Association of maternal and cycle-related factors to pregnancy and neonatal outcome when excluding paternal factors by using donor sperm: a retrospective cohort study

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Running title: Maternal and cycle-related factors to fertility

Abstract

Objective: Find out the maternal and cycle associated factors that affect the obstetric outcomes when excluding the paternal effect.

Design: A retrospective cohort study.

Setting: Public fertility center.

Population: 1098 couples that received fresh IVF/ ICSI cycle with donor sperm.

Methods: The associations of maternal factors and cycle-related parameters with obstetric outcomes were depicted as adjusted odds ratios (aOR) / adjusted β and 95% confidence intervals (CI).

Main outcome measures: Obstetric outcome.

Results: Women over 35 years had a distinctly decreased pregnancy and live birth rate (aOR 0.904; 95% CI 0.317--2.584 and aOR 0.905; 95% CI 0.398--2.768). Abortion and multiple ART-cycles were linked with increased risks of pregnancy failure, the oocyte retrieval and transferred embryo numbers improved pregnancy incidence. Blastocyst transfer increased both the probability of pregnancy and live birth (aOR 1.77; 95% CI 1.035--3.026 and aOR 1.364; 95% CI 1.041--1.788). For newborn, the negative contribution of transferred embryo number to birth weight and length was observed (adjusted β -0.30; 95% CI -0.533 to -0.277 and adjusted β -0.197; 95% CI -0.789 to -0.332). Female BMI and endometrial thickness on hCG day both had a positive effect on birth weight and length.

Conclusions: Oocyte number, transferred embryo number, and blastocyst transfer were positively associated with pregnancy or live birth. Females over 35 had an abortion, and multiple ART cycles experience linked to failure pregnancy. The transfer of multiple embryo poses a threat to newborn body weight and length, while higher mother BMI and endometrial thickness had positive influence.

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Tweetable abstract: Age, obstetrics history were negatively linked to pregnancy and newborn; No. of oocyte/embryo，and embryo type were positive.

Keywords:
Donor sperm, Maternal factors, Cycle-related factors, Obstetrics outcomes

Introduction

In 1784, Spallanzani first established that the physical contact between the oocyte and the sperm was the prerequisite of embryo development (Radhey Shyam Sharma, 2018). Since then, a serious of pioneering efforts on animals were done to establish artificial insemination as a practical procedure (W. Ombelet and J. Van Robays, 2015). However, the real beginning of assisted reproduction technology (ART) in humans appeared until 1943 (A F Guttmacher, 1943). With the development of semen preparation techniques, finally, in 1978, Steptoe and Edwardsthe made a breakthrough that the first baby of in-vitro fertilization (IVF) was born. From then on, the real start of a new era of ART began.

It is a comprehensive awareness that the physiological changes that occur in women are potentially detrimental to fertility, pregnancy, and infant because women undertake a more critical role in fertility, from providing oocyte to maintain nutrition and provide a house for fetal development. There were some researches involved in the effect of maternal factors on fertility, such as age; it was well-known that advanced female age caused poor fertility include reduced ovarian reserve and decreased oocyte/embryo competence due to increased incidence of aneuploidies and decreased mitochondrial activity (Danilo Cimadomo, 2018). Female BMI was positively associated with pregnancy hypertensive disorder and gestational diabetes mellitus but negatively related to preterm birth (M A Q Mutsaerts, 2014). Maternal obesity led to a wide range of structural anomalies during fetal development (Katherine J Stothard, 2009). The lifestyle, such as female smoking, resulted in delayed implantation and increased preterm birth rate (A M Z Jukic et al, 2011). However, when investigating the effect of maternal factors on fertility, none of these studies exclude the paternal contribution to embryo development, pregnancy outcome, and neonatal conditions. Paternal conditions also contribute to fertility and offspring. According to data from over 40 million births at Stanford University, advanced paternal age increased pregnancy and infant risks because of sperm DNA mutations (N Phillips, 2019; Stacy Colaco and Denny Sakkas, 2018). Besides, an unhealthy lifestyle, like smoking, impaired male fertility through increasing germ cell DNA damage, and aneuploidies (Marc A Beal, 2017). In general, maternal and paternal factors both affect fertility.

Using donor sperm was firstly documented in the early 1880s (Gregoire AT and Mayer RC, 1965). Until 1953, the first infant reported conceived with frozen donor sperm was born (Steinberger E, 1973). The appearance of donor sperm technology beneficial those nonobstructive azoospermia and oligospermia couples who experienced failure of testicular or epididymal surgical specimen (N Rives, 2012). In the meaning while, donor sperm also provides an ideal opportunity to rule out

possible male factors that affect embryo development, pregnancy, and neonatal condition as the donor sperm has nearly equal good quality.

