Effect of advanced paternal age on reproductive outcomes in IVF cycles of non-male-factor infertility: a retrospective cohort study

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Advanced paternal age has been overlooked, and its effect on fertility remains controversial. Previous studies have focused mainly on intracytoplasmic sperm injection (ICSI) cycles in men with oligozoospermia. However, few studies have reported on men with semen parameters within reference ranges. Therefore, we conducted a retrospective cohort study analyzing the reproductive outcomes of couples with non-male-factor infertility undergoing in vitro fertilization (IVF) cycles. In total, 381 cycles included were subgrouped according to paternal age (<35-year-old, 35–39-year-old, or ≥40-year-old), and maternal age was limited to under 35 years. Data on embryo quality and clinical outcomes were analyzed. The results showed that fertilization and high-quality embryo rates were not significantly different (all P > 0.05). The pregnancy rate was not significantly different in the 35–39-year-old group (42.0%; P > 0.05), but was significantly lower in the ≥40-year-old group (26.1%; P < 0.05) than that in the <35-year-old group (40.3%). Similarly, the implantation rate significantly decreased in the ≥40-year-old group (18.8%) compared with that in the <35-year-old group (31.1%) and 35–39-year-old group (30.0%) (both P < 0.05). The live birth rate (30.6%, 21.7%, and 19.6%) was not significantly different across the paternal age subgroups (<35-year-old, 35–39-year-old, and ≥40-year-old, respectively; all P > 0.05), but showed a declining trend. The miscarriage rate significantly increased in the 35–39-year-old group (44.8%) compared with that in the <35-year-old group (21.0%; P < 0.05). No abnormality in newborn birth weight was found. The results indicated that paternal age over 40 years is a key risk factor that influences the assisted reproductive technology success rate even with good semen parameters, although it has no impact on embryo development.

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INTRODUCTION

With the implementation of the “three-child” policy and the increase in divorce/remarriage rates, delayed childbearing has become a societal trend that has resulted in age-related fertility decline. It is generally accepted that female age is negatively correlated with embryonic development and pregnancy outcome. However, the male partner contributes half of the genetic material of the embryo, providing a haploid genome. Spermatozoa have crucial roles related to embryogenesis, which may influence assisted reproductive technology (ART) outcomes; however, studies investigating the effects of paternal age on ART outcomes are limited and controversial.

A survey of 1976 women who controlled for maternal age found that the pregnancy rate was lower (52.9% vs 76.8%) for advanced paternal age (≥45 years) than that for young paternal age (<25 years).1 Several investigators have suggested that the fertilization rate,2–4 embryo quality,1 pregnancy rate,1,3,5–8 live birth rate,5 and miscarriage rate1 are all negatively impacted by advancing paternal age. However, other studies did not observe any effect of paternal age on the rates of fertilization, pregnancy,1,5,7,8 live birth,1,5,8,13,14 or miscarriage.1,3,5,6,11,16,17,18 These discrepancies could be partially explained by the different study protocols or populations used, accounting for confounding factors, such as female age, ART treatment, and semen quality. Most of these results originated from intracytoplasmic sperm injection (ICSI) cycles in which the sperm parameters were abnormal. Limited and inconclusive medical literature exists on the effect of paternal age on in vitro fertilization (IVF) cycles in which most men have good semen parameters.

The purpose of this study was to investigate the potential effect of advanced paternal age on embryo development and clinical outcomes in an IVF setting at the Reproductive Medicine Center, Zhongshan Hospital, Fudan University (Shanghai, China). Strict inclusion and exclusion criteria were used in this retrospective cohort study. First, we chose couples in which the maternal age was <35 years, and all couples were older than 20 years. Second, females with primary or secondary

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infertility diagnosed with tubal disease were included. Third, all the cycles were conventional IVF cycles in which paternal semen parameters of all male partners met the 5th edition of the World Health Organization (WHO) criterion 2010 with minor modification. We hypothesized that reproductive outcomes would be affected by paternal age. The variables that we analyzed were the IVF laboratory outcomes (including the rates of fertilization, cleavage, transferable embryo, and blastocyst formation) and the clinical outcomes (including rates of pregnancy, implantation, live birth, and miscarriage), among other outcomes.

