Gynecology and Obstetrics Clinical Medicine 1 (2021) 197–204

Contents lists available at ScienceDirect

Gynecology and Obstetrics Clinical Medicine

journal homepage: www.keaipublishing.com/en/journals/gynecology-and-obstetrics-clinical-medicine

Research article

The interaction effect between advanced paternal age and paternal obesity is associated with the low implantation rate in couples with unexplained recurrent pregnancy loss

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ARTICLE INFO

Keywords:
Advanced paternal age
Implantation rate
Live birth rate
Obesity
Preimplantation genetic test
Unexplained recurrent pregnancy loss

ABSTRACT

Objective: To explore the roles of advanced paternal age (APA) and abnormal paternal weight on embryo quality and pregnancy outcomes for unexplained recurrent pregnancy loss (uRPL) couples who underwent preimplantation genetic testing for aneuploidies (PGT-A).

Methods: This study included 779 uRPL couples who underwent their first PGT-A cycles between 2014 and 2018. Male patients' aging and nutritional status were quantified by paternal age and body mass index (BMI). Routine semen parameters and sperm DNA fragmentation index (DFI) were used to reflect the seminal quality. Blastocyst formation rate and aneuploidy rate were used to reflect the embryo quality. Cycle cancellation rate, implantation rate, pregnancy loss rate, and live birth rate were measured to evaluate the treatment efficiency from IVF. To remove the interference of maternal age, only the women younger than 38 years old were included. After univariate screening, interaction tests were performed in a generalized linear model (GLM) to further examine the effects of paternal age and BMI on each outcome indicator.

Results: In the total population (779 cycles), there were no statistical differences in aneuploidy rate, cycle cancellation rate, implantation rate, pregnancy loss rate, and live birth rate, whether stratified by paternal age or paternal BMI. Similar results occurred in the younger men (<40 y.o., 633 cycles). Conversely, among the men with advanced age (≥40 y.o., 146 cycles), there were statistical differences between the three BMI groups in four semen parameters (total sperm number, total motility, progressive motility, and total motile sperm count), implantation rate, and live birth rate. After interaction testing, the results of GLM suggested that the interaction effect between APA and paternal obesity was associated with the low implantation rate of uRPL couples.

Conclusions: For the uRPL couples seeking for PGT-A treatment, if the male patients have both advanced age and obesity, their spouses are at higher risks for embryo implantation failure.

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Publishing services by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.gocm.2021.10.003
Received 12 July 2021; Received in revised form 6 October 2021; Accepted 20 October 2021
Available online 27 November 2021

1. Introduction

Throughout the past three decades, couples in developed countries have been delaying parenthood to ever-later ages. This trend has become so pervasive that demographers proposed a term for it, 'postponement transition',[1] and an analogous phenomenon has emerged in developing Chinese society. As we focus on maternal factors, it also matters to attend to the effects of paternal factors on the efficacy of in vitro fertilization (IVF), the safety of peripartum, and the health of the progeny. The proportion of couples planning to have children at advanced ages is increasing in China in particular due to the 'Two/Three-Child Policy', but the majority of these couples may not be aware of the increased risks of infertility and adverse reproductive outcomes.

