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EMBRYO BIOLOGY

# Chromosomal polymorphisms are independently associated with multinucleated embryo formation

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**Abstract**
*Purpose* The purpose of this study is to explore the factors associated with embryo multinucleation, particularly focused on the influence of parental chromosomal polymorphisms in embryo multinucleation.
*Methods* This is a retrospective case-control study involving 1260 infertile couples undergoing their first IVF/ICSI cycles. Couples were screened for abnormalities in their karyotype and were evaluated for blastomere persistence of multinucleation. Demographic characteristics, stimulation protocol, and pregnant outcomes were analyzed using logistic regression analysis.
*Results* The level of basal FSH was lower in the multinucleated embryos group (5.37 vs 5.72 IU/L). The Multinucleated embryos group received less gonadotropins (1788.5 vs 1891.3 IU), and the level of LH on day of HCG triggering was lower (1.09 vs 1.30 IU/L). More oocytes were recovered in the multinucleated embryos group (11.51 vs 9.23). Chromosomal polymorphisms were seen in at least 1 out of 163 (12.9%) couples. Multivariate logistic regression analysis revealed that chromosomal polymorphisms were independently associated with an increase in the occurrence risk of multinucleated embryos (OR = 1.61, 95% CI, 1.06–2.44) in the first IVF/ICSI cycle. The miscarriage rate in the multinucleated embryos group was 10% higher than that of the control group.
*Conclusions* Chromosomal polymorphisms were independently associated with multinucleation embryo formation. A higher LH level on the day of HCG triggering was associated with a decreased chance of multinucleation.

**Keywords** In vitro fertilization · Chromosomal polymorphism · Multinucleated embryo

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## Introduction

A blastomere containing more than a single interphase nucleus is defined as being multinucleated [1]. The presence of multinucleated blastomeres (MNB) in the cleaving embryo has been associated with poor embryo development and adverse in vitro fertilization (IVF) outcomes [2, 3]. The incidence of MNB has been reported to vary between 15 and 40% [4–6]. In recent years, with the application of time-lapse monitoring systems, more MNB embryos were identified. However, most of these reports were not identifying embryos at ESHRE/ALPHA consensus embryo evaluation times [7], which implies that the actual occurrence may be higher.

Kligman et al. reported that 74.5% of multinucleated embryos were chromosomally abnormal compared to 32.3% of non-multinucleated embryos [8]. Similar results were reported by Yilmaz et al., whose research indicated that the majority of the MNB embryos were genetically abnormal [6]. Ambroggio et al. suggested the multinucleated embryos should not be recommended for transfer in IVF cycles [9].

The mechanism of multinucleation is unclear. Jackson et al. found higher rates of multinucleation present in cycles with

both the higher E2 levels and increased number of oocytes recovered [4], and concluded that multinucleation in normally fertilized embryos is associated with an accelerated ovulation induction response. De Vincentiis et al. found embryo multinucleation rates increase when in vitro matured oocytes are used instead of in vivo matured oocytes [10]. De Cassia reported a higher incidence of MNB embryos with the use of gonadotropin-releasing hormone agonists, male factor infertility, and in cycles with higher number of retrieved oocytes [11]. None of these researchers investigated the karyotypes of the infertile couple with respect to multinucleation formation.

Chromosomal polymorphisms are the variants in the chromosomal heterochromatin region, usually occurring 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. Pericentric inversions on chromosomes 9[inv(9)] are also categorized as polymorphisms [12]. Such polymorphisms are generally considered "normal" karyotypes [12]. However, more and more studies indicate that chromosomal polymorphisms may be associated with certain clinical problems such as abnormal spermatogenesis [13], infertility [14, 15], and recurrent miscarriages [16, 17]. Several recent studies found that chromosomal polymorphic variants were associated with higher rate of chromosomal abnormalities among blastomeres at the cleavage stage [18–20].

Therefore, we hypothesized that couples with chromosomal polymorphisms might experience a higher rate of embryo multinucleation. The aim of this paper was to analyze the factors associated with MNB with particular attention to chromosomal polymorphisms.

