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ASSISTED REPRODUCTION TECHNOLOGIES

# Effect of male age on the outcome of in vitro fertilization: oocyte donation as a model

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**Abstract**
*Purpose* To assess the influence of male age on the outcome of oocyte donation cycles.
*Materials and methods* A total 103 oocyte donation cycles of 70 couples (male aged 26 to 57) were examined, all of which were performed with conventional in vitro fertilization using fresh ejaculation sperm. Main outcome measures were fertilization rate, clinical pregnancy, live birth rates and pregnancy loss.
*Results* A total 122 cryopreserved embryo transfer were performed, resulting in 34 cycles resulted in clinical pregnancy and 27 live births. No significant correlation was found between male age and fertilization rate. No significant difference was found in male age between the patients who achieved clinical pregnancy and live birth and those who did not. All the pregnancy loss occurred in cycles where the male was older than 37, however, when the cycles were divided into two groups according to whether or not male age older than 37, no statistically significant difference was not found in pregnancy loss rate.
*Conclusions* Aging of the male has no significant impact on fertilization, pregnancy or live birth in oocyte donation cycles, but may be associated with pregnancy loss.

**Keywords** In vitro fertilization · Live birth rate · Male age · Oocyte donation · Pregnancy loss

*Capsule* No significant effect of male age was found on fertilization, pregnancy or live birth in 103 oocyte donation cycles from a Chinese population.

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## Introduction

Today, more and more couples tend to delay childbearing toward later stages in their lives, and as a result, more concerns are now given to the effect of aging on the fecundity of couples. The contribution of advancing female age to pregnancy outcomes is well documented, yet knowledge on effects of male age is much less [1, 2]. Studies have shown that semen parameters, such as volume [3], total sperm count [4], sperm DNA integrity [3, 5] and so on, would diminish with the increase of male age, which may undermine male fecundity. However, semen parameters are only indirect indicators of male fecundity and cannot reflect the effect of aging on male fecundity directly. Meanwhile, for some reasons, female age is highly correlated with male age, and as a result, it is difficult to evaluate the relative contribute of aging on male fecundity by examining the reproductive outcomes in the whole population or in couples undergoing infertility treatment. Oocyte donation program is a unique assisted reproductive technology in which all the oocytes are collected from young women, while sperm are from men of different ages. Thus, it may be used as a good model to analyze the relationship between male age and reproductive outcomes with less confounding from female age. The aim of this study was to evaluate the effect of male age on reproductive outcome by examining 103 oocyte donation cycles from a Chinese population.

## Materials and methods

### Population

This retrospective study was approved by the institutional Review Board of Tongji Hospital, Wuhan, China. One

hundred and three cycles of 70 couples undergoing oocyte donation-in vitro fertilization program at the Reproductive Medicine center of Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China, from January 2004 to December 2009 were involved. The mean age of recipients was 39.8±6.9 (26 to 53), all the recipients had a diagnosis of diminished ovarian reserve, poor response to ovary simulation, chromosome aberration, or age greater than 42 years.

The mean age of male partners was 41.5±6.7 (26 to 57). Fresh sperm samples were used in all the involved cycles. Oocyte donation cycles using cryopreserved sperm and the cycles in which intracytoplasmic sperm injection was used were not included in this study.

According to Chinese law, only the women undergoing ovary stimulation because of infertility were allowed to donate their redundant oocytes to other patients who need donor oocytes. All the donors in this study were less than 37 years old at the time of oocyte retrieval and met the screening criteria as outlined by the Ministry of Health of the People's Republic of China. All donors had normal FSH concentrations (<10 mIU/mL), a basal antral follicle count >12, and normal gonadotropin responsiveness with peak E2 >pg/mL. They had their serum HIV antibody examined before and 6 months after oocyte retrieval, and both of the results were negative. All the involved donors achieved clinical pregnancy in the current or following IVF-ET cycles.

