Effects of chromosomal abnormalities on pregnancy outcomes in female undergoing artificial insemination with donor’s sperm

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Abstract
This study aimed to evaluate the clinical characteristics, pregnancy outcomes and prognostic factors for pregnancy of female with chromosomal abnormalities (CA) after artificial insemination with donor’s sperm (AID) treatment.

A retrospective case-control study was analyzed by using the data of 29 female patients with CA and 116 controlled patients with normal karyotype (1:4 ratio) who underwent AID cycles at Guangdong Family Planning Special Hospital from January 2011 to December 2017. In all cases, reproductive histories were collected, and the cytogenetic analysis was performed by Trypsin-Giemsa banding and karyotyping. The embryos were fertilized via intracervical or intrauterine insemination. Clinical characteristic variables were compared.

The prevalence of CA was found to be 0.29% in the whole AID population. The live birth rates of CA group and controlled group were 41.4% and 31.0% (P = .29) respectively. Compared to normal karyotype group, patients with CA showed higher rate of primary infertility (93.1% vs 75.9%, P = .049); Multivariate analysis demonstrated that ovarian stimulation (odds ratio, 3.055; 95% confidence interval, 1.421–6.568; P = .004) was associated with adverse pregnancy outcomes in female patients with AID treatment.

For the infertility CA patients who were phenotypically normal, AID was a suitable choice, whereas ovarian stimulation results in an improvement in the pregnancy rate.

Abbreviations: AI = artificial insemination, AID = artificial insemination with donor’s sperm, ART = assisted reproductive technology, CA = chromosomal abnormalities, CI = confidence intervals, ICI = intracervical insemination, ICSI = intra-cytoplasmic sperm injection, IUI = intrauterine insemination, IVF-ET = in-vitro fertilization and embryo transfer, NK = normal karyotype, OR = odd ratios.

Keywords: artificial insemination with donor’s sperm, chromosomal abnormality, clinical characteristics, infertility, pregnancy outcomes

1. Introduction
Infertility was estimated to affect 8% to 12% of reproductive-aged couples worldwide.[1,2] Approximately 50% of infertility cases were caused by genetic defects.[3] Among the various genetic causes of infertility, chromosomal abnormalities (CA) have been proven to be the main factors.[3] Previous researches showed that the prevalence of CA among patients with infertility varied from 1.05 to 17%.[4,5] CA could occur in any pregnancy,[6,7] and arise more frequently among women than men.[6] CA can be divided into autosomal abnormalities and sex abnormalities groups,[6] and different subtypes were associated with distinct clinical characteristics depending on the karyotype and the genetic background of the patients.
In the past 2 decades, more and more CA carriers resorted to assisted reproductive technology (ART), such as artificial insemination (AI), in-vitro fertilization and embryo transfer (IVF-ET), intra-cytoplasmic sperm injection, and preimplantation genetic diagnosis (PGD). Among various ARTs, AI is an easy, cost-effective, and noninvasive technique. As the first-line treatment for couples with male factor or unexplained infertility, AI appears to have high pregnancy rate (24%–80%). and most of the previous investigations on AI were based on patients undergoing artificial insemination by donor’s (AID) or their husband’s sperm. However, so far there has been very limited data regarding the fertility outcomes of CA patients undergoing AID, and important information on the difference between CA and normal karyotype (NK) group in the clinical characteristics and outcomes is lacking.

The present study aimed to investigate the effects of CA on clinical characteristics, pregnancy outcomes, and access the prognostic factors for pregnancy in women undergoing artificial insemination with donor’s sperm. To the best of our knowledge, this is the first study focusing on the effects of chromosomal abnormalities on pregnancy outcomes in female undergoing artificial insemination with donor’s sperm. Our results highlight the importance of detecting CA before the AID treatment, and could help women and gynecologists to make informed decisions about their choices for AID treatment.