The purpose of the current study was to determine the potential maternal factors that affect the pregnancy and obstetrics outcome in donor cases. Unlike traditional studies that not exclude the male factors when calculating the female effect on ART, our research aims to provide more precise and valuable parameters for clinical reference.

Material and method

Study population and design

This study was a retrospective cohort study from the Northwest Women's and Children's Hospital, Xi'an, People's Republic of China. Our center has a unique sperm bank in the northwest of China. The study was approved by Ethics Review Board of the hospital. The participants signed the informed consent. We studied obstetrics and neonatal outcome of a total of 1098 fresh embryo transfer donor sperm cycles in our ART center with a complete record under either IVF or ICSI manner between Jan 2015 and Dec 2019. Baseline characteristics, cycle characteristics, and obstetric and neonatal data were extracted from the hospital's electronic medical record. All study data were collected by authorized staff and stored in a restricted directory on the hospital's network system.

Associated parameters: Infertility reason of recipient males, the semen parameter of donors were analyzed. The maternal factors such as female age, infertility type, infertility years, past obstetric history, body mass index (BMI), endometrial thickness on hCG trigger day, gained oocyte number, gonadal hormone concentration was considered. Also, cycle-related parameters such as cycle number, fertilization type, embryo type, transferred embryo number were included. The baseline level of these parameters and the association to obstetrics and neonatal outcome were calculated and depicted.

Inclusion criteria: Fresh IVF or ICSI cycle with donor sperm, women age from 20 to 50, regular menstrual cycle (interval 25–35 days) with at least one year of infertility.

Exclusion criteria: Freeze embryos cycles, conventional IVF, or ICSI without donor sperm, artificial inseminations with donor sperm (AID).

Donor sperm processing

The donor sperm processing was according to the primary guidelines of anonymous sperm donor selection, published by the National Health Commission of the People's Republic of China (http://www.nhc.gov.cn/). (I) The age of male donors should be between 22 and 45; (II) Only donors with the good physical, psychological condition and have no history of genetic disease of themselves and also their family members should be considered; (III) Liquefaction time should be less than 60 minutes, sperm concentration $\geq 60\times10^6$/ml, the percentage of progressive sperm (PR) ≥60% and

percentage of normal morphology >30%; (IV) post-thaw PR sperm viability should ≥40%, and PR sperm content should not less than 12×10^6/ml; (V) potential donors must undergo laboratory testing to exclude the sexually transmitted infections and genetic diseases, including; HIV-1 and -2, syphilis, gonorrhea, chlamydia, mycoplasma, hepatitis B and C, cytomegalovirus, toxoplasma gondii, rubella virus, herpes simplex virus types 1 and 2, and karyotype analysis. Only when donors passed all tests, their semen samples were cryopreserved and stored for at least a 6-month quarantine period to allow for HIV re-screening before being real qualified donor semen. After that, the donation process began, and one semen sample provided to at most five female recipients.

Ovarian stimulation and embryo transfer

A combination of GnRH agonist/GnRH antagonist and recombinant FSH protocols were used as an ovarian stimulation protocol. When ultrasound monitoring found the patient had three follicles large than 18 mm diameter, HCG was administered to trigger ovulation. Oocyte retrieval was performed 36h after HCG injection by ultrasonography-guided aspiration. Then the gained oocyte was fertilized with donor sperm at 5000 sperm per oocyte by IVF or one donor sperm per oocyte through Intra cytoplasm sperm injection (ICSI).

The fertilized one-cell embryo was cultured in G-1TM PLUS and then G-2TM PLUS (Vitrolife) medium. Day 1 morning checked the successful fertilization sign of two pronuclei (2PN), Day 3, and Day 5 morning assessed the cleavage stage embryo and blastocyst quality by morphology grading. On Day3 except for the grade IV embryos (2--3 blastomeres, uneven and nonhomogeneous blastomeres ≥3, > 20% cytoplasmic fragmentation) were unavailable, embryos of other grades were used for further culture to form blastocyst or transferred directly according to the patient's willing. On Day 5 morning, the quality of the blastocyst was re-evaluated. Embryos of grade III were defined as the blastocele enlarge to the whole embryos; grade IV was classified as enlarged blastocele with a diameter over than original embryo, and also thinner zona pellucida; grade V referred to the status that trophectoderm hatch out but not fully released, grade VI indicated embryos that break entirely away from zona pellucida. Embryos that been accessed into the above four grades were qualified for transfer. The delayed development embryo below-grade III would still in culture for at most one day further.