## Materials and methods

### Study population

One thousand two hundred sixty infertile couples who had embryos cultured from their first IVF/ICSI cycle between January 2011, and December 31, 2015 in our clinic were enrolled in the study. All couples underwent karyotype screening before IVF treatment. Those with normal karyotype or chromosome polymorphisms were included in the study. Exclusion criteria included couples with abnormal karyotypes, couples with poor ovarian reserve, and couples stimulated by mini-stimulation protocol or oocyte retrieval after natural cycle.

### Karyotypic analysis

Karyotypic analysis was carried out using cultured lymphocytes from peripheral blood. The lymphocyte chromatin was stained with the G-banding technique after 68–72 h of culture. At least 20 meta-phases were evaluated for each case, and five meta-phases were karyotyped using light microscopy. The banding resolution was 320–400 bands per haploid set (BPHS). C-banding staining methods were added when necessary to assist with karyotypic analysis.

### Classification of polymorphic variations

According to the International System for Chromosome Nomenclature 2013 [21], visualized polymorphic variations were recorded: polymorphic variations in the length of the centromeric heterochromatin on the long arms of chromosomes 1, 9, and 16 (1qh+, 9qh+, and 16qh+); size of satellites (ps+); and lengths of stalks (pstk+/−) of the acrocentric (acro) chromosomes (chromosomes in D and G genomes); the pericentric inversion of chromosomes 1, 9, and Y were 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 culturing artifacts in a majority of meta-phases studied. When heteromorphisms were detected, all karyotypes were examined by two independent laboratory technicians to minimize uncertainty and variable results.

### Controlled ovarian stimulation

The patients underwent either GnRH agonist or GnRH antagonist protocol for ovulation induction. The initial doses were based on antral follicle counts, female age, and basal FSH. The subsequent doses were adjusted according to follicle growth and serum estradiol levels. Final follicular maturation was triggered with human chorionic gonadotropin (HCG) or recombinant HCG (rHCG alpha) when at least three leading follicles reached a mean diameter ≥ 18 mm. Transvaginal oocyte retrieval was scheduled 34–38 h later.

### Embryo culture and multinucleation assessment

Conventional IVF or ICSI was performed 4–6 h after the oocyte retrieval. The fertilization check was done at 16–18 h after insemination. The presence of two pronuclei was considered normal fertilization. Cleavage stage embryos were assessed for cell stage, percent fragmentation, multinucleation, and blastomere symmetry according to embryo evaluation times proposed by ESHRE/ALPHA consensus [1]. A blastomere containing more than a single interphase nucleus on day 2 (44 ± 1 h post-insemination) was defined as being multinucleated [1] and those in which there was 0 or 1 nucleus per blastomere were termed control embryos. Each embryo was double checked by two embryologists. (Fig. 1)The highest quality embryos were defined as optimal day 3 embryo (68 + 1 h post-insemination) with 8 equally sized mononucleated blastomeres, with < 10% fragmentation [1].

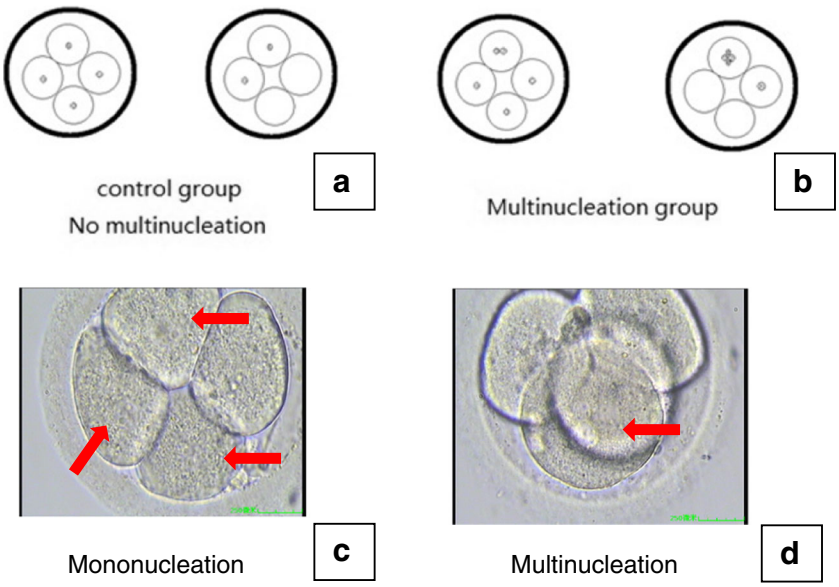


**Fig. 1** Analog diagram of embryo without multinucleation **a**; analog diagram of multinucleation **b**; actual picture of embryo without multinucleation (mononucleation in blastocysts), magnification = ×400 **c**; actual picture of embryo with multinucleation, magnification = ×400 **d**