2. Methods

This retrospective case–control study was performed using the data of 29 female patients with chromosomal anomalies and 116 control cases (randomly selected from patients with a normal karyotype according to the age at a ratio of 1:4) who underwent AID cycles. Based on their chromosome status, patients were compared regarding clinical characteristic (including age, cycle of AID, tubal patency status, type of infertility, pregnancy outcome, stimulation protocol, and insemination technology), pregnancy outcomes, and prognostic factors for pregnancy of female with CA after AID.

2.1. Patient recruitment

This study was performed in a large cohort of 10122 patients (including chromosomal anomalies and control group) who were treated by AID at Guangdong Family Planning Special Hospital from January 2011 to December 2017. Patients with chromosomal polymorphism were excluded from this analysis. For all included women, the potency of at least one fallopian tube was diagnosed by hysterosalpingography or laparoscopy, and their spouses had azoospermia. According to the regulations of AID indications and contraindications issued by Ministry of Public Health of China, written informed consent were obtained before the AID treatments. Ethical approval was released by the Ethical Committee of Guangdong Family Planning Special Hospital (EC2020.21).

2.2. Karyotype analysis of chromosome G-banding

For patients treated in our center, genetic testing is a routine procedure. Therefore, the karyotype analysis of chromosome G-banding was carried out for all the AID patients included in this study. According to the standard cytogenetic protocols, metaphase chromosomes were prepared from the peripheral blood cultures. Subsequently, the chromosomal anomalies status of the carriers was accessed by Trypsin-Giemsa banding at around 550-band level. Thirty metaphases were analyzed for all the patients, and an enlarged 100 metaphases were investigated if abnormalities or mosaicism appeared. Sex chromosome mosaics occurring at a level <10% were defined as minor mosaicism or low-level mosaicism. CA was defined according to the HGVS guidelines version 15.11 (https://www.HSVS.org/varnomen) and the karyotype description was recorded.

2.3. Sex hormonal level testing and specific antigen measures

For the evaluation of ovarian reserve and detection of additional endocrinological abnormalities, a full baseline hormonal test, including basal follicle-stimulating hormone (bFSH), basal bioactive luteinizing hormone (bLH), basal prolactin (bPRL), basal estradiol (bE2), and basal testosterone (bT), was carried out by highly sensitive electrochemiluminescent immunoassay at the second or third day of menstruation (early follicular phase). In addition, antisperm antibody and endometrial antibody were tested by enzyme-linked immunosorbent assay on the same day with sex hormonal testing.

2.4. Selection of ovulation methods

During natural cycles, AID was used as the initial treatment for ovulating women. For the patients with at least two unsuccessful AIDs by natural cycle and/or patients with ovulation failure, ovulation stimulation was applied, and the cycle of AID treatment was terminated if the number of dominant follicles exceeded four.

2.5. Sperm source and sperm processing

All sperm samples were obtained from the Human Sperm bank of Guangdong Province.

2.6. AID treatment

AID treatment was performed during natural cycles or induced ovulation cycles when the diameter of the dominant follicle was >18 mm and the endometrial thickness exceeded 7 mm. The embryos were fertilized via intracervical insemination (ICI) or intrauterine insemination (IUI). After the AID treatment, routine luteal phase support was established. IVF-ET treatment was usually applied for patients with 3 to 4 unsuccessful AID cycles, or other cycles according to the patients’ practical situation.

2.7. Outcome measures

Serum β-hCG levels were measured 14 days after AID treatment, and transvaginal ultrasound was performed in patients with positive findings for β-hCG 5 weeks after the AID treatment. Clinical pregnancy was considered when gestational sacs were observed on ultrasound. All pregnant patients were followed up until the end of delivery. According to the definition by the WHO, infertility was classified as primary or secondary. Primary infertility referred to women who have been unable to become pregnant or unable to carry a pregnancy to a live birth, whereas secondary infertility was defined as the inability to become pregnant or the inability to carry a pregnancy to a live birth following a previous pregnancy.
2.8. Statistical analysis