**PATIENTS AND METHODS**

**Patients**

This retrospective cohort study was conducted at the Reproductive Medicine Center, Zhongshan Hospital, Fudan University. Data from all IVF treatment cycles using fresh spermatozoa from January 2017 to December 2019 were analyzed. Repeat IVF cycles for the same couple during this study period were not considered. To avoid sampling bias in the analysis, we studied ART data by performing quality control. The inclusion criteria were as follows: female under 35 years old with normal baseline ovarian reserve (follicle-stimulating hormone [FSH] <10 mIU ml⁻¹, antral follicle count [ AFC ] >8, and anti-Müllerian hormone [ AMH ] >1.1 ng ml⁻¹); conventional IVF cycles with indications for female factors in which the female partners had bilateral tubal obstruction or the absence of both tubes; 18.5 kg m⁻² body mass index (BMI) ≤25 kg m⁻²; and frozen embryo transfer. According to paternal age, the study population was divided into three subgroups: <35-year-old, 35–39-year-old, and ≥40-year-old. IVF was considered only for couples in which the male partner’s semen parameters fell into the following ranges: ejaculate volume ≥1.5 ml; sperm concentration ≥15 × 10⁶ ml⁻¹; total sperm count ≥39 × 10⁶; percent motility ≥40%; progressive motility ≥20% (grade A); morphology (percentage of normal forms) ≥4%; and vitality (percentage of alive sperm) ≥58% with reference to the 5th edition of the WHO criterion 2010 with minor modification. Cases using donor’s sperm were excluded from the analysis. To minimize the influences on the results of various subfertility factors, spouses with genetic diseases were excluded, and females with uterine malformations, infections, endometriosis, polycystic ovary syndrome (PCOS), autoimmunity, endocrine diseases, or metabolic diseases were not included. Men with BMI >30 kg m⁻² or other factors that affect sperm vitality (living habits including smoking, alcohol, and psychological stress) were excluded. Written informed consent was obtained from all couples, and the study was approved by the Ethics Committee of Zhongshan Hospital, Fudan University (approval No. B2020-321R).

**Semen analysis and preparation**

Semen samples were collected by masturbation into a sterile container after 2–7 days of ejaculatory abstinence on the day of oocyte retrieval. After the semen was completely liquefied for 30–60 min, sperm samples were analyzed (volume, concentration, motility, and morphology) following the 5th edition of the WHO criterion 2010 and recorded. In general, raw semen specimens with >5 × 10⁶ total motile sperm were considered adequate for conventional IVF. Sperm were selected by discontinuous density gradient centrifugation (DGC), and the bottom fraction was aspirated, washed with 2 ml of sperm washing medium (SAGE, Trumbull, CT, USA) and centrifuged (Universal 320, Hettich, Tuttingen, Germany) at 300g for 8 min. Motile spermatozoa were recovered from the tube bottom, suspended in 0.5 ml of G-IVF™ PLUS (Vitrolife, Göteborg, Sweden) fertilization medium, and incubated at 37°C for at least 2 h.

**Stimulation protocol and IVF**

For young patients with normal ovarian reserve, controlled ovulation stimulation was performed with the gonadotropin-releasing hormone (GnRH) antagonist (0.25 mg; Cetrorelixum, Merck Serono, SA, Switzerland) protocol. Ultrasound monitoring was performed, and serum estradiol (E₂) levels were measured during ovarian stimulation. When at least two follicles’ diameters ≥18 mm, human chorionic gonadotropin (hCG; Lizhu Pharmaceutical Trading Co., Zhuhai, China) at 5000–10 000 IU was administered to trigger ovulation. Cumulus oocyte complexes (COCs) were retrieved via ultrasound guidance approximately 36 h after hCG administration.