### Embryo transfer and clinical outcomes

The embryo transfer procedure was performed on day 3. Only the first embryo transfer cycle was evaluated. The control group consisted of cycles in which only control embryos were produced and transferred. The multinucleated group consisted of cycles in which multinucleated embryos were present in the cohort embryos, but only the sibling control embryos were transferred. None of the multinucleated embryos were transferred.

Serum beta-HCG levels were measured 14 days after transfer. A clinical pregnancy (CP) was defined as visualization of gestational sac on ultrasound 4 weeks after embryo transfer. The implantation rate (IR) was calculated by dividing the number of implanted embryos by the number of embryos transferred. Pregnancy termination before 12 weeks of gestational age was considered as early miscarriage; implantation outside of the uterus was defined as ectopic pregnancy and the live birth rate was defined as the proportion of IVF cycles reaching embryo transfer that resulted in the birth of at least one live-born child.

### Statistical analyses

All data analyses were performed using SPSS 20.0. Data are presented as mean ± standard deviation (SD) for continuous variables, and number of subjects ($n$) and percentage (%) for categorical variables.

Firstly, we performed univariate analyses using a two-sample $t$ test. Rates and proportions were compared between groups using chi-square test. We selected variables with $P < 0.10$ in univariate analyses to include in our multivariate analyses. Multivariate logistic regression analysis was carried out to determine the influencing factors associated with multinucleated embryos. We also controlled for female age in the logistic regression. Odds ratio (OR) and its 95% confidence interval (95% CI) was used to present the association.

## Results

### Comparison demographic characteristics of study population

The study included 1260 couples undergoing their first IVF/ICSI treatment among which 188 (14.9%) couples had multinucleated embryos. There were no significant differences between subjects with regard to female age, BMI, mean infertility duration, type of infertility, and cause of infertility. The basal FSH level was significantly lower in the multinucleated embryos group than that of the control group (5.37 vs 5.72 IU/L, $P = 0.007$) (Table 1).

### Stimulation cycle characteristics of study population

The stimulation protocol (GnRH agonist vs antagonist) was not significantly different between two groups, but multinucleated embryos group received less gonadotropin than the control group. The level of LH on day of HCG triggering was significantly lower in multinucleated embryos group ($P = 0.001$), while the stimulation days and estrogen level on the day of HCG triggering were not different between the two groups (Table 2).

**Table 1** Demographic characteristics of study population

| | Multinucleated embryos group ($N$ = 188) | Control group ($N$ = 1072) | $P$ value |
|---|---|---|---|
| Female age(years) | 31.28 ± 4.25 | 31.17 ± 4.15 | 0.732 |
| Female BMI (kg/m$^2$) | 21.45 ± 2.83 | 21.28 ± 2.76 | 0.439 |
| Infertility duration | 4.08 ± 2.77 | 3.97 ± 3.03 | 0.643 |
| Type of infertility | | | 0.544 |
| Primary infertility | 109(58.0%) | 596(55.6%) | |
| Secondary infertility | 79(42.0%) | 476(44.4%) | |
| Basal FSH (IU/L) | 5.37 ± 1.47 | 5.72 ± 1.66 | 0.007 |
| Cause of infertility | | | |
| Female factor | | | 0.378 |
| Tubal factor | 117(62.2%) | 705(65.8%) | |
| Endometriosis | 17(9.0%) | 86(8.0%) | |
| Anovulation | 6(3.2%) | 49(4.6%) | |
| Male factor | 91(47.3%) | 535(49.9%) | 0.516 |

## Frequency of chromosomal polymorphisms and the relationship with multinucleated embryos

Chromosomal polymorphisms were seen in a total of 163 (12.9%) couples. Variants were present in 85 (6.7%) men and 88 (6.9%) women, while 10 couples had chromosomal polymorphic variants in both partners. The proportion of main polymorphic variants detected in men and women is shown in Table 3. The prominent variants observed in men were 21pstk+ (23.9%), 1qh+ (21.2%), and Inv(9)(p12q13) (18.8%). The main chromosomal variants in women were Inv(9)(p12q13) (27.3%), 21pstk+ (19.3%), and 1qh+ (18.2%).