Statistical analysis was performed using SPSS version 24.0 (IBM Corporation, Armonk, New York). The association between clinical characteristics and CA status was analyzed using independent samples t test for continuous variables and the Pearson or Fisher-exact test for categorical variables. Odd ratios (OR) and 95% confidence intervals were used to depict the association between the predictors and pregnancy outcomes. Pregnancy success referred to pregnancy with delivery of a healthy baby. The area under receiver-operating characteristic curve was used to determine the best serum basal hormone level (eg, bFSH, bLH, bE2, bT) for predicting pregnancy outcome. Univariate and multivariate analysis (LR backward stepwise) were carried out by binary logistic regression to identify clinicopathological factors that influencing the pregnancy outcomes. Variables with P value <.5 in univariate analysis were included in the multivariate analysis. For all tests, P value <.05 was considered as statistically significant.

3. Results

3.1. Patients’ clinical characteristics

The clinical characteristics of the 145 patients were listed in Table 1. The median age was 33 (range, 22–42), and 320 AID cycles were used for 145 patients’ treatment, the average cycle was 2.26 (range, 1–5 cycle). Compared with normal chromosomes carriers, CA patients (29/145, 0.29%) had higher incidence of primary infertility (93.1% vs 76.7%, P = .049), similar clinical pregnancy rate (41.4% vs 33.6%, P = .434), and similar live birth rate (41.4% vs 31.0%, P = .29). No significant difference was found among the 3 groups in terms of age, cycles of AID, tubal patency status, stimulation protocol, or fertilization method.

3.2. 29 Abnormal chromosomes cases

For the 29 CA carriers aged from 23 to 41 years, the median age at diagnosis was 33. In this subcohort, 20 (69%) presented with a history of primary infertility, and 9 (31%) had a history of secondary infertility; 26 (90%) showed regularity menstrual cycle, and 3 (10%) presented oligomenorrhea. After AID treatment, 12 (41.4%) patients got pregnant and 17 (58.6%) patients were nonpregnant. Then 7 of the 17 nonpregnant patients underwent IVF-ET treatment, and 3 of them got pregnant. Those CA patients underwent 1 to 4 cycles of AID, and the average cycle was 2.44. The live birth rates of CA and control groups were calculated to be (12/69) 17.3% and (36/253) 14.2% (P = .577), respectively. The CA carriers went through menarche between the ages of 11 and 17, and the median menarche age was 14. Detailed information could be found in Table 2.

In the 29 abnormal chromosomes patients, 23 (78.9%) exhibited various degrees of mosaicism: 45,X/46,XX in 31.0%, 45,X/46,XX/47,XXX in 17.2%, 46,XX/47,XXX in 27.6%, and 47,XX+/mar /46,XX in 3.4%. Most of the mosaicism (17/23) patients showed minor mosaicism. Among the 23 mosaicism cases, 10 (45.5%) carriers were pregnant. Additionally, 6 (20.7%) of 29 patients were diagnosed with autosomal chromosome abnormalities, and 2 (33.3%) out of them got pregnant. More details were showed in Table 3.

3.3. Sex hormonal level and specific antigen

The ROC curve was plotted to determine the cutoff value of the hormone. The threshold values of bFSH, bLH, bE2, and bT were calculated to be 8.105 mU/mL, 8.105 mU/mL, 91.93 pmol/L, 0.345 nmol/L, respectively, which were used to evaluate the different hormones’ status (low level, less than the threshold value; high level, no less than the threshold value). Based on the different hormones’ status, patients were compared regarding chromosome status and pregnancy outcomes. The patients’ sex hormonal level and specific antigen of the 145 AID cases were summarized in Table 4. Compared with NK group, no significant difference in terms of bFSH, bLH, bE2, bT, AsAb, or endometrial antibody was observed in CA group.

3.4. Other history diseases of AID patients

No significant difference was found between the CA and NK groups in terms of chromosomes status or pregnancy outcomes, regardless of the history of polycystic ovaries, endometrial polyp, endometriosis, uterine fibroids, pelvic inflammation, hyperprolactinemia, or ovarian tumor (Table 5).