The effects of increased maternal age on reproductive capacity have been widely studied. Advanced maternal age (AMA) has been shown to negatively influence the quality of oocytes and final pregnancy outcomes, while increasing the likelihood of aneuploid (hypohaploid or hyperhaploid) in oocytes due to the dysfunction of spindle pole body (SPB) in cytoplasm.[2,3] However, it has been shown that increased aneuploidy rate is also associated with males over 40 years old, and that paternal causes have been hypothesized to be responsible for up to 50% of subfertility cases, with 31.5% being attributed solely to the male.[4] Paternal age may have a critical impact on embryo aneuploidy rate, though likely not as pronounced an effect as maternal age.[5] Among couples undergoing IVF using donor oocytes, younger paternal age is associated with a higher incidence of live birth and a lower incidence of spontaneous abortion.[6] Increasing paternal age has been shown to decrease embryo quality. Because of the lack of a direct correlation between male and female partner ages, it is likely that decreased embryo quality is not solely caused by increased maternal age. Furthermore, increasing paternal age may be associated with genetic disorders, such as achondroplasia.[7] However, these conclusions are not universal and contrary studies have been published.[8] One study showing limited association between paternal age and live births in cohort of donor cycles suggested that paternal age is just a weak factor.[9] In addition, another study found that when the oocyte donor was <36 years of age, the paternal age does not affect reproductive outcomes, indicating that intracytoplasmic sperm injection (ICSI) and oocyte quality can jointly overcome the lower reproductive potential of semen from older men.[10] Concurrently, in standard IVF and ovum donation cycles, there is no clear association between embryo quality and paternal age.[11] Importantly, while paternal aging is associated with a significant decline in total sperm count, this change does not appear to affect fertilization and live birth rates in the oocyte donation model.[12] Though it has been well established that increasing maternal age adversely affects IVF outcomes, the effects of paternal age are still controversial, especially in population who have history of unexplained recurrent pregnancy loss (uRPL). Due to the disagreements among the current literatures, we are eager elucidate effects of paternal age on embryo quality and pregnancy outcomes in a uRPL population that underwent PGT-A.

Abnormal weight, whether for males and females, not only contributes to an increased risk of chronic diseases but also increases susceptibility to reproductive complications. Aside from extremely underweight men (specifically, the thinnest 1%) who themselves have increased risks of infertility, the infertility rate in men increases with an elevated body mass index (BMI).[13] It has been demonstrated that an increased BMI is inversely correlated with clinical pregnancy and live birth rates per assisted reproductive technology (ART) treatment cycle.[14] In addition, both under and over paternal weights exhibit adverse effects on sperm count.[15] Furthermore, a high paternal BMI increases the BMI of the offspring, suggesting an adverse effect of increased paternal BMI on progeny health.[16] Conversely, some studies have found that semen quality was not affected by the BMI of male partners in subfertile couples.[17] It is worth noting that most overweight/obese men do not experience significant fertility problems, suggesting the correlation between paternal BMI and fertility problems may be weak.[18] These contradictive conclusions create an obtuse situation and provide no previous clarity as to whether the uRPL of obese men could improve fecundity by weight control.

The definition of RPL varies by country and society. Briefly, its etiologies can be attributed to six aspects, including genetics, anatomy, endocrinology, infections, immunes, and prothrombotic state (PTS). Notably, there still are many causes of RPL that remain unknown. As such, it is difficult to conclusively predict which ART is the best option to achieve pregnancy, factoring in cost and invasiveness of the procedure, for unexplained infertile patients having consistently normal semen analysis results.[19] Infertility affects up to 15% of couples at childbearing age and exhibits adverse impacts on their life quality. The identification of potentially modifiable risk factors may guide some patients to achieve their reproductive goals more easily. To this end, using large-scale and comprehensive information of couples seeking PGT-A treatment in our center, we herein studied the associations between paternal age and BMI on embryo quality and pregnancy outcomes to provide consultation guidance for uRPL couples.

2. Material and methods

2.1. Study population

The first PGT-A cycles of 779 uRPL couples at the Center for Reproductive Medicine, Shandong University from June 2014 to June 2018 were included in this retrospective study. Only the first embryo transfer in each ovarian stimulation was included. Since the diagnosis of uRPL was based on a set of checks covering all possible causes, couples who were unable to determine the cause of pregnancy loss due to the absence of necessary inspections were also excluded from this study. The flow chart was shown in Fig. 1.

It is essential to eliminate the interferences of maternal factors in andrology research, especially those that are closely related to study outcomes—such as maternal age. Therefore, our study attempted to separate age-related decline in the quality of oocytes and embryos by only studying women younger than 38 years old. This age cut-off was selected based on evidence from previous studies demonstrating that the average rate of follicular exhaustion, cycle cancellation rate, and blastocyst aneuploidy rate were all significantly increased in female population after the age of 38.[20–23]

Ethics approval

This study was approved by the Ethics Committee of Reproductive Medicine Center of Shandong University. Informed consent was obtained from all participants.