Statistical analysis

Descriptive statistical analysis was performed on the main maternal and ART characteristics. Continuous data were presented as the number of cases, the mean value ± standard deviation (SD). Categorical data are presented as percentage (%). χ^2 test or Fisher exact test for categorical variables, and ManneWhitney U test or Student t test. Binary logistic regression and multivariate linear regression analysis adjusted by covariates were used to identify variables related to clinical pregnancy, live birth,

neonatal weight, and length, and indicated as adjusted Odds ratios (aOR) or adjusted β and 95% confidence intervals (CI). P < .005 "*"; P < 0.01 "**"; P < 0.001 "***".

Results

Maternal demographic

The infertility reason for male (Table S1) and the baseline semen parameter of donor sperm (Table S2) were showed in supplementary material. The demographic characteristics of 1098 female recipients were included in TableI. According to age, the females were divided into four groups 20-25, 26-30, 31-35, 36-50. The inter-group differences of various maternal factors were depicted by comparing with the age of 20-25. From TableI, the primary female age that received donor sperm administration was 26-30, which occupied 52.09%. Among the whole population, primary infertility holds the major reason for infertility; however, with the increasing of maternal age, the proportion of secondary infertility increased, especially in the age group over 35, primary and secondary infertility achieved almost equal. The infertility years was significantly increased from 2.5±1.41 in age between 20-25 to 13.86±6.03 in age over 35. Accordingly, the cumulative gravidity and the parity were increased evidently with advanced maternal age, while the cumulative abortion proportion was decreased with maternal age from 80.77% to 21.05%. This result indicates, on the one hand, the prolonged reproductive period in the advanced maternal age group increases the chance of pregnancy and delivery; on the other hand, maybe because of treatment during the reproductive period, the miscarriage rate is significantly decreased in advanced age group compare with younger ladies. Regarding physiological characteristics, female BMI was increased in older women over 35 years old at 24.1±2.65 kg/m^2. The endometrial thickness on hCG trigger day decreased gradually with age; females in 36-50 had the thinnest endometrium of 11.2±2.33 mm. Interestingly, the gonadal hormone concentration among each group showed no differences. In brief, Females who accept donor sperm therapy always have an age-dependent increase in secondary infertility proportion, infertility years, complex past obstetrics history, BMI, and thinner endometrial thickness on hCG trigger day.

Embryo development, pregnancy, and delivery

As shown in TableII, most females were under their first donor ART cycle in each age bracket, but the proportion of people who experienced more than one cycle was gradually increased with age. The oocytes retrieval decreased with age increased, from 10.54±4.271 to 7.9±4.39, indicating the age-dependent impairment of ovarian response to super-ovulation. The fertilization rate was equal in each age group, almost 80%. Day 3 cleavage embryos and Day 5 blastocysts were both transferred with no noticeable amount differences among each age except in age above 35. In this group, more Day 3 cleavage embryos were transferred maybe because less available embryo could develop to the blastocyst stage in the advanced maternal age group. Around 40% of people transferred one embryo, the rest 60% transferred two embryos, scarce people received three embryos. The clinical pregnancy rate was 67.89% in age 20-25, 68.18% in age 26-30, and 70.36% in 31-35 without a difference, but when females

age over 35 years, the pregnant rate significantly decreased to 53.01%. In accordance with the pregnant rate, the live birth rate also reduced from 58.42% in the age group 20-25 to 31.33% in age over 35; In women younger than 35, the live birth rate had no significant difference. The birth weight and length had no apparent differences among each age group. In summary, the cycle number, oocyte number, D3 embryo proportion, pregnant rate, the live birth rate have a significant difference in the age group 35-50 compare with other ages.