The COCs were subsequently incubated in G-IVF™ PLUS (Vitrolife) fertilization medium droplets (2 COCs per 30 μl drop) covered with paraffin oil in a 5% CO₂ incubator at 37°C. After 4–5 h of incubation, IVF was performed by adding sperm (sperm motility percentage ≥90%) separated by a Percoll density gradient to the fertilization medium droplets (5 × 10⁴ sperm per 30 μl drop) and further incubated. Oocytes with two pronuclei (2PN) were considered normally fertilized 16–19 h after insemination. Embryos were cultured sequentially with G-1™ PLUS and G-II™ PLUS media (Vitrolife) with 5% Serum Substitute Supplements (SSS, Irvine Scientific, Santa Ana, CA, USA) in a tri-gas (6% CO₂, 5% O₂, and 89% N₂) incubator at 37°C. Cleavage-stage embryos were scored and graded on day 3 (based on the number of blastomeres, cell symmetry, and fragmentation) according to Cutting’s criteria. Grade I–III cleavage-stage embryos were identified as transferable embryos. Grade I–II embryos were defined as high-quality embryos, and they met the following criteria: (1) 6–8 cells at 3 days after fertilization; (2) homogeneous blastomeres; and (3) fragmentation <10%. In general, two high-quality cleavage-stage embryos were cryopreserved, and excess transferable embryos were further cultured to day 6. The blastocyst stage was assessed according to Gardner’s criteria. Embryos were frozen by vitrification following standard protocols using a Kitazato vitrification kit (Cryotop Safety Kit, Kitazato, Japan) with high-security vitrification straws (Cryo Bio System, LAigle, France). Embryos with better grades were of higher priority for transfer over the following frozen-thawed embryo transfer (FET) cycles.

**FET cycle protocol**

FET was performed during hormonal treatment cycles in our center. Briefly, from day 3 of the menstrual cycle, patients received oral estradiol valerate (EV; Progynova, Bayer, Leverkusen, Germany) daily. B-ultrasound monitoring was performed approximately 10 days after medication. When the endometrial thickness reached ≥7 mm, vaginal progesterone (60 mg per day) was administered. Cleavage-stage embryos or blastocysts were thawed and transferred on day 3 or day 5 of corpus luteum transformation. After thawing, embryos were cultured for at least 2 h in G-II™ PLUS medium (Vitrolife) with a 5% SSS (Irvine Scientific) droplet at 37°C until transfer.

**Follow-up**

Serum β-hCG was detected 2 weeks after embryo transfer. A clinical pregnancy was diagnosed when a gestational sac and fetal heartbeat were identified via ultrasound approximately 28 days after transfer. The implantation rate was calculated by dividing the total number of gestational sacs and fetal heartbeats detected by the total number of transferred embryos. The miscarriage rate was defined as the number of cases with pregnancy loss before 20 weeks divided by the number of clinical pregnancies. The live birth rate was defined as the total number of cases with at least one baby born divided by the total number of...
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RESULTS

Characteristics and comparison of the study population

Our analytical cohort included 1203 couples who underwent IVF treatment. Of these, 409 were selected to be recruited (Figure 1). After exclusion of cycles with oocyte vitrification (n = 7), donor sperm (n = 11), or total fertility failure (n = 10), 381 IVF cycles were included in the study. Cycles were divided into three subgroups: paternal age <35 years (261 cycles), 35–39 years (72 cycles), and ≥40 years (48 cycles). Of these, 16 cycles had no ET, 2 cycles had no follow-up data, and 363 IVF cycles were followed up. For all cycles, male age was considered to be that on the day of egg retrieval.

The baseline characteristics of the patients are presented in Table 1. The age range was 20–34 years for women and 20–50 years (mean ± standard deviation (s.d.)) as expected, since paternal age was the criterion used for classifying the groups. Maternal average age was similar for the three groups (all P > 0.05). Females in the three groups had BMI within the normal range (18.5 kg m\(^{-2}\) ≤ BMI ≤ 25 kg m\(^{-2}\)). There were no significant differences in terms of the duration of infertility, pattern of infertility, basal serum hormones (FSH, luteinizing hormone (LH), estradiol (E\(_2\)), progesterone (P), and other clinical characteristics among the three groups (all P > 0.05).