2.2. Diagnostic criteria

There is still controversy over the definition of advanced paternal age (APA).[24] In most previous studies, the APA cut-off ranged from 40 to 50 years old.[25] Given the male subjects in previous studies were generally older than 40, we chose 40 years as our age cut-off. The criteria of weight for male subjects in this study was based on the health industry standard (WS/T 428–2013), issued by the Chinese Ministry of Health in 2013 (http://hbba.sacinfo.org.cn/stdDetail/89b7c7f055ed8bc30a138baca3132e2e).

The American Society for Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE) defined a diagnosis of RPL after the loss of two or more pregnancies.[26,27] However, many couples did not receive necessary inspections or professional medical guidance before trying to conceive naturally, we hence defined RPL as the same couple suffering three or more consecutive pregnancy losses before the 24th week of gestation in this study, which was consistent with the experts' consensus published in the ***Chinese Journal of Obstetrics and Gynecology*** in 2016 (http://rs.yiigle.com/resource_static.jspx?contentId=865493).

Couples with the following conditions were excluded: Chromosomal abnormalities (G-banding karyotype analysis), Monogenic disorders (https://www.omim.org/statistics/entry), Antiphospholipid antibody

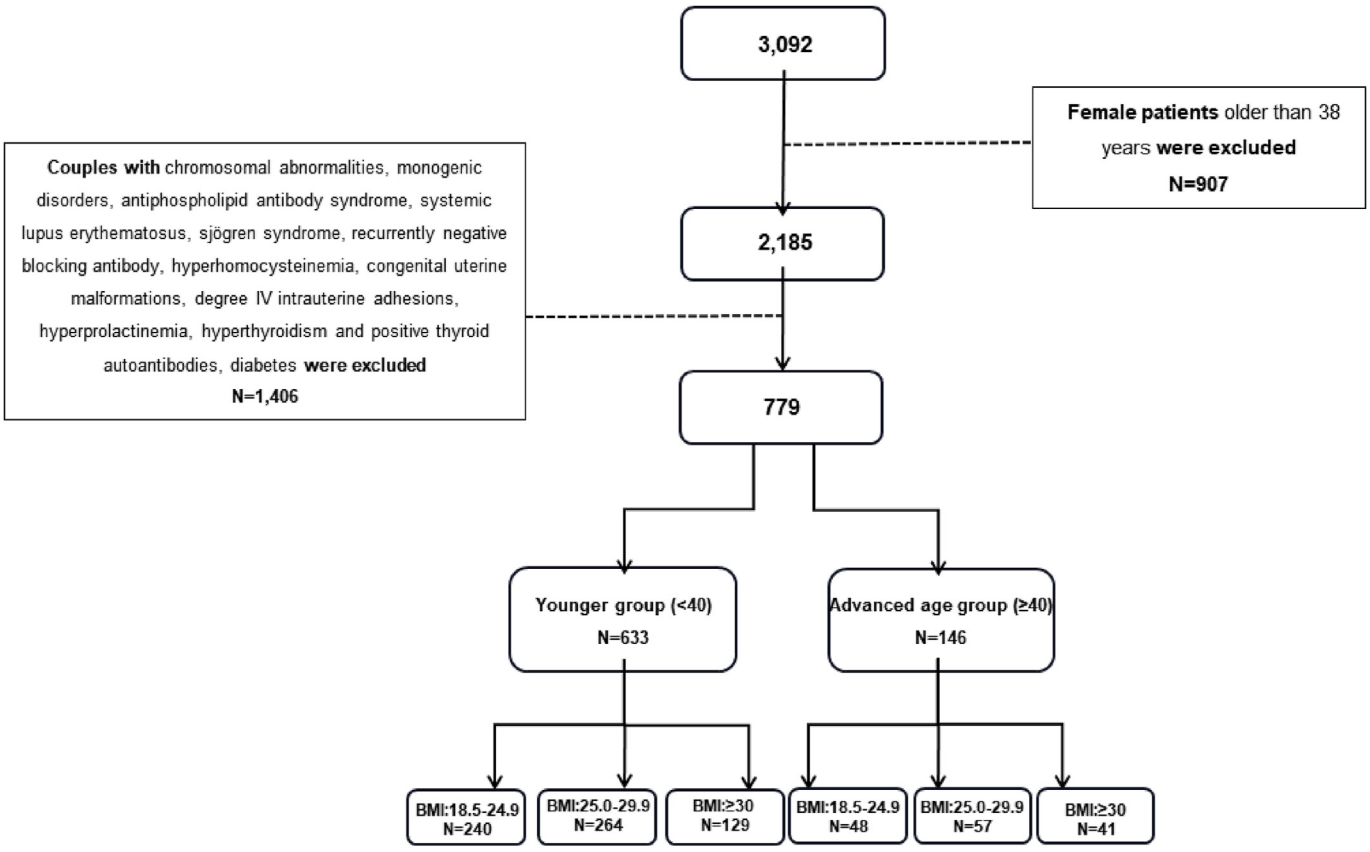

Fig. 1. Flow chart of the study populations.

syndrome (revised Sydney criteria in 2006[28]), Systemic lupus erythematosus (SLICC in 2012[29]), Sjögren syndrome (ACR/EULAR in 2016[30]), Recurrently negative blocking antibody after intervention, Hyperhomocysteinemia (>15 μmol/L), Congenital uterine malformations (bicornuate, didelphic, unicornuate, arcuate), Degree IV intrauterine adhesions,[31] Hyperprolactinemia (>25 ng/ml), Hyperthyroidism and positive thyroid autoantibodies (ATA in 2016[32]), Diabetes (ADA in 2012[33]), Fig. 1.

2.3. Clinical route

