate and OPR using the binary logistic regression model.

	Clinical pregnancy rate		Ongoing pregnancy rate		Early miscarriage rate	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Female chromosomal polymorphic variations carrier (Group 1)[a]	0.993 (0.627−1.571)	0.974	1.034 (0.652−1.640)	0.887	1.210 (0.353−4.148)	0.761
Male chromosomal polymorphic variations carrier (Group 2)[a]	1.310 (0.955−1.795)	0.094	1.245 (0.907−1.708)	0.175	1.620 (0.774−3.392)	0.201
IVF or ICSI	1.294 (0.950−1.764)	0.102	1.288 (0.943−1.758)	0.112	0.824 (0.342−1.986)	0.667
Female partner age	1.053 (0.914−1.215)	0.474	0.984 (0.852−1.136)	0.827	1.121 (0.761−1.652)	0.564
Women basal FSH (IU/l)	1.224 (0.949−1.579)	0.120	1.248 (0.965−1.614)	0.091	0.889 (0.438−1.802)	0.743
Protocol of stimulation[b]	0.428 (0.325−0.563)	<0.0001	0.423 (0.319−.561)	<0.0001	1.844 (0.887−3.835)	0.101
Sperm parameters	1.098 (0.920−1.310)	0.299	1.094 (0.916−1.307)	0.321	1.099 (0.661−1.825)	0.716
Dosage of Gn[c]	0.739 (0.562−0.971)	0.030	0.758 (0.574−1.001)	0.051	1.106 (0.522−2.342)	0.792
E_2 level on HCG day (pg/ml)	0.941 (0.817−1.084)	0.402	0.932 (0.808−1.076)	0.338	0.922 (0.619−1.374)	0.690
Thickness of endometrium on HCG day (cm)	1.267 (0.878−1.829)	0.205	1.438 (0.981−2.108)	0.062	0.622 (0.257−1.504)	0.292
No. of oocytes obtained	1.033 (0.855−1.247)	0.738	1.040 (0.860−1.258)	0.682	0.861 (0.511−1.450)	0.573
No. of high-quality embryos transferred[d]	1.574 (1.345−1.843)	<0.0001	1.490 (1.269−1.749)	<0.0001	1.099 (0.674−1.793)	0.705

[a]OR: odds for the outcome of Groups 2 and 3 compared with Group 1.
[b]OR: relative change in odds on outcome comparing the short-term ovarian stimulation protocol to the long-term ovarian stimulation protocol.
[c]OR: relative change in odds for the outcome when the dosage of Gn is increased.
[d]OR: relative change in odds for the outcome when the number of high-quality embryo transferred is increased by 1 SD.

although it did not reach statistical significance. Since many independent factors are related to the outcome of IVF or ICSI treatment, such as the sperm parameters mentioned above, a binary logistic regression method was used to evaluate the odds of chromosomal hetermorphism expression. Some variables in this model were continuous quantitative variables, but the effect of a one-unit change in these variables had no practical meaning. Therefore, they were transformed to categorical variables. The ratios of these variables among the three groups were initially compared, and no differences were found. Then, using a logistic regression model, it was found that there were three confounding factors including ovarian stimulation protocol, dosage of Gns and number of high-quality transferred embryos. After accounting for the effect of these factors, no adverse effect of chromosomal polymorphism variation on the outcome of IVF/ICSI treatment was found.

Among the infertile couples who received the first IVF/ICSI treatment in our study, Y chromosome heteromorphism was the most prevalent chromosome heteromorphism in the male partner (7.84%). The heteromorphism changes of the Y chromosome included increasing secondary constriction of the long arm (or the so-called heterochromatic regions) and microdeletion of the Y chromosome. The impacts of small Y chromosome, Y chromosome azoospermia factors microdeletion and microdeletion of the heat-shock-protein gene on infertility have also attracted attention (Nagvenkar *et al.*, 2005; Vinci *et al.*, 2005). Some authors reported studies of the relationship between Y chromosome microdeletion and the outcome of ICSI treatment. For example, Kihaile *et al.* (2005) compared reproductive outcomes following ICSI treatment between those with Y chromosome microdeletions and those with intact Y chromosomes in Japanese and African men with azoospermia and oligozoospermia. They found that there were no significant

differences in fertilization, blastocyst development, implantation and pregnancy rates between the two groups. Choi *et al.* (2004) also reported the outcomes of ICSI cycles in 17 patients with Y microdeletions. They found a trend toward lower fertilization rates in patients with Y microdeletions but it did not reach statistical significance, while the CPR of the microdeletion group was similar to that of controls. Two other articles also reported no impact of Y chromosome microdeletion (Silber *et al.*, 1998; van Golde *et al.*, 2001).

In the present data, 13.04% of male partners displayed chromosomal polymorphism variations, which had no adverse effect on pregnancy rates. Clinical and OPRs of Group 3 (male carriers of chromosomal heteromorphism) showed no significant differences. Thus, we postulated that chromosomal heteromorphism in infertile men may have no negative effect on IVF/ICSI treatment, which was consistent with the results reported previously.