Prognostic factors associated with pregnancy and live birth

Relationships between maternal or cycle-related factors and clinical pregnancy or live birth were presented in TableIII. In the left column were the maternal factors and cycle associated factors, followed by the count of subjects in each stratum. These were evaluated by the fully adjusted models assessing the aOR of each element to clinical pregnancy and live birth. After adjustment for potential confounders (Female age, infertility type, infertility years, past obstetric history, female BMI, endometrial thickness on hCG day, retrieved oocyte number, ART cycle number, fertilization type, transferred embryo type, transferred embryo number). As observed, relative to the reference group of women aged 20-25 years, women aged above 35 years had a distinctly higher risk of low clinical pregnancy and live birth (aOR 0.904; 95% CI 0.317--2.584 and aOR 0.905; 95% CI 0.398--2.768). In contrast, the infertility type, the infertility years, did not affect clinical pregnancy and live birth in the statistic. Regarding past obstetrics history, when taken no prior abortion history as a reference, the aOR of abortion once to clinical pregnancy was 0.571; 95% CI 0.332--0.983, which means prior abortion may adversely affect the fertility competent of women. Concerning cycle-related parameters, the risk of experiencing two cycles to pregnancy was aOR 0.434; 95% CI 0.250--0.753 compared with the first ART cycle, cycle number change had no effect on live birth. Besides, the oocyte retrieval number improved the incidence of clinical pregnancy but not live birth, aOR of clinical pregnancy was 1.053; 95% CI 1.005--1.104. For the cycle-related factors, in the fully adjusted model, Day 5 blastocyst both significantly increased the probability of successful pregnancy and live birth (aOR 1.77; 95% CI 1.035--3.026 and aOR 1.364; 95% CI 1.041--1.788), as did transferred embryo number (aOR 2.06; 95% CI 1.279--3.32) for clinical pregnancy but not for live birth. The risk estimates did not vary appreciably across models when there was an adjustment for multiplicity. In summary, females aged over 35, prior abortion history and multiple preceding ART cycles increase the risk of clinical pregnancy failure, while the retrieved oocyte number, embryo number, and blastocyst transfer other than cleavage-stage embryo improve the incidence of clinical pregnancy. In contrast, live birth is negatively impaired by advanced maternal age and enhanced by blastocyst transfer.

Prognostic factors associated with the birth length and weight

Neonatal body conditions were indicated in TableIV. The adjustment of confounders was like that in pregnancy and live birth. Transferred embryo numbers pose a significantly higher risk of birth weight loss (adjusted β -0.303; 95% CI -0.533 to -

0.277), as well as birth length decreasing (adjusted β -0.197; 95% CI -0.789 to -0.332). In contrast, female BMI contributes positive effect on birth weight (adjusted β 0.146; 95% CI 0.014 -- 0.047) and birth length (adjusted β 0.096; 95% CI 0.007--0.078). To endometrial thickness on hCG trigger day, the endometrial thickness was positively correlated with birth weight and length (adjusted β 0.098; 95% CI 0.002 -- 0.241 and adjusted β 0.086; 95% CI 0.004 -- 0.099, respectively).

Discussion

This is a single center's retrospective analysis study evaluating the obstetric and neonatal outcome of donor sperm therapy patients. As we know, the reproduction result comes from both maternal and paternal factors; sometimes, it is ambiguous to identify which one is dominated. Donor semen had relatively consistent good quality after the screening. We speculated that the paternal factor was almost equal among different donor semen. Thus maternal factors that affect obstetric and neonatal outcomes should be conspicuous after statistical calculation. According to our result, advanced maternal age, incremental prior abortion times, and an increasing number of the ART cycle are associated with a higher risk of clinical pregnancy failure; besides, females aged over 35 years are also negatively linked to live birth. In regards to cycle-related parameters, retrieved oocyte number, transferred embryo number hold a positive effect on clinical pregnancy; besides, selecting Day 5 blastocyst transfer both benefit clinical pregnancy and live birth. For the neonatal outcome, more transferred embryo numbers resulted in lower newborn weight and length**.** In contrast, maternal BMI and endometrial thickness on hCG trigger day both had a positive contribution to neonatal weight and length.

People select donor sperm as ART therapy always had severe semen abnormal. According to our result, paternal azoospermia and couple factor contribute to the most population of donor sperm treatment. It has been demonstrated that donor sperm could improve fertilization, clinical pregnancy rate, and live birth without an increase of miscarriage, preterm births, or low birth weights (Yang Yu, 2018; Bo Yu, 2018; Sabrina A Gerkowicz, 2012).

Maternal factors were commonly accepted has a vital effect on fetal, obstetrics, and neonatal outcome. The causal pathways that underlie these associations have been studied but not definitively understood in humans. Although some researches have illustrated part of the maternal and lab ART cycle factors that may have an association with obstetrics and neonatal outcome, fewer studies stand on the respect of excluding or homogenization paternal factor to explore the real contribution of maternal and specific laboratory factors in the ART outcome. Retrospective research at Atlanta University used the inverse ideal with our study, found neither advancing male age, elevated BMI, nor poor sperm quality were associated with outcomes in the frozen donor oocyte cycle (Gianmartin Cit, 2019). This research was the first to use donor sperm to exclude the paternal effect on the ART outcome and specifically focused on maternal and cycle parameters.