The time interval from birth date to retrieval date of semen or oocytes during this in vitro fertilization and embryo transfer (IVF-ET) cycle was defined as the age of each couple. Each couple's height and weight were measured on the day that they decided to start their first PGT-A cycle at our center.

Semen samples were obtained aseptically into a plastic container by masturbating from male patients with abstinence periods of 2–7 days in a private room near the laboratory department. The detection of samples was performed by experienced laboratorians. Semen volume was measured by weighing. Sperm concentration, total sperm number (semen volume × sperm concentration), total motility (progressive motility, PR + non-progressive motility, NR), and total motile sperm count (total sperm number × total motility) were calculated and recorded by a cytometer. Sperm DNA fragmentation index (DFI) was obtained by the method of sperm chromatin structure assay (SCSA) using flow cytometry analysis (BD FACSC alibur; DFI View Alpha10.01.388329.1(C) 2010).

Controlled ovarian hyperstimulation (COH) was performed as per standard clinical procedure at our center. The protocols in detail had been described in our previous reports.[34–36] hCG at a dose of 4000–10000 IU was administered when at least two follicles grew to 18 mm in diameter; 34–36 h later, oocytes were retrieval using transvaginal ultrasound-guided follicular aspiration. All oocytes were fertilized by ICSI and the zygotes were cultured in vitro until the blastocyst stage (5–6 days). Blastocyst scoring was conducted according to morphological standard.[37] 4–20 trophoblast cells were drilled from high-quality blastocysts (>BC) and then biopsied. While waiting for the PGT results, all blastocysts were vitrified and cryopreserved. Additionally, for couples with six or more high-quality blastocysts, a decision about the number of biopsied embryos was made in advance, based on the economic ability and willingness of this couple.