18.1% (34/188) of couples with multinucleated embryos had chromosomal polymorphic variants compared to 12.0% (129/1072) in the control group ($P$ = 0.023). The proportion of chromosomal anomalies in males was higher in the multinucleated embryos than that of the control group (10.1 vs 6.2%, $P$ = 0.046). A similar trend was observed for females but the difference was non-significant ($P$ = 0.230) (Fig. 2).

## Multivariate analysis of factors associated with multinucleated embryos

Multivariate logistic regression analysis revealed that chromosomal polymorphism was associated with an increase of 61% in the occurrence risk of multinucleated embryos (OR = 1.61, 95% CI, 1.06–2.44) in the first IVF/ICSI cycle, after adjusting for female age, basal FSH, LH level on day of HCG, and total gonadotropin dose. Females with higher level of basal FSH and LH level on day of HCG were at lower risk of having multinucleated embryos (OR = 0.89 and 0.71, respectively). An increase of 750 IU (e.g., 75 IU increased for each day, and lasted for 10 days) in total gonadotropin dose was associated with a decrease of 18% in the occurrence risk of multinucleated embryos (OR = 0.82, 95%CI, 0.68–0.99). Older females tended to have higher risk of having multinucleated embryos but the association was not statistically significant ($P$ = 0.158) (Table 4).

**Table 2** Stimulation cycle characteristics of multinucleated embryo group and control groups

| | Multinucleated embryos group ($N$ = 188) | Control group ($N$ = 1072) | $P$ value |
|---|---|---|---|
| Stimulation protocol | | | 0.767 |
| GnRH agonist protocol | 176(93.6%) | 1017(94.9%) | |
| GnRH antagonist protocol | 12(6.4%) | 55(5.1%) | |
| Stimulation days | 11.38 ± 2.11 | 11.53 ± 2.26 | 0.407 |
| Total gonadotropin dose (IU) | 1788.5 ± 609.2 | 1891.3 ± 758.7 | 0.041 |
| E2 level on day of HCG (pmol/L) | 11,220.2 ± 5215.8 | 10,670.3 ± 5167.9 | 0.147 |
| LH level on day of HCG (IU/L) | 1.09 ± 0.65 | 1.30 ± 1.00 | 0.001 |
| Type of insemination | | | 0.369 |
| IVF | 133(70.7%) | 792(73.9%) | |
| ICSI | 55(29.3%) | 280(26.1%) | |

**Table 3** Frequency of chromosomal polymorphism variation

| Karyotypes | Females with chromosome polymorphisms ($N$ = 88) | Males with chromosome polymorphisms ($N$ = 85) |
|---|---|---|
| (1, 9, 16) qh+ | | |
| 1qh+ | 16 (18.2%) | 18 (21.2%) |
| 9qh+ | 8 | 4 |
| 16qh+ | 2 | 1 |
| D/G genomes | | |
| 13pstk+ | 4 | 0 |
| 13pstk- | 0 | 1 |
| 14pstk+ | 4 | 3 |
| 14ps+ | 0 | 1 |
| 15ps+ | 1 | 2 |
| 15pstk+ | 4 | 2 |
| 21ps+ | 1 | 0 |
| 21pstk+ | 17 (19.3%) | 21 (23.9%) |
| 22ps+ | 2 | 1 |
| 22pstk+ | 3 | 7 |
| 22pstk- | 0 | 1 |
| Inv | | |
| Inv(1)(p13q21) | 1 | 0 |
| Inv(9)(p12q13) | 24 (27.3%) | 16 (18.8%) |
| Inv(Y)(p11.2q11.2) | – | 5 |
| Multiple variation | 1[a] | 2[b] |

[a] 46,XX,16qh+,22pstk+

[b] 46,XY,inv.(9)(p12q13),21pstk+; 46, XY,1qh+,15ps+

### Quality of embryos and pregnancy outcome

More oocytes were recovered but the high-quality embryo rate was lower in the multinucleated embryos group than that of the control group ($P$ = 0.001 and 0.015, respectively). There were no significant differences in the fertilization rate and mean number of embryos transferred between groups ($P$ = 0.626 and 0.333, respectively) (Table 5).