3.5. Predictors for pregnancy outcomes

In multivariate analysis, only ovarian stimulation protocol (hazard ratio, 3.055; 95% confidence interval, 1.421–6.568; P = .004) was found to be independent prognostic factor for adverse pregnancy outcomes in 145 AID patients. All calcu-
lations were adjusted by abnormal chromosomes, ovarian stimulation protocol, bFSH, bLH, and bT (Table 6).

4. Discussion

Infertility, a disease with increasing prevalence,[1,2] has complicated causes and therefore requires complex treatments.[17] Chromosomal abnormalities (CA) were considered as the main genetic causes of infertility.[3] In present research, the live birth rates of CA and control group were (12/29) 41.3% and (36/116) 31.0% ($P = .29$). The live birth rates of CA and control group based on all cycles were calculated to be (12/69) 17.3% and (36/253) 14.2% ($P = .577$), respectively. In most studies,[18–20] the live birth rates of CA were lower than the control group. However, in our study, the live birth rates of CA group were higher than NK group, although the difference was not significant. The possible reasons might be, first, in our study, we focused on infertile patients who conferred to AID treatment. However, other studies were carried out on the infertile patients who referred to other ART (such as IVF-ET, intra-cytoplasmic sperm injection, preimplantation genetic diagnosis).[10,18,21] The patients with normal karyotype usually have better pregnancy outcomes than CA carriers after ART treatment.[21] Second, most of previous reports were carried out on couples,[10,18,20–23]

### Table 2
Clinical characteristics and chromosome karyotype of 29 chromosomes abnormalities patients.

| Age | Chromosome karyotyping | Cycle | Pregnancy outcome | Type of infertility | Menstrual cycle | Menarche |
|-----|------------------------|-------|-------------------|---------------------|-----------------|----------|
| 21–25 | mos 45.X(26)/46.XX [4] | 2     | Nonpregnancy      | Secondary           | Oligomenorrhea  | 15       |
|      | 46.XX,inv(9)p12q21(2) | 1     | Live birth        | Secondary           | Oligomenorrhea  | 13       |
| 26–30 | mos 46.XX [28]/47.XXX [2] | 4     | Live birth        | Primary             | Regularity      | 14       |
|      | mos 47.X[3]/46.XX [62] | 4     | Non-pregnancy     | Primary             | Regularity      | 13       |
|      | mos 47.XXX [3]/46.XX [58] | 3     | Nonpregnancy      | Primary             | Regularity      | 14       |
|      | mos 47.XXX [3]/46.XX [58] | 3     | Nonpregnancy      | Secondary           | Regularity      | 16       |
|      | mos 45.X(4)/46.XX [26] | 3     | Live birth        | Secondary           | Regularity      | 15       |
|      | 46.XX,inv(2)(q31q35) | 1     | Nonpregnancy      | Primary             | Regularity      | 12       |
|      | 47.XX, +mar[23]/46.XX [51] | 3     | Nonpregnancy      | Secondary           | Regularity      | 14       |
|      | 46.XX,inv(7)p11q34 | 3     | Live birth        | Primary             | Regularity      | 12       |
|      | mos 45.X[3]/46.XX [99] | 2     | Live birth        | Primary             | Regularity      | 12       |
|      | mos 45.X[3]/46.XX [47] | 1     | Live birth        | Primary             | Regularity      | 16       |
|      | mos 45.X[3]/46.XX [76] | 3     | Nonpregnancy      | Primary             | Regularity      | 15       |
| 31–35 | mos 45.X(2)/46.XX [48] | 1     | Live birth        | Primary             | Regularity      | 12       |
|      | 46.XX [94]/47.XXX [6] | 2     | Live birth        | Primary             | Regularity      | 15       |
|      | 46.XX,inv(2)p11q13 | 3     | Nonpregnancy      | Secondary           | Regularity      | 14       |
|      | mos 45.X [7]/46.XX [41]/47.XXX [2] | 4     | Nonpregnancy     | Secondary           | Regularity      | 13       |
|      | mos 46.XX [47]/47.XXX [3] | 3     | Live birth        | Primary             | Oligomenorrhea  | 17       |
|      | mos 46.XX [96]/47.XXX [4] | 4     | Live birth        | Primary             | Regularity      | 15       |
|      | 46.XX,inv(1)p13q21 | 1     | Nonpregnancy      | Primary             | Regularity      | 13       |
|      | mos 47.XXX [4]/45.XX [46.XX [88] | 2     | Nonpregnancy      | Secondary           | Regularity      | 13       |
|      | 46.XXX [4]/45.XXX [83] | 2     | Nonpregnancy      | Secondary           | Regularity      | 13       |
| 36–40 | 46.XX,?dup(1)(q11q12),inv(1)p13q21 | 3     | Nonpregnancy      | Secondary           | Regularity      | 12       |
|      | mos 45.X[3]/47.XXX [3]/46.XX [83] | 1     | Nonpregnancy     | Primary             | Regularity      | 15       |
|      | mos 46.XX [48]/47.XXX [2] | 2     | Nonpregnancy      | Primary             | Regularity      | 15       |
|      | mos 45.X[7]/47.XXX [5]/46.XX [88] | 2     | Live birth        | Primary             | Regularity      | 13       |
|      | mos 45.X[9]/46.XX [93] | 2     | Nonpregnancy      | Primary             | Regularity      | 16       |