In total, 1521 euploid embryos were detected from the 2715 embryos screened with array comparative genomic hybridization (array-CGH: SurePlex whole genome amplification kit, fluorescent labeling system, 24sure microarray; Illumina, San Diego, CA, USA; InnoScan 900, Innopsys, FR; BlueFuse Multi software, BlueGnome) and next-generation sequencing (NGS: Miseq, Illumina, San Diego, CA, USA). The diagnosis was confirmed by four experienced genetic technicians. For the array-CGH results, euploid embryos were defined as chromosome ratios within a 0.3 ± log2 ratio. Aneuploid embryos with a ratio greater than +0.3 log2 ratio were categorized as trisomic, while those with a ratio less than −0.3 log2 were classified as monosomic. For NGS results, gains (partial or full) and losses (partial or full) were defined as a shift of the dots above or below the copy number state of 2.7 or 1.3. Embryos were diagnosed as aneuploid if the chromosomal copy number (CNV) measures deviated from the default copy number.[38,39]

The cycle would be canceled if euploid embryos were not obtained after all high-quality blastocysts were tested. At the second (or later) menstrual cycle after the retrieval of oocytes, the endometrium was prepared[36,40] and only one blastocyst was selected for transfer according to the geneticists' advice. Clinicians decided whether to administer endometrial scratching in the last menstrual cycle prior to this transfer based on the failure ART history of this couple. Progesterone was used for luteal phase support after the transfer. Biochemical pregnancy was

defined as serum β-hCG ≥25mIU/ml; measured 2 weeks after the transfer. Clinical pregnancy was defined as a gestational sac in utero and was detected by ultrasonic scanning 35 days after the transfer. Successfully embryo implantation was confirmed by ultrasound scanning for gestational sac with a fetal heartbeat after 6–9 weeks of pregnancy. Live birth was defined as the delivery of a viable infant after 28 weeks or more of gestation post-transplant.

The following ratios were used in this study: DFI>30% (men with DFI>30%/men underwent DFI test), blastocyst formation rate (usable blastocyst/2 PN), blastocyst aneuploidy rate (aneuploidy/total biopsied), cycle cancelation rate (cycle without transplantable embryo/cycle with COH), implantation rate (fetal heartbeat/euploid embryo transfer), pregnancy loss rate (biochemical and clinical loss/positive β-hCG), and live birth rate (live birth/euploid embryo transfer).

2.4. Statistic approach

Normality was examined for continuous variables via the Kolmogorov-Smirnov test. Data were presented as mean ± standard deviation (SD) for variables normally distributed, and median (interquartile range, IQR) for those not normally distributed. Categorical variables were presented as the percentage (no./total no.). Groups of three were compared using the analysis of variance (ANOVA) or Kruskal-Wallis H-test for continuous variables and the Chi-square test or Fisher's exact test for categorical variables. According to the Bonferroni correction level (level of significance=0.0167), pairwise comparisons were conducted within groups. A generalized linear model (GLM, type: logistic regression) was established to assess the interactions between the two evaluating variables on embryo quality or pregnancy outcomes after adjusting for confounding variables (men with DFI>30%, maternal age, maternal BMI, times of previous miscarriages, maternal FSH; maternal AMH; maternal AFC; women with PCOS, protocols of endometrial preparation, serum E2 of transformation day, the endometrium thickness of transfer day, history of endometrium scratching). Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were obtained and the P-value of <0.05 was considered statistically significant. SPSS 25.0 (Chicago, IL, USA) and Graphpad Prism 8.1 (Graphpad Software, San Diego, CA, USA) were used to achieve all the above.

3. Results

When the total population (779 cycles) was stratified by paternal age, there were no statistical differences between the younger and advanced age groups in the indicators of embryo quality and pregnancy outcomes, except for blastocyst formation rate (younger age vs advanced age: 58.1% vs 54.6%, P=0.043), Fig. 2. When the total population was stratified by paternal BMI, there were no statistical differences among three BMI groups in the indicators of embryo quality and pregnancy outcomes, except for pregnancy loss rate (P=0.047), Fig. 3. After pairwise comparisons, the pregnancy loss rate in the obese group was higher than that in the normal and overweight groups (obese vs normal: 63.89% vs 49.70%, P=0.021; obese vs overweight: 63.89% vs 50.88%, P=0.033), though these differences were not significant at Bonferroni's correction test level, Fig. 3.

To further investigate the roles of APA and abnormal paternal weight in uRPL, male patients were divided into two groups: younger age (<40 y.o., 633 cycles) and advanced age (≥40 y.o., 146 cycles), and three BMI groups: normal (18.5–24.9 kg/m^2, 288 cycles), overweight (25–29.9 kg/m^2, 321 cycles), obese (≥30 kg/m^2, 170 cycles). The baseline data of their female spouses were presented in Supplement Table 1. see website (https://www.sciencedirect.com/science/article/pii/S2667164621000506).