Among the 188 subjects in the multinucleated embryo group, 3 cases had no viable embryos to transfer and in 2 cases, the embryos were stored without transfer, resulting in a final total of 183 transfer cycles. In the control group, 20 cases had no viable embryos and 9 cases had embryos stored without transfer, resulting in 1043 transfer cycles in all. The data for transfer cycles showed that the miscarriage rate in multinucleated embryos group was 10% higher than the control groups ($P$ = 0.002), while there were not significant differences in other outcomes between two groups ($P$ > 0.05) (Table 6).

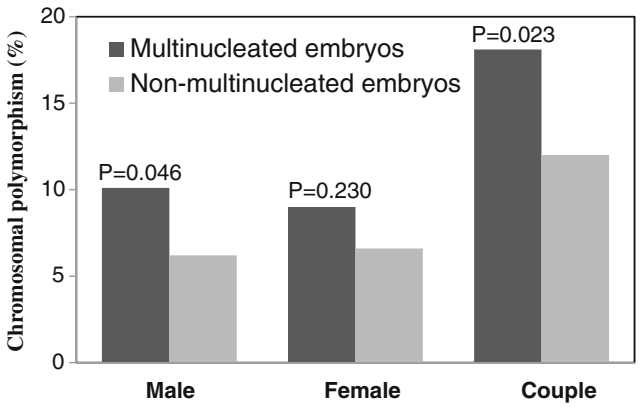


**Fig. 2** The occurrence rate of chromosomal polymorphism in couples with and without multinucleated embryos

## Discussion

Normal human embryos have a single nucleus per blastomere; however, sometimes blastomeres are present with more than one nucleus per cell. Multinucleation is an abnormality described in cleaving embryos, and it has been correlated with increased rates of aneuploidy and chromosomal abnormalities [6, 8].

Several previous studies have discussed the mechanism of multinucleation formation, and concluded that the factors that contribute to multinucleation formation are mainly encountered during the treatment procedure. De Cassia et al. [11] found that a higher incidence of MNB embryos arose when using gonadotropin-releasing hormone agonists in the IVF/ICSI cycles; however, in our study, no difference was found between the multinucleation rate when comparing GnRH agonist and antagonist protocols. We noted similar results to Kyrou et al. who analyzed the embryos by preimplantation genetic screening and found there was no difference in the proportion of abnormal blastomeres when using gonadotropin-releasing hormone (GnRH) agonist, or antagonist protocol [22].

Previous studies [4, 11] found that higher E2 levels and the increased numbers of oocytes recovered were associated with multinucleation formation, and concluded that multinucleation in normally fertilized embryos is associated with an accelerated ovulation induction response [4]. Similar results were presented in our study, as we found the basal FSH and total gonadotropin dose were lower than that of the control group but the number of oocytes recovered was higher than that of the control group. The stimulation duration in our study was similar between the two groups which differed from a previous study [4]. Jackson et al. speculated the multinucleation formation was associated with an accelerated ovulation induction response. We further hypothesize that the difference may be explained by lower FSH accompanied with better ovarian reserve, therefore requiring less gonadotropins and producing more oocytes.

In our study, we found that lower LH level on the day of HCG was correlated with multinucleation. We found no previous study that reported the LH level on the day of HCG

**Table 4** Multivariate analyses of the effect of factors on multinucleated embryos

| Factors | Odds ratio(OR) | 95%CI | *P* value |
|---|---|---|---|
| Age (years) | 1.03 | 0.99–1.07 | 0.158 |
| Chromosomal polymorphism | 1.61 | 1.06–2.44 | 0.027 |
| Basal FSH(IU/L) | 0.89 | 0.79–0.99 | 0.040 |
| LH level on day of HCG (IU/L) | 0.71 | 0.57–0.89 | 0.002 |
| Total gonadotropin dose (750 IU) | 0.82 | 0.68–0.99 | 0.046 |

triggering in multinucleated and normal embryos. The reason for this phenomenon is unknown.