### In vitro fertilization

A long ovarian stimulation protocol was applied to all of the donors. Briefly, the donors were given 0.1 mg GnRHa (Ferring, Kiel, Germany) daily from midluteal phase of last menstrual cycle and 150 IU recombined human FSH (Serono, Geneva, Switzerland) 3 days after menstrual onset until oocyte retrieval. A single 10,000 IU dose of recombined HCG (Serono, Geneva, Switzerland) was administered when Ultrasonographic criteria for follicular maturity were met. Thirty-six hours after HCG administration, oocyte retrieval was performed by transvaginal ultrasound–guided follicular aspiration.

Ejaculated semen samples were obtained by masturbation after abstinence of 2 to 7 days on the day of oocyte retrieval. After liquefaction, the samples were evaluated according to WHO criteria (1999) [6]. Then the samples were prepared by a swim-up purification step. Specimens with more than 2 million motile spermatozoa per ejaculate and abnormal morphology <95% were considered to be proper for conventional IVF. The oocytes were inseminated with 50,000 to 100,000 progressive motile sperm 4 to 6 h after oocyte retrieval. Specimens not suitable to conventional IVF were referred to ICSI and the cycles were not included in this study.

Fertilization was evaluated 16 to 18 h after insemination by the confirmation of two distinct pronuclei. The embryos were then transferred into culture medium (G-1, Vitrolife AB, Sweden); embryonic division and morphology were evaluated every 24 h until 3 days after oocyte retrieval when embryos were considered suitable for cryopreservation by vitrification with cryotop (Kitazato, Japan). All the usable embryos were cryopreserved in liquid nitrogen. The embryos were considered to be safe to transfer only when a second negative HIV antibody screening result from the donor was acquired.

### Embryo transfer

Hormone replacement cycles were adopted in the recipients to prepare the endometrial. Briefly, from the fifth day of the menstrual cycle, 4 mg of estradiol valerate was given orally every day, and from the 9th day, the dose of estradiol valerate was increased to 6 mg. On the 13th day, thickness of endometrial was examined by ultrasound and the dose of estradiol valerate was modified according the development of endometrial and progesterone was added when the endometrial thickness was suitable. All recipients' endometrial thickness was larger than 8 mm before embryo transfer.

Fertilization rate was calculated as the ratio of number of embryos to the number of mature oocytes. Plasma beta-hCG was measured 14 days after embryo transfer, and a rising HCG concentration indicate the establishment of pregnancy. Clinical pregnancy [7] (CP) was defined as a pregnancy diagnosed by Ultrasonographic visualization of one or more intrauterine gestational sac with heart beat about 4 weeks after embryo transfer. Clinical pregnancy rate [7] was calculated as the number of clinical pregnancies expressed per 100 oocyte donation cycles. Spontaneous pregnancy loss [7] was defined as the loss of a clinical pregnancy before 20 completed weeks of gestational age. Pregnancy loss rate was calculated as the number of pregnancies loss in per 100 pregnancies.

### Statistics

Statistical analyses, including student's *t* test, Chi-squared test and Pearson correlation, were carried out with SPSS 10.0 (SPSS, Chicago, IL). All hypothesis testing was two-sided with a probability value of 0.05 deemed to be significant.

## Results

Our patient population consisted of 70 patients undergoing 103 donor oocyte cycles. The mean age of the recipients was 39.8±6.9 (26 to 53). And the mean age of the male partners

was 41.5±6.7 (26 to 57). The mean age of the donors was 27.2±3.5 (22 to 37). The mean number of mature oocytes donated per cycle was 6.3 (5 to 10) and the number of usable embryos per cycle was 3.8 (1 to 8). The average fertilization rate was 78.8%±18.6% (20% to 100%). A total 125 cryopreserved embryo transfer cycles were performed and clinical pregnancy was achieved in 34 recipients, resulting live birth in 27 couples.