### Table 3
Different chromosomal abnormalities type of 29 patients.

| Chromosomal abnormalities | Karyotype | No. cases, n (%) | Live birth n (%) |
|---------------------------|-----------|------------------|------------------|
| Sex chromosome abnormalities |           |                  |                  |
| Mosaic                    | mos 45.X/46.XX | 9 (31.0%)     | 4 (44.4%)       |
|                           | mos 45.X/46.XX/47.XXX | 5 (17.2%)    | 2 (40%)         |
|                           | mos 46.XX/47.XXX | 8 (27.6%)     | 4 (50%)         |
|                           | mos 47.XXX,+mar/46.XX | 1 (3.4%)      | 0               |
| Autosomal chromosome abnormalities |           |                  |                  |
| Inversion                 | 46.XX,inv(1)p13q21 | 1 (3.4%)      | 0               |
|                           | 46.XX,inv(2)p11q11q13 | 1 (3.4%)     | 0               |
|                           | 46.XX,inv(2)p31q35 | 1 (3.4%)      | 0               |
|                           | 46.XX,inv(7)p11q34 | 1 (3.4%)      | 0               |
|                           | 46.XX,inv(9)p12q21.2 | 1 (3.4%)    | 1 (100%)        |
|                           | 46.XX,?dup(1)(q11q12),inv(1)p13q21 | 1 (3.4%) | 1 (100%)        |
whereas our study focused on a cohort of female infertility patients. It has been reported that the live birth rate of male CA infertility was usually lower than female. \[23\] Moreover, CA patients showed more primary infertility (93.1% vs 75.9%, \(P = .049\)) than NK group, which is consistent with previous reports.\[24,25\] It has been reported that CA could lead to primary infertility by affecting ovarian development, maturation of oocytes, and fertilization competence.\[24\]

As n useful tool, karyotype testing has been widely applied to investigate CA and their impact on human reproduction.