With regard to chromosomal polymorphisms, during the last years, there have been published many articles with conflicting views on the clinical effect of chromosome variants. In our study, we found that the total chromosomal polymorphism rate was 12.9% in infertile couples. Similar with the results by Gorskaya et al. who reported the frequency of variants in the couples with primary infertility was 14% [23]. However, Sheroy et al. reported chromosomal polymorphism existed in 25.41% of couples with primary infertility, with a corresponding rate of 15.16% in fertile couples [15]. Data from 19,950 women demonstrated a significantly higher incidence of chromosomal polymorphisms in total infertile patients compared with the control group [24]. Meanwhile, dates from male infertile patient were more elaborate. Gao et al. investigated karyotype in 16,294 male infertile patients and found the rates of chromosomal polymorphism are 5.36% in normal semen group and 25.51% in light oligoasthenospermia group [25]. Stratified sampling found that there is no significant correlation between autosomal polymorphisms and male infertility, but Yqh +/− may be responsible for Y chromosome microdeletion and male infertility. [26–28].

The effects of chromosomal polymorphisms on IVF outcomes are controversial as well. Some studies stated chromosomal polymorphisms had no apparent adverse effect in IVF treatment [12, 13], whereas other studies drawn different conclusions. Ni et al. investigated couples with chromosomal polymorphisms in male partners and found they had poor pregnancy outcomes after IVF treatment manifesting as high cumulative early miscarriage rate and low live birth rate after a complete cycle [29]. Xu et al. reported chromosomal polymorphisms in either male or female carriers seemed to have adverse effects on IVF/ICSI-ET outcomes; and they analyzed in detail that chromosomal polymorphisms in male carriers affected outcomes mainly by decreasing the rates of fertilization, embryo cleavage, good-quality embryos, clinical pregnancies, ongoing pregnancies, and deliveries as well as increasing the biochemical pregnancy rate; chromosomal polymorphisms in female carriers affected outcomes only by lowering the embryo cleavage rate. The mean fertilization rate of couples with male chromosomal polymorphisms carriers undergoing IVF was significantly lower than that in those undergoing ICSI (61.1 vs 66.5%) [30]. The same result was proved by Liang et al.; they found that male chromosomal polymorphisms adversely influence fertilization rates of IVF cycles [31].

Fewer studies focus on the relationship of chromosomal polymorphism on the embryo quality. Garcia-Guixé et al. analyzed 95 embryos (15 IVF-PGD cycles) from couples showing a karyotype with a polymorphism by FISH, and found increased aneuploidy in preimplantation embryos from carriers of chromosomal variants, concretely those with heterochromatin and/or satellite polymorphisms [19]. In our study, this is the first time it has been reported that couples with chromosomal polymorphisms also appears to have increased multinucleated embryos during IVF treatment. The possible mechanisms were explained by a previous study. Sperm FISH analysis revealed an increased rate of aneuploidy in men with heterochromatin polymorphism [32]. Similar study by Morales et al. found that the frequency of infertile men with increased rates of sperm aneuploidy was higher among polymorphism carriers. (37.7 vs 16.3%), proved a relationship between polymorphisms and aneuploidy in spermatozoa and embryos [33].

This result may explain some surprising results of the previous studies. Some previous studies reported that chromosomal polymorphism had a high incidence rate in infertile couples [15, 24, 25] and couples with recurrent miscarriage [16, 17, 34, 35];

**Table 5** Quality of embryos for couples with and without chromosomal polymorphism

| | Multinucleated embryos group (*N* = 188) | Control group (*N* = 1072) | *P* value |
|---|---|---|---|
| Oocytes recovered | 11.51 ± 6.11 | 9.23 ± 5.30 | 0.001 |
| Fertilization rate (%) | 81.36 (1694/2082) | 81.82(7871/9620) | 0.626 |
| Top quality embryo rate (%) | 32.01(478/1493) | 35.34(2368/6701) | 0.015 |
| No. of transferred embryos | 2.02 ± 0.60 | 1.97 ± 0.63 | 0.333 |