In the three paternal age groups, the sperm parameters were within the normal ranges according to the WHO criterion 2010 with minor modifications. In the three paternal age groups, semen volumes, sperm concentrations, sperm motility, progressive motility (PR), and nonprogressive motility (NR) were not significantly different (all P > 0.05). In addition, the numbers of retrieved oocytes and metaphase II oocytes did not significantly differ among the three groups (all P > 0.05).

Sperm characteristics of the cohort

As shown in Table 2, the sperm parameters were within the normal ranges according to the WHO criterion 2010 with minor modifications. Specifically, in the three paternal age groups, semen volumes, sperm concentrations, sperm motility, progressive motility (PR), and nonprogressive motility (NR) were not significantly different (all P > 0.05).

Table 1: Characteristics of the in vitro fertilization cycle in the different paternal age groups

| Characteristic                        | <35-year-old | 35–39-year-old | ≥40-year-old |
|--------------------------------------|--------------|----------------|-------------|
| IVF cycles (n)                        | 261          | 72             | 48          |
| Paternal age (year), mean±s.d.        | 30.7±2.3     | 36.3±1.3a      | 44.4±5.7ab  |
| Maternal age (year), mean±s.d.        | 30.8±2.9     | 31.2±1.8       | 31.6±2.2    |
| Pattern of infertility               |              |                |             |
| Primary infertility, % (xtotal)       | 49.0 (128/261) | 45.8 (33/72)  | 41.7 (20/48) |
| Secondary infertility, % (xtotal)     | 51.0 (133/261) | 54.2 (39/72)  | 58.3 (28/48) |
| Duration of infertility (year), mean±s.d. | 2.8±2.0    | 2.5±1.3        | 2.5±1.6     |
| Maternal BMI (kg m\(^{-2}\)), mean±s.d. | 22.1±1.8    | 22.2±1.7       | 21.6±1.9    |
| AFC (n), mean±s.d.                    | 12.6±4.7     | 12.5±4.8       | 13.6±4.5    |
| Basal FSH (mIU ml\(^{-1}\)), mean±s.d.| 6.8±1.5      | 6.7±1.8        | 7.1±1.8     |
| Basal LH (mIU ml\(^{-1}\)), mean±s.d. | 5.0±2.1      | 5.4±2.4        | 4.9±2.2     |
| Basal estradiol (pmol l\(^{-1}\)), mean±s.d.| 39.6±16.5  | 39.5±17.2      | 40.7±15.6   |
| Basal progesterone (nmol l\(^{-1}\)), mean±s.d.| 0.2±0.1    | 0.1±0.9        | 0.2±0.1     |
| Retrieved oocytes (n), mean±s.d.     | 10.4±6.8     | 9.1±6.2        | 11.0±6.0    |
| Metaphase II oocytes (n), mean±s.d.   | 9.3±6.1      | 8.3±5.8        | 9.5±5.2     |

Table 2: Semen parameters of in vitro fertilization cycles in the different paternal age groups

| Semen parameter                        | <35-year-old | 35–39-year-old | ≥40-year-old |
|----------------------------------------|--------------|----------------|-------------|
| IVF cycles (n)                          | 261          | 72             | 48          |
| Volume (ml), mean±s.d.                 | 2.9±1.2      | 2.6±0.9        | 2.6±1.0     |
| Concentration (x10\(^{6}\)  ml\(^{-1}\)), mean±s.d.| 62.5±20.3   | 66.0±18.7      | 60.6±17.9   |
| Total motility (%), mean±s.d.          | 54.4±10.4    | 53.1±11.2      | 52.2±8.7    |
| Progressive motility (%), mean±s.d.    | 27.7±8.0     | 25.7±8.5       | 25.5±5.6    |
| Nonprogressive motility (%), mean±s.d. | 26.7±6.7     | 27.4±7.1       | 26.7±6.5    |
| Normal morphology (%), mean±s.d.       | 6.3±2.2      | 6.4±1.9        | 7.0±1.8     |