### Table 4
Sex hormonal level and specific antigen of 145 AID patients.

| Patient Characteristics | Normal Karyotype (n = 116) | Chromosomal Abnormality (n = 29) | Live Birth (n = 48) | No Live Birth (n = 97) | \(P^\ast\) | \(P^{\ast\ast}\) |
|------------------------|---------------------------|---------------------------------|---------------------|------------------------|---------|---------|
| bFSH, mIU/mL           | 6.25 ± 2.08               | 6.67 ± 3.07                     | 6.57 ± 1.89         | 6.22 ± 2.49            |         |         |
| Low level              | 97 (78.2%)                | 27 (21.8%)                      | 43 (34.7%)          | 81 (65.3%)             | .194    | .328    |
| High level             | 19 (90.5%)                | 2 (9.5%)                        | 5 (23.8%)           | 16 (76.2%)             |         |         |
| bLH, mIU/mL            | 4.42 ± 2.56               | 4.94 ± 3.21                     | 4.50 ± 2.35         | 4.54 ± 2.87            |         |         |
| Low level              | 109 (80.7%)               | 26 (19.3%)                      | 47 (34.8%)          | 88 (65.2%)             | .413    | .108    |
| High level             | 7 (70.0%)                 | 3 (30.0%)                       | 1 (10.0%)           | 9 (90.0%)              |         |         |
| bE2, pmol/L            | 114.3 ± 58.82             | 108.71 ± 52.78                  | 120.20 ± 65.89      | 109.71 ± 52.94         |         |         |
| Low level              | 40 (74.1%)                | 14 (25.9%)                      | 21 (38.9%)          | 33 (61.1%)             | .169    | .254    |
| High level             | 76 (83.5%)                | 15 (16.5%)                      | 27 (29.7%)          | 64 (70.3%)             |         |         |
| bT, nmol/L             | 1.05 ± 0.67               | 0.88 ± 0.63                     | 1.11 ± 0.78         | 0.97 ± 0.60            |         |         |
| Low level              | 12 (70.6%)                | 5 (29.4%)                       | 9 (52.9%)           | 8 (47.1%)              | .302    | .064    |
| High level             | 104 (81.3%)               | 24 (18.8%)                      | 39 (30.5%)          | 89 (69.5%)             |         |         |
| AsAb                   | Negative                  | 102 (79.1%)                     | 27 (20.9%)          | 43 (33.3%)             | .740    | .867    |
| Positive               | 14 (87.5%)                | 12 (12.5%)                      | 5 (31.25%)          | 11 (68.75%)            |         |         |
| EmAb                   | Negative                  | 88 (80.0%)                      | 22 (20.0%)          | 39 (35.5%)             | 1.000   | .286    |
| Positive               | 28 (80.0%)                | 7 (20.0%)                       | 9 (25.7%)           | 26 (74.3%)             |         |         |

Values are expressed by mean ± standard deviation or n (%).

* Chromosomal abnormality versus normal karyotype.

† Nonpregnancy and adverse-pregnancy versus pregnancy.

AsAb = antisperm antibody, bE2 = basal estradiol, bFSH = basal follicle stimulating hormone, bLH = basal bioactive luteinizing hormone, bT = basal testosterone, EmAb = endometrial antibody.

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### Table 5
Other disease of 145 AID patients.

| Patient Characteristics | Normal Karyotype (n = 116) | Chromosomal Abnormalities (n = 29) | Live Birth (n = 48) | No Live Birth (n = 97) | \(P^\ast\) |
|------------------------|---------------------------|---------------------------------|---------------------|------------------------|---------|
| Polycystic ovaries, n (%) | No                       | 112 (80.0%)                     | 28 (20.0%)          | 48 (34.3%)             | 92 (65.7%) .171 |
| Yes                    | 4 (80.0%)                 | 1 (20.0%)                       | 0                   | 5 (100.0%)             |         |
| Endometrial polyp, n (%)         | No                       | 109 (79.0%)                     | 29 (21.0%)          | 47 (34.1%)             | 91 (65.9%) .425 |
| Yes                    | 7 (100.0%)                | 0                               | 1 (14.3%)           | 6 (85.7%)              |         |
| Endometriosis, n (%)          | No                       | 111 (79.9%)                     | 28 (20.1%)          | 47 (33.8%)             | 92 (66.2%) .664 |
| Yes                    | 5 (83.3%)                 | 1 (16.7%)                       | 1 (16.7%)           | 5 (83.3%)              |         |
| Uterine fibroids, n (%)         | No                       | 108 (79.4%)                     | 28 (20.6%)          | 46 (33.8%)             | 90 (66.2%) .718 |
| Yes                    | 8 (88.9%)                 | 1 (11.1%)                       | 2 (22.2%)           | 7 (77.8%)              |         |
| Pelvic inflammation, n (%)      | No                       | 115 (79.9%)                     | 29 (20.1%)          | 47 (32.6%)             | 97 (67.4%) .331 |
| Yes                    | 1 (100.0%)                | 0                               | 1 (100.0%)          | 0                      |         |
| Hyperprolactinemia, n (%)       | No                       | 107 (78.7%)                     | 29 (21.3%)          | 45 (33.1%)             | 91 (66.9%) 1.000 |
| Yes                    | 9 (100.0%)                | 0                               | 3 (33.3%)           | 6 (66.7%)              |         |
| Ovarian tumor, n (%)           | No                       | 114 (79.7%)                     | 29 (20.3%)          | 48 (33.6%)             | 95 (66.4%) 1.000 |
| Yes                    | 2 (100.0%)                | 0                               | 0                   | 2 (100.0%)             |         |