In our result, clinical pregnancy and live birth rates were negatively correlated with advanced maternal age over 35. The majority of studies proved that advanced maternal age decreased the natural conception rate and ART (S J Chua, 2020; Samantha C Lean, 2017). A meta-analysis including 63 cohort studies and 12 case-control studies demonstrated advanced maternal age increased the risk of stillbirth (OR 1.75; 95% CI 1.62 -- 1.89), the risks of fetal growth restriction, neonatal death, Neonatal Intensive Care Unit admission, and gestational diabetes mellitus (Jordana Leader, 2018). These findings are backing up our results. Further, preceding abortion history for once led to adverse clinical pregnancy in our conclusion, while abortion for twice or more had no such effect, it may be ascribed to fewer subjects in this group; thus, the tendency was interrupted (Rebecca F, 2017). Fewer reports had mentioned this effect. Besides, the oocyte number retrieved and transferred embryo number significantly positively associated with clinical pregnancy rate. It is easy to understand; more oocytes mean the more significant possibility to gain available embryos. However, multiple embryo transfer had been demonstrated to have a higher pregnancy rate but pose health risks for both mothers and infants. A survey among 317,829 ART-exposed births verified multiple births explaining 36% of the congenital disabilities (Dmitry M Kissin, 2015). This consensus demonstrated well the significant improvement of pregnancy rate but not live birth rate in two embryo transfer group compared with single embryo transfer. Based on the binary logistic regression, the blastocyst embryo transfer had a more practical effect on clinical pregnancy and live birth than cleavage-stage embryo transfer. Refer to previous studies; some opinions showed no superiority of blastocyst compared with cleavage-stage embryo transfer in clinical practice by meta-analysis. However, the author also admitted that the quality of the evidence for the primary outcomes was low, additional well-designed RCTs were still needed before robust conclusions can be drawn (W P Martins, 2017; Demián Glujovsky, 2016; Wenhao Shi, 2019).

In regards to neonatal condition association factors, consistent with previous studies, low birth weight was more likely among double embryo transfer singletons born than non-ART infants and single embryo transfer (Petra De Sutter, 2006; Angela S Martin, 2017). Our finding suggested that the increase of transferred embryo numbers contributed to a higher risk of lower birth weight also happened in the donor sperm cycle. The discovery of the positive effect of maternal BMI and endometrial thickness on hCG trigger day on birth weight or length in the donor cycle were also verified by other researchers in spontaneous pregnancy and stander ART process (Austrida Gondwe, 2018. Morgane Ballon, 2019; Edson Borges Jr, 2019; Rebecca Moffat et al., 2017).

Strengths and Limitations

One of the strengths of our study is that our data are from a single ART center. All the donor recruitment and screening, ovarian stimulation, embryo culture, and analysis

were conducted at a single center, thus eliminating significant variation in clinical protocols, embryo handling, et al.; as confounding factors.

The main limitation of our study is that the number of subjects in advanced maternal age was relatively low. This may affect some of the statistical significance calculations that lead to interruptions of age-dependent tendencies. The other regrettable matter is that the data of frozen embryo transfer of donor was not included due to objective reason. In the future, the advance will be made to enlarge the sample size in each group and consider the effect of frozen embryo transfer.

Conclusions

Our findings analyze maternal and cycle-related parameters to obstetrics and neonatal outcome in the view of homogenizing paternal factors by using donor sperm. The positive factors like retrieved oocyte number, embryo number, and blastocyst transfer to clinical pregnancy or live birth were confirmed. In the meaning well, adverse factors such as female age over 35 years, preceding abortion history, and multiple ART cycles to clinical pregnancy outcomes were also proved. For neonatal, the number of transferred embryos leads to decreased newborn body weight or length, which is in converse with the effect of female BMI and endometrial thickness on hCG trigger day to newborn conditions. Our data add to the general knowledge of maternal and cycle-related factors on clinical pregnancy, obstetrics, and neonatal outcome. These findings may assist in providing preconception counseling to paternal infertility families who wish to have children.

Declaration

Authors'roles

Study concept, design, and supervision: Juanzi SHI; Acquisition of data, Statistical analysis and interpretation of data, and Drafting of the manuscript: Qian LI; Critical revision of the manuscript for important intellectual content: Wenhao SHI, Xia XUE, Xi Tong LIU; Obtained funding: Wenhao SHI, Juanzi SHI.

Conflict of interesting

The authors declare that they have no conflicts of **interest.**

Availability of Data and Materials

The data underlying this article are available in the article and in its online supplementary material

Ethics Approval

The supporting institutions of Northwest Women's and Children's Hospital have received ethics approval from 2001. All procedures performed in this study followed the ethical supporting-- "Review table of the Clinical Application and Ethics

Committee of Northwest Women's and Children's Hospital-2019013" from 20191218 onward.

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