a,b The results are significantly different (P<0.05) compared with those of the <35-year-old group; the results are significantly different (P<0.05) compared with those of the 35–39-year-old group. IVF: in vitro fertilization; s.d.: standard deviation; BMI: body mass index; AFC: antral follicle count; FSH: follicle-stimulating hormone; LH: luteinizing hormone.
Embryo quality and clinical outcomes in IVF cycles

In total, 381 cycles (261 cycles in the <35-year-old paternal age group, 72 cycles in the 35–39-year-old group, and 48 cycles in the ≥40-year-old group) were included in our statistical analysis, as shown in Table 3. Among the different paternal age groups, there were no significant differences in terms of the fertilization rate, transferrable embryo rate, high-quality embryo rate, or blastocyst formation rate (all \( P > 0.05 \)). However, the fertilization rate was slightly lower (73.9% vs 77.2%), while the high-quality embryo rate was higher (44.9% vs 40.0%) in the ≥40-year-old group than in the <35-year-old group. The blastocyst formation rate was higher in the 35–39-year-old and ≥40-year-old groups than that in the <35-year-old group. We found no statistically significant effects of paternal age on fertilization or embryo development among the different paternal age groups when the paternal age was <35 years, 35–39 years, and ≥40 years (all \( P > 0.05 \)).

After exclusion of 16 cycles with no embryo transfer (11 cycles in the <35-year-old paternal age group, 3 cycles in the 35–39-year-old group, and 2 cycles in the ≥40-year-old group) and 2 cycles with no follow-up data, a total of 363 FET cycles were followed up, as presented in Table 4. Of these cycles, the percentages of cleavage-stage embryo transfer cycles, blastocyst transfer cycles, and two-step embryo transfer (cleavage-stage and blastocyst) among the three groups, were not significantly different (all \( P > 0.05 \)). The number of embryos transferred in each of the three groups was also not significantly different (\( P > 0.05 \)). However, we found a statistically significant lower implantation rate for men aged ≥40 years (18.8%) compared to the groups of men aged <35 years (31.1%) and 35–39 years (30.0%; both \( P < 0.05 \)). For men <35 years old, the pregnancy rate was 40.3%; for men of 35–39 years old, the pregnancy rate was 42.0%; but for men ≥40 years old, the pregnancy rate dropped to 26.1%. Chi-squared analysis revealed that there were no significant differences in the pregnancy rate between the 35–39-year-old group and the <35-year-old group (\( P > 0.05 \)). There was a significant decline in the pregnancy rate in the ≥40-year-old group compared with the 35–39-year-old and <35-year-old groups (both \( P < 0.05 \)). A declining trend in the live birth rate was noted in the paternal age subgroups (all \( P > 0.05 \)). In addition, compared with that in the <35-year-old group (21.0%), the miscarriage rate in the 35–39-year-old group (44.8%) was significantly higher (\( P < 0.05 \)). The miscarriage rate in the ≥40-year-old group was also higher, but there was no significant difference (\( P > 0.05 \)). Moreover, there were no significant differences in the perimatur or multiple pregnancy rates among the different paternal age groups (all \( P > 0.05 \)). Ectopic pregnancy was only found in the <35-year-old group.