No significant correlation was found between male age and fertilization rate ($r$=−0.003, $P$=0.977). No significant difference was found in male age, recipient age or the donor age when the cycles were divided into two groups according to whether a clinical pregnancy was achieved, or according to whether a live birth was achieved (Table 1)

Altogether there were 7 clinical pregnancies resulted in spontaneous abortion in the period of 8th to 12th weeks after embryo transfer, all of them occurred in cycles where the male partner was older than 37. However, no statistically significant difference was found in pregnancy loss rate between the groups ($P$=0.075), when the men were divided into two groups according to whether or not older than 37. No significant difference was found between the groups in clinical pregnancy rate or in live birth rate either ($P$=0.790 and $P$=0.486, respectively) (Table 2).

**Table 2** Pregnancy outcomes in different male age groups

| Male age | CP rate | Live birth rate | Pregnancy loss rate |
|---|---|---|---|
| ≤37 | 31.0% (9/29) | 31.0%(9/29) | 0%(0/9) |
| >37 | 33.8%(25/74) | 24.3%(18/74) | 28.0%(7/25) |
| *P* value | 0.790 | 0.486 | 0.075 |

## Discussion

In oocyte donation cycles, all oocytes involved were collected from donors less than 37 years old. In addition, although undergoing infertility treatment, all donors achieved the clinical pregnancy in the current or following ART cycles, which could confirm their fecundity. Thus, the influence of oocyte quality on the outcomes of ART was minimized. On the other hand, many studies have shown that the age of recipients has little impact on embryo implantation and clinical pregnancy in oocyte donation cycles. Consequently, sperm quality would be one of the most critical factors relating to outcomes of oocyte donation cycles. And the impact of age on male fecundity could be evaluated by analyzing in vitro fertilization rate and pregnancy of men with different ages without the interference of female age.

In this study, no correlation between male age and outcomes of in vitro fertilization was found. Also, when the cycles were divided into two groups according to whether a clinical pregnancy was achieved, or according to whether a live birth was achieved, no significant difference was found in male age between the groups, indicating that male aging would not lead to severe influence on fertilization or embryo development in vitro, which was similar to studies of other authors [8–10], but disagreed with founding of other authors [11, 12].

Some studies from Europe and USA found that the increase of male age may lead to elevation of miscarriage rate after ART [4, 11–13]. We also found that male aging may be related to the increase of pregnancy loss in the first trimester of pregnancy. In the patients involved in this study, early pregnancy loss occurred only in couples where the males were older than 37. Given the founding that sperm DNA integrity would decrease with the increase of male age [5] and may be related to pregnancy loss [14, 15], and the fact that the small sample size of this study may obscure the actual effect of male age, the relationship between male age and pregnancy loss needs to be verified by further studies, even though the difference of pregnancy loss rate between the age groups was not statistically significant.

To our knowledge, this is the first report about the impact of age on the male fecundity using an oocyte donation model in Asian population. It was found in this study that advancing male age may have little impact on the outcomes of oocyte donation cycles, such as in vitro fertilization rate and clinical pregnancy, but may be related to early pregnancy loss. However, as a retrospective study, the sample size of this study is relatively small, and further studies, especially prospective studies with larger sample size, are needed.

**Table 1** Age of male, recipient and donor in different groups according to CP and live birth

| Age(yr) | Groups divided according to CP | | | Groups divided according to live birth | | |
|---|---|---|---|---|---|---|
| | CP | Non-CP | *P* | Live birth | Non-live birth | *P* |
| Male | 41.9±6.3 | 41.6±6.8 | 0.738 | 41.3±6.6 | 41.6±6.8 | 0.870 |
| Recipient | 39.8±6.7 | 39.8±7.1 | 0.993 | 39.4±6.6 | 39.9±7.1 | 0.739 |
| Donor | 27.6±2.9 | 26.9±3.7 | 0.865 | 27.4±3.1 | 27.1±3.6 | 0.679 |

*CP* Clinical pregnancy

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