After thorough analysis of the karyotypes of chromosomal heteromorphism in male carriers, 60% of male carriers expressed heteromorphism of the Y chromosome, but only seven men were defined as Yqh−, while 93.55% had Yqh+. Thus, it appears that the potential impact of Yqh+ on the outcome of IVF−embryo transfer treatment should not be ignored. Antonelli *et al.* (2000) performed cytogenetic analysis in 333 males with oligozoospermia and NOA who were receiving IVF−embryo transfer treatment and found that too many DNA repeats at specific regions of the Y chromosome may impact on the pairing and synapsis of X and Y chromosomes during meiosis. Minocherhomji *et al.* (2009) believe that the increase in the long arm of the Y chromosome may inhibit the expression of genes of spermatogenesis by position-effect variegation (PEV) and decrease reproductive capacity. However, some other researchers do not agree. For example, Kalantari *et al.* (2001) analyzed the chromosomes and semen of infertile males, and they concluded that Y chromosome

heteromorphism did not directly affect the sperm count. The conflicting results indicate that more data are needed to elucidate the effect of Y chromosomal heteromorphism on spermatogenesis. In the present study, with a high percentage of Yqh+ among male chromosomal heteromorphism carriers, Yqh+ appeared to have no adverse effect on the pregnancy rate following IVF/ICSI treatment, despite a potential impact on spermatogenesis.

In addition to Y chromosomal heteromorphism, there were other heteromorphism types related to infertility: heteromorphisms shown by short-arm regions of D and G group chromosomes and heteromorphisms shown by paracentric long-arm regions of chromosomes 1, 9 and 16, and inv(9) (Madon et al., 2005; Sahin et al., 2008; Minocherhomji et al., 2009).

The increase in the length of the secondary constriction in the long arm of chromosomes 1, 9 and 16 is also common in chromosome polymorphism variations. Minocherhomji et al. (2009) found that both infertile men and infertile women more frequently had a 9qh+ karyotype. In the present study, the secondary constriction in the long arm of chromosomes 1, 9 and 16 was most common in females with chromosome polymorphism variation (3.29%), and was close to that in males with polymorphism variation (1.97%). The structure and function of these duplicate DNA sequences are still unknown. The repeat in the distal chromosome segments may cause clinical symptoms because of increased, highly repetitive, DNA sequences (Hennig, 1999; Broccoli, 2004). Recent studies indicate that heterochromatin in chromosomal polymorphism variations regulates gene expression by an epigenetic mode, e.g. reversible transformation between heterochromatin (non-coding DNA sequences) and euchromatin (expressed DNA sequences) (Frenster and Herstein, 1973; Nakatsu et al., 1974). One other interesting point about heterochromatin is known as positive-effect variegation, which is defined as silencing of a normally expressed gene when it is brought into juxtaposition with pericentric heterochromatin (Cryderman et al., 1998).

D-genome and G-genome chromosomes are the other kinds of common chromosome heteromorphisms. They were also common in the present study (2.17% in men and 1.77% in women). These heteromorphisms show increased heterochromatin at the chromosome telomere, the short arm of the chromosome, and the centromere, as well as variants at the NOR. Heterochromatin located in centromeres has an essential role in spindle attachment and chromosome movement, meiotic pairing and sister chromatid cohesion (Karpen and Endow, 1998). When chromatin variation occurs in these regions, it causes defects in centromere function and kinetochore assembly, difficulty in homologous chromosome pairing, and impacts on cell division. All of the above abnormalities could affect gamete formation.

In the present study, both Groups 2 and 3 (infertile couples with chromosomal heteromorphism) showed similar CPRs and OPRs compared with the normal group. This finding suggests that, although variations in heterochromatin could affect gametogenesis and lead to infertility, the efficiency of IVF–embryo transfer treatment would not be affected. For instance, OAT and azoospermia due to a decline in spermatogenesis could be resolved by the ICSI technique, and ovarian stimulation could circumvent impaired oogenesis. In the present study, logistic analysis also indicated that heteromorphism carriers could achieve the same pregnancy rate as normal karyotype couples, when two or three high-quality embryos were transferred.

In addition to the above result, one other interesting finding in the present study was that the couples with male chromosomal heteromorphism, but not female carriers, displayed a trend toward a higher early miscarriage rate (10.31 versus 6.84%, $P > 0.05$), and this phenomenon was more obvious in those couples with normal sperm parameters (12.00% of Group 3 versus 5.42% in control group, $P > 0.05$). Some authors pointed out that 41% of recurrent miscarriages are aneuploid, and 15% of patients will have repeat aneuploidy (Carp et al., 2008). In a study by Yakin et al. (2005), sperm fluorescent in situ hybridization (FISH) analysis in NOA or OAT men with 9qh+ revealed an increased rate of sperm aneuploidy. The incidence of Y chromosomal polymorphism variation was increased in male partners of infertile couples in the present study, which could have caused the slightly higher early miscarriage rate, even in those without sperm parameter abnormality. Thus, in the future, more sensitive techniques are needed to identify minor chromosomal variation, e.g. analysis of aneuploidy in gametes by FISH or other molecular genetics methods.

In summary, although past literature has indicated that there may be some relationship between polymorphic variants of chromosomes and infertility, the clinical and OPRs of IVF/ICSI treatment in the present study did not appear to be affected; only the early miscarriage rate of male carriers tended to be higher. However, the number of heteromorphism carriers was insufficient. Furthermore, the chromosome analysis method in the present study had a 400–550 BPHS banding resolution; so some potential variations could not be distinguished from common polymorphism variations. Therefore, a greater number of samples and more sensitive techniques are needed for further study.

Authors' roles

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