Birth and newborn outcomes after FET treatment

FET cycles were calculated as the number of enrolled patients who underwent the first FET treatment. Table 5 shows the delivery and newborn characteristics after FET treatment. In total, of 141 pregnancy cycles, 92 singleton infants and 16 twin infants were analyzed. The birth weight of singletons in the ≥40-year-old group significantly decreased compared with that in the <35-year-old and 35–39-year-old groups (both \( P > 0.05 \)), but was within the normal range (2500–4000 g). Regarding the twin birth weight, there was no significant difference between the <35-year-old and 35–39-year-old groups (\( P > 0.05 \)), but all newborns were at the lower limit of normal (2500–4000 g). In addition, in the ≥40-year-old group, there were no twin births. No abnormality in birth weight was found among the three groups. However, due to the limited number of cases, additional data are required before a conclusion regarding the influence of advanced male age (≥40 years) can be drawn.

| Table 3: In vitro fertilization laboratory data of three different paternal age groups |
|-----------------------------------------------|
| **IVF laboratory data** | **Paternal age groups** | **<35-year-old** | **35–39-year-old** | **≥40-year-old** |
| ------------------------ | ------------------------ | --------------- | ------------------ | --------------- |
| IVF cycles (n)           |                         | 261             | 72                | 48              |
| Oocytes retrieved (n)    |                         | 2715            | 616               | 525             |
| Normal fertilization (2PN) |                       | 77.2  (2096/2715) | 77.1  (475/616) | 73.9  (388/525) |
| Cleavage, % (n/total)    |                         | 97.9  (2044/2096) | 97.9  (465/475) | 98.7  (383/388) |
| Effective embryo, % (n/total) |                   | 73.3  (1499/2044) | 77.6  (361/465) | 78.1  (299/383) |
| High-quality embryo, % (n/total) |                  | 40.0  (818/2044) | 41.3  (192/465) | 44.9  (172/383) |
| Blastocyst formation rate, % (n/total) |          | 51.6  (636/1232) | 57.6  (160/278) | 55.5  (127/229) |
| **Note:** IVF: in vitro fertilization; 2PN: two pronuclei |

| Table 4: Clinical outcomes in three different paternal age groups after frozen-thawed embryo transfer treatment |
|---------------------------------------------------------------|
| **Clinical outcome** | **<35-year-old** | **35–39-year-old** | **≥40-year-old** |
| FET cycles (n)       | 248             | 69               | 46               |
| Transferred embryos (n) | 341           | 100              | 64               |
| Cleavage-stage embryo transfers, % (n/total) | 47.5 (162/341) | 58.0 (58/100) | 43.8 (28/64) |
| Blastocyst transfers, % (n/total) | 43.7 (149/341) | 38.0 (38/100) | 53.1 (34/64) |
| Cleavage-stage and blastocyst transfers, % (n/total) | 8.8 (30/341) | 4.0 (4/100) | 3.1 (2/64) |
| Transferred embryos (n), mean±s.d. | 1.4±0.5       | 1.5±0.5         | 1.4±0.5         |
| Clinical pregnancy, % (n/total) | 40.3 (100/248) | 42.0 (29/69) | 26.1 (12/46) |
| Implantation, % (n/total) | 31.1 (106/341) | 30.0 (30/100) | 18.8 (12/64) |
| Live birth, % (n/total) | 30.6 (76/248) | 21.7 (15/69) | 19.6 (9/46) |
| Miscarriage, % (n/total) | 21.0 (21/100) | 44.8 (13/29) | 25.0 (3/12) |
| Preterm birth, % (n/total) | 17.1 (13/76) | 26.7 (4/15) | 22.2 (2/9) |
| Multiple pregnancy, % (n/total) | 9.0 (9/100) | 6.9 (2/29) | 8.3 (1/12) |
| Ectopic pregnancy, % (n/total) | 3.0 (3/100) | 0 (0/29) | 0 (0/12) |
| Birth defects, % (n/total) | 0 (0/76) | 0 (0/15) | 0 (0/9) |