AID = artificial insemination with donor’s sperm.

* Chromosomal abnormality versus normal karyotype.

† Nonpregnancy and adverse-pregnancy versus pregnancy.
Particularly, in this cohort, 23 of the 29 CA carriers were identified to be mosaicism. This is consistent with previous reports,[14] in which mosaicism was demonstrated to be the most prevalent anomaly mainly found in women. 73.9% (17/23) of the mosaic carriers showed high proportion of 46,XX, and 10 of the 23 carriers were pregnant. Although female with mosaicism are usually infertile or subfertile, they might have the chance to give birth to normal healthy child by ART.[26] In present study, 14 patients of the mosaicism patients were diagnosed as Turner Syndrome (TS), 64% (9/14) showed 45,X/46,XX, whereas 36% (5/14) displayed 45,X/46,XX/47,XXX. This demonstrated 45,X/46,XX as common karyotypes (15%-23%) in varying degrees of TS mosaicism, which is consistent with previous reports.[27] As has been reported previously, the pregnancy rate increased with the 46,XX ratio of the mosaic carrier,[28] so the type of CA could be used to predict the pregnancy rate.[8] It was suggested that full preconception evaluation of karyotype was needed before AID treatment.[29] The inversion was reported to make structural changes without loss or gain of genetic material, the risk of pregnancy loss in patients with an inversion was found to be lower than those with other CAs.[26] In the present study, inversion of chromosome 1, 2, 7, 9 was identified, as has been frequently observed in previous study.[30,31] Of note, inversion of chromosome 9 is the most common inversion in human chromosomes.[30] Previous researches have demonstrated an increased risk of infertility in carriers with inversion variants.[30,32] In our study, only patients with inversion of chromosome 1 and 9 finally got pregnancy by AID, indicating that inversion 2 and 7 might play a role in the etiology of subfertility. According to previous report, the inversions of chromosome were not related to unbalanced rearrangements in offspring,[33] but certain breakpoints of inversions were associated with reproductive pathology, and thus affect pregnancy outcomes.[34]

Another interesting observation of this work regards the predictors for pregnancy outcomes. After adjusting for abnormal chromosomes, ovarian stimulation protocol, bFSH, bLH, and bT, CA was not found to be correlated with pregnancy failure, which is in contrast to previous studies.[34,35] The possible reasons might be: in our study, the main CAs were mosaic and inversion, which were associated with lower risk of pregnancy loss compared to other CAs[26,28]; the 29 CA patients in this study showed phenotypically normal, and therefore they had higher chance of getting pregnant by AID.

In our study, the AID patients ovulated by natural cycle had lower pregnancy rate with respect to those treated by ovarian stimulation. This is consistent with previous reports in which ovarian stimulation resulted in a higher pregnancy rate in AID patients.[11,36] Ovarian stimulation treatment can increase the number of mature follicles, improve the follicles development, and consequently lead to higher pregnancy rate.[37]

Our results might be affected by the following limitations: this is a retrospective case–control study; the number of patients is limited. Evaluation results showed that we were successful in

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**Table 6**

Analysis of the predictor for pregnancy outcomes in 145 AID patients.