**Table 6** Pregnancy outcome for multinucleated embryos group and control group

| | Multinucleated embryos group ($N$ = 183) | Control group ($N$ = 1043) | $P$ value |
|---|---|---|---|
| Pregnancy rate | 60.7(111/183) | 55.9(583/1043) | 0.231 |
| Implantation rate | 40.1(142/354) | 40.8(801/1965) | 0.819 |
| Miscarriage rate | 21.6(24/111) | 11.1(65/583) | 0.002 |
| Ectopic rate | 3.6(4/111) | 3.8(22/583) | 0.931 |
| Live birth rate | 45.4(83/183) | 47.6(496/1043) | 0.582 |

However, there was no apparent adverse effect in IVF treatment in some studies [12, 13]. The possible mechanism is couples with chromosomal polymorphic variants may be more likely to produce sperm [32, 33] and embryos [33] with abnormal chromosomal components; these abnormal chromosomic embryos can be seen as multinucleation in embryo culture [6, 8]; furthermore, these embryos may lead to infertility and/or recurrent miscarriage in natural conception. However, during IVF, because more embryos are prepared after ovarian stimulation, we can select suitable embryos (e.g., without multinucleation) to transfer even in couples with chromosomal polymorphic variants. In routine practice, multinucleated embryos identified were seldom transferred. In the study of Hong et al., they only compared the pregnancy outcome of the first embryo transfer cycle, and nearly no multinucleation embryos were transfer at that time, so they found no significant differences between the chromosomal polymorphic variants group and the control group [12], whereas in the study of Ni et al., they investigated a complete cycle of IVF treatment, when good-quality embryos were exhausted and multinucleation embryos would be transferred, so they found poor pregnancy outcomes in cumulative early miscarriage rate and low live birth rate between the two groups [29].

In our study, the high-quality embryo rate in the multinucleated embryo group was lower than the control group. This was due to the embryo evaluation criterion established by ESHRE/ALPHA [1]. Following this criterion, multinucleated embryos are not considered high-quality embryos. We also noted that although the pregnancy rate was not significantly different between the two groups, the miscarriage rate was higher in multinucleated embryo group. This phenomenon may be due to the fact that the multinucleation rate would have been higher using time-lapse evaluation [7]; the embryo transferred might be multinucleated embryos which were not identified by conventional embryo evaluation and made the miscarriage rate rise.

There were some limitations in this study. Firstly, we only evaluated multinucleation on a single day (day 2 post-insemination) of the embryo culture without employing time-lapse recording of embryo culture. This strategy could result in missing some multinucleated embryos. Additionally, in this study, chromosomal polymorphisms in males or females both tended to increase the occurrence risk of multinucleated embryos in univariate analysis, while the effect estimated by multivariate analysis was non-significant for chromosomal polymorphisms in women (OR = 1.31, 95% CI, 0.74–2.32) or male (OR = 1.69, 95% CI, 0.98–2.89) separately, perhaps because of the inadequacy of sample size for gender-specific analyses. We did not detect any differences in the strength of association between specific types of polymorphisms and the risk of multinucleation. Obviously, a larger sample could help identify the potential effect of specific types of chromosomal polymorphisms in females and males separately. Thirdly, it remains unclear whether multinucleation was a repetitive phenomenon and occurred repeatedly in persons with polymorphic variants. Further analysis of more samples from couples with polymorphic variants and repeated treatment cycles would help further understand the effects of polymorphic variants on multinucleated formation. Fourthly, if available, embryos donated for research from couples with and without chromosomal polymorphisms would be very useful to more accurately count multinucleation in cleavage embryos.

## Conclusion

In couples undergoing IVF, chromosomal polymorphisms were independently associated with multinucleateated embryo formation. Higher numbers of oocytes retrieved were associated with a higher incidence of multinucleation. A Higher LH level on the day of HCG triggering was associated with a decreased chance of multinucleation.

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**Authors' roles** Study was conceived by L.S. and Z.H.C; H.W., L.Y., and J.L. collected the clinic data; C.X. Yi performed chromosome karyotypes analysis and C.Q.OU. performed the statistical analyses. L.S., C.Q.OU., Z.H.C., and C.X. Y. wrote the first draft. L.S. and C.Q.OU. are in charge of the revisions of the paper. All authors approved the final manuscript.

**Compliance with ethical standards** This study was approved by the Medical Ethics Committee of Guangzhou Women and Children's Medical Center.

**Conflict of interest** The authors declare that they have no conflict of interest.

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