\( a \)The results are significantly different (\( P<0.05 \)) compared with those of the <35-year-old age group; \( b \)The results are significantly different (\( P<0.05 \)) compared with those of the 35–39-year-old group. s.d.: standard deviation; FET: frozen-thawed embryo transfer
Our study revealed that the pregnancy and implantation rates were significantly lower in couples with male partners aged ≥40 years. Our findings are basically consistent with the same results. In addition, some results have suggested that increased paternal age has a detrimental effect on ART outcomes.4,18 Van Opstal et al.21 showed that advanced male age negatively affects the probability of reaching the eight-cell stage at day 3 in couples undergoing IVF treatment. In contrast, we did not find an effect of male age on transferable embryos or high-quality embryos. Of note, in this study, we found that male paternal age ≥40 years with normal semen parameters did not affect blastocyst formation rates. This result contrasts the general belief that the women’s age has an impact on the overall blastocyst rate.39 In addition, some studies have suggested that increased paternal age negatively influences embryo development.3,5 Conversely, other research suggested that increased paternal age did not affect embryo quality at the cleavage stage (days 2–3) but did significantly decrease blastocyst formation.25 The summarized findings were from studies that selected mainly ICSI cycles in which males were oligozoospermic.4,18 In addition, most studies have limitations in that they did not separate IVF cycles from ICSI cycles. These results cannot clearly explain whether semen sperm parameters or male age affects reproductive outcome, as controversial results regarding the effect of male aging on sperm parameters have been reported.5,26

Some studies have suggested that increased paternal age has a detrimental effect on ART outcomes.4,12,13,27–29 Our study revealed that the pregnancy and implantation rates were significantly lower for couples with male partners aged ≥40 years. Our findings are basically consistent with the same results.4 A retrospective study of 1938 conventional IVF cycles showed that male age had a detrimental effect on pregnancy rates in cases of paternal age ≥40 years, but only when women were aged >35 years.30 We did not study women older than 35 years. Due to the small number of cases currently analyzed, more data and larger numbers are required for analysis. In addition, in 2016, a study using the large anonymized Human Fertilisation and Embryology Authority (HFEA) national database collected over two decades demonstrated that there is no evidence to show that live birth and miscarriage occurrence are affected by the sperm donor age of 41–45 years (both P < 0.05), in which the older sperm donor population with normal sperm parameters was selected.31 In a cohort study based on 71 937 couples, no association was found between paternal age and the risk of birth defects. Two studies from a prospective study of 5121 couples and a cohort of 17 000 intruterine insemination (IUI) cycles found that the miscarriage rates in male partners older than 35 years were significantly increased compared with partners of men younger than 35 years after controlling for maternal age.32,33 Our study suggested that the miscarriage rate for paternal age from 35–39 years was significantly higher (P < 0.05) than that for paternal age <35 years, and there were no abnormalities in the birth weights of newborns among the different paternal age groups. Although there was a declining trend in the live birth rate, there was no significant difference among groups. As no birth defects were recorded, a statistical analysis was not possible. Because of the small number of cases in the older group, more neonatal data need to be analyzed.

Most of the previous studies controlled for female age using the oocyte donation model. Despite using young donor eggs in those studies, the results of the studies are also inconclusive.34–36,42 Some suggested that increased paternal age was associated with poor pregnancy outcomes in an oocyte donation model,41 and others suggested that there was no association.14 Because there were confounding uncontrolled variables, the age of the maternal recipients has not been addressed in analyses of many oocyte donating cycles,36 although the age of the oocyte can be controlled. Thus, the effects of oocyte age and recipient age may be distinct. The recipient’s uterine receptivity is a factor that usually cannot be controlled. In addition, the interaction between paternal age and maternal age could not be evaluated, but only the ages of male partners of the recipients and the donors’ ages were evaluated. One study reported that live birth rates declined with increasing paternal age, but this association was greatly attenuated when adjusted for recipient age.29 The results demonstrated a decline in live birth rates with increasing paternal age and an increased incidence of pregnancy loss when controlling for recipient endometrial preparation.2