|                     | Univariate analysis | Multivariate analysis |
|---------------------|---------------------|-----------------------|
|                     | P       | OR   | 95.0% CI | P       | OR   | 95.0% CI |
| Abnormal chromosomes|         |      |          |         |      |          |
| Yes vs no           | .292   | 0.638 | 0.276–1.472 | .236   | 0.577 | 0.232–1.433 |
| Age, y              |         |      |          |         |      |          |
| ≥35 vs <35          | .78    | 1.139 | 0.456–2.846 | —       | —     | —         |
| Cycle of AID        |         |      |          |         |      |          |
| >3 Cycles vs ≤3 cycles | .084 | 1.904 | 0.918–3.948 | —       | —     | —         |
| Tubal patency status |         |      |          |         |      |          |
| Unilateral vs bilateral | .55 | 0.779 | 0.344–1.765 | —       | —     | —         |
| Type of infertility |         |      |          |         |      |          |
| Secondary vs primary | .791   | 1.126 | 0.469–2.703 | —       | —     | —         |
| AsAb                |         |      |          |         |      |          |
| Positive vs negative | .867   | 1.1   | 0.359–3.367 | —       | —     | —         |
| EmAb                |         |      |          |         |      |          |
| Positive vs negative | .289   | 1.587 | 0.676–3.723 | —       | —     | —         |
| Fertilization method |         |      |          |         |      |          |
| ICI                 | .374   | Ref. level | —       | —       | —     | —         |
| IUI (IUI vs IC)     | .163   | 2.539 | 0.685–9.408 | —       | —     | —         |
| IUI + ICI (IUI + ICI vs IC) | .967 | 1.02 | 0.396–2.630 | —       | —     | —         |
| Stimulation protocol |         |      |          |         |      |          |
| OSC vs NC           | .009   | 2.586 | 1.271–5.261 | 0.004   | 3.055 | 1.421–6.568 |
| bFSH, mU/mL         | .332   | 1.699 | 0.583–4.953 | 0.092   | 2.806 | 0.844–9.332 |
| High level vs low level | .142 | 4.807 | 0.591–39.101 | 0.104   | 5.868 | 0.695–49.547 |
| bLH, mU/mL          | .255   | 1.508 | 0.743–3.063 | —       | —     | —         |
| High level vs low level | .071 | 2.567 | 0.922–7.149 | 0.096   | 2.621 | 0.843–8.147 |

AID = artificial insemination with donor’s sperm, AsAb = antisperm antibody, bE2 = basal estradiol, bFSH = basal follicle stimulating hormone, bLH = basal bioactive luteinizing hormone, bT = basal testosterone, EmAb = endomethal antibody, IC = intracervical insemination, IUI = intrauterine insemination, NC = natural cycle, OSC = ovarian stimulation cycle.
achieving our research goals in terms of internal validity, whereas the external validity of the present study was limited by selection bias and sample size. Further studies with larger sample size should be conducted to verify the external validity of the present study. Nevertheless, a well-defined cohort of CA patients was recruited and strict inclusion criteria were applied. The included female patients were artificially inseminated using the sperm of the donors instead of their husbands to avoid the influence of the possible anomalies in their husbands. Therefore, we focused on the effects of chromosomal abnormalities on pregnancy outcomes only in female undergoing artificial insemination with donor’s sperm. Moreover, all the data were from one clinical center to ensure the homogeneity of the patients’ clinical data.

In conclusion, mosaics are the most prevalent anomaly in CA patients who showed more primary infertility than NK patients. CA had no significant effect on pregnancy outcomes after carriers receive AID treatment. During the AID treatment, appropriate ovarian stimulation could increase the opportunity for CA patients to get pregnant. The results highly suggest AID, as an easy and economic ART treatment, was a suitable choice for the infertility CA patients who were phenotypically normal (eg, patients with low-level mosaic CA or inversion of chromosome). Our study provides useful information for gynecologists and CA patients to make informed decisions about their choices for AID treatment.

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Author contributions

GS and YL contributed to the conception and design of the study. The clinical data were analyzed and collected by HJ, LC, LL, and QC. YL and TS analyzed and interpreted the data. The manuscript was drafted by YL and revised by GS and TS. All authors read and approved the final manuscript.

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