The mechanism for the decline in the pregnancy and implantation rates and increase in the abortion rate as a consequence of advanced paternal age remains to be explained. Male genetic alterations in germ cells of older fathers could be a factor.4,35 The effect of paternal age on aneuploidy remains debatable. The analyzed result from 11 535 pregnancies obtained by IUI cycles using donor spermatozoa showed that the risk of trisomy 21 for the fetus increased when the donor was 38 years old.36 Previous studies also reported that an older male may contribute to a higher prevalence of aneuploid embryos, which may also explain the increased spontaneous abortion rate in advanced paternal age.37 Moreover, increased DNA fragmentation in sperm has also been linked to a lower clinical pregnancy rate,38 lower embryo quality,39 and higher risk of pregnancy loss.40 Sperm DNA damage has already been shown to be elevated in advanced male age.41 Genetic changes in the sperm of older men, including mutations or alterations in genetic imprinting, can be induced by environmental or biological factors in sperm cells.42 A higher frequency of sperm chromosome numerical and structural aberrations has been described in advancing paternal age.43–45 Cytogenetic analysis of semen samples from donors has demonstrated the increased risk of numerical and structural aberrations in males of 59–74 years old compared with males 23–39 years old.44 Changes in sperm epigenetic modifications such as methylation have also been

Table 5: Birth and newborn characteristics after frozen-thawed embryo transfer treatment

| Variable                  | <35-year-old | 35–39-year-old | ≥40-year-old |
|---------------------------|--------------|----------------|-------------|
| Pregnancy cycles (n)      | 100          | 29             | 12          |
| Live births (n)           | 82           | 17             | 9           |
| Singleton babies (n)      | 70           | 13             | 9           |
| Male babies (n)           | 34           | 12             | 6           |
| Female babies (n)         | 36           | 1              | 3           |
| Birth weight of singletons (g), mean±s.d. | 3370±500 | 3530±410 | 2920±790* |
| Twin babies (n)           | 12           | 4              | 0           |
| Male babies (n)           | 6            | 3              | 0           |
| Female babies (n)         | 6            | 1              | 0           |
| Birth weight of twins (g), mean±s.d. | 2590±200 | 2500±310 | None        |

*The results are significantly different (P<0.05) compared with those of the <35-year-old group; a the results are significantly different (P<0.05) compared with those of the 35–39-year-old group. s.d.: standard deviation.
shown to be related to male infertility. These findings might point to the possible influence of sperm DNA on ART outcomes, even when sperm parameters are normal (as in this study).

CONCLUSIONS
Similar to maternal age over 35 years, a paternal age of 40 years has been proposed as the cutoff age after which poor reproductive outcomes and problems in offspring likely occur, and paternal age is a key risk factor that influences infertility even with good semen parameters. Sperm parameters may not reflect sperm function, and the contribution of advanced male age to sperm DNA integrity and sperm genetic and epigenetic states should be considered. The American Society for Reproductive Medicine has established age of 40 years as the upper age limit for sperm donors.

This study has limitations due to its monocentric nature and small sample sizes. This research is a retrospective study in which single-factor data analysis was performed. Other factors that affect the vitality of sperm (living habits including smoking, alcohol, and psychological stress) were not included in the scope of this study. Our preliminary results remain indicative and worth verifying in larger cohorts. Additional prospective studies are needed to further investigate male age and its potential effects on reproductive outcome.

AUTHOR CONTRIBUTION
XD, SYL and HJS contributed to the current study hypothesis and modifications of the manuscript. XD and SYL contributed to clinical diagnosis and oversaw patient recruitment. XML and YBL performed the statistical analyses and contributed to the writing of the manuscript. XML, YBL, XC, DDZ, and ML contributed to data collection. TCZ contributed to the information register. All authors read and approved the final manuscript.

COMPETING INTERESTS
All authors declare no competing interests.

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## Supplementary Table 1: Logistic regression analysis for clinical pregnancy outcome in the study

| Independent variables            | OR (95% CI)       | P    |
|---------------------------------|-------------------|------|
| Maternal age (year)             | 0.947 (0.872–1.029) | 0.197|
| BMI (kg m\(^{-2}\))             | 0.990 (0.889–1.103) | 0.856|
| Duration of infertility (year)  | 1.031 (0.917–1.160) | 0.607|
| Number of transferred embryos per cycle | 0.970 (0.626–1.505) | 0.893|

BMI: body mass index; OR: odds ratio; CI: confidence interval