In the younger population, the male patients in normal weight group were younger (31.4 ± 4.0 vs 32.3 ± 3.9, P=0.007) than those in overweight group. The proportion of male patients whose DFI >30% in obese group was higher than that in normal weight group (41.0% vs 17.3%, P=0.005). For the semen parameters, there had statistical differences between three BMI groups in semen volume (P=0.043) and progressive motility (P=0.036). Pairwise comparisons showed statistically significant difference in progressive motility (37.1 vs 39.5, P=0.012) between the normal and over weight groups. Besides, no statistically significant differences were found in embryo and outcome indicators (blastocyst formation rate, aneuploidy rate, cycle cancellation rate, implantation rate, pregnancy loss rate, live birth rate), Table 1.

Within the population with advanced age, there had statistically significant differences between the three BMI groups in total sperm number (P<0.001), total motility (P=0.010), progressive motility (P=0.021), and total motile sperm count (P<0.001). After pairwise comparisons, there were substantial differences between the obese group and the normal weight group in all the above semen parameters. The blastocyst formation rate (P=0.509), aneuploidy rate (P=0.434), cycle cancellation rate (P=0.261), and pregnancy loss rate (P=0.094) did not differ statistically among three BMI groups. However, the differences in implantation rate (P=0.001) and live birth rate (P=0.047) were statistically significant. Between-group comparisons showed that the implantation rate in obese group was significantly lower than that in normal and over weight group (obese vs normal: 46.4% vs 86.8%, P <0.001; obese vs overweight: 46.4% vs 78.4%, P=0.008), Table 1, Fig. 3.

The results of the GLM revealed the interactions between paternal age and paternal BMI on embryo implantation status. After stratifying by paternal age, for every one-unit increase in BMI in older men, the risks of

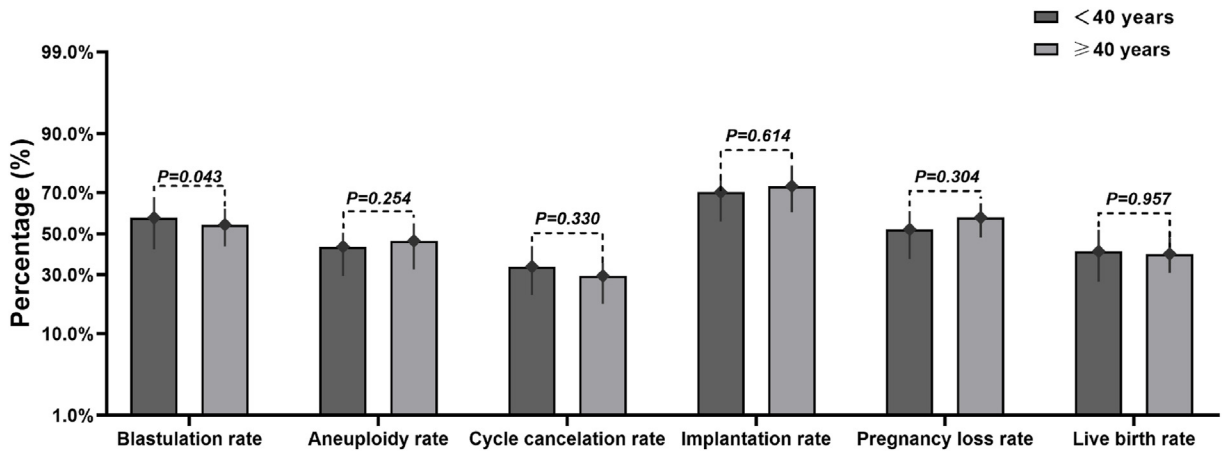

Fig. 2. The main cycle indicators when the total population was stratified by paternal age. As shown above, there is no statistically significant difference in the other five ratios except blastocyst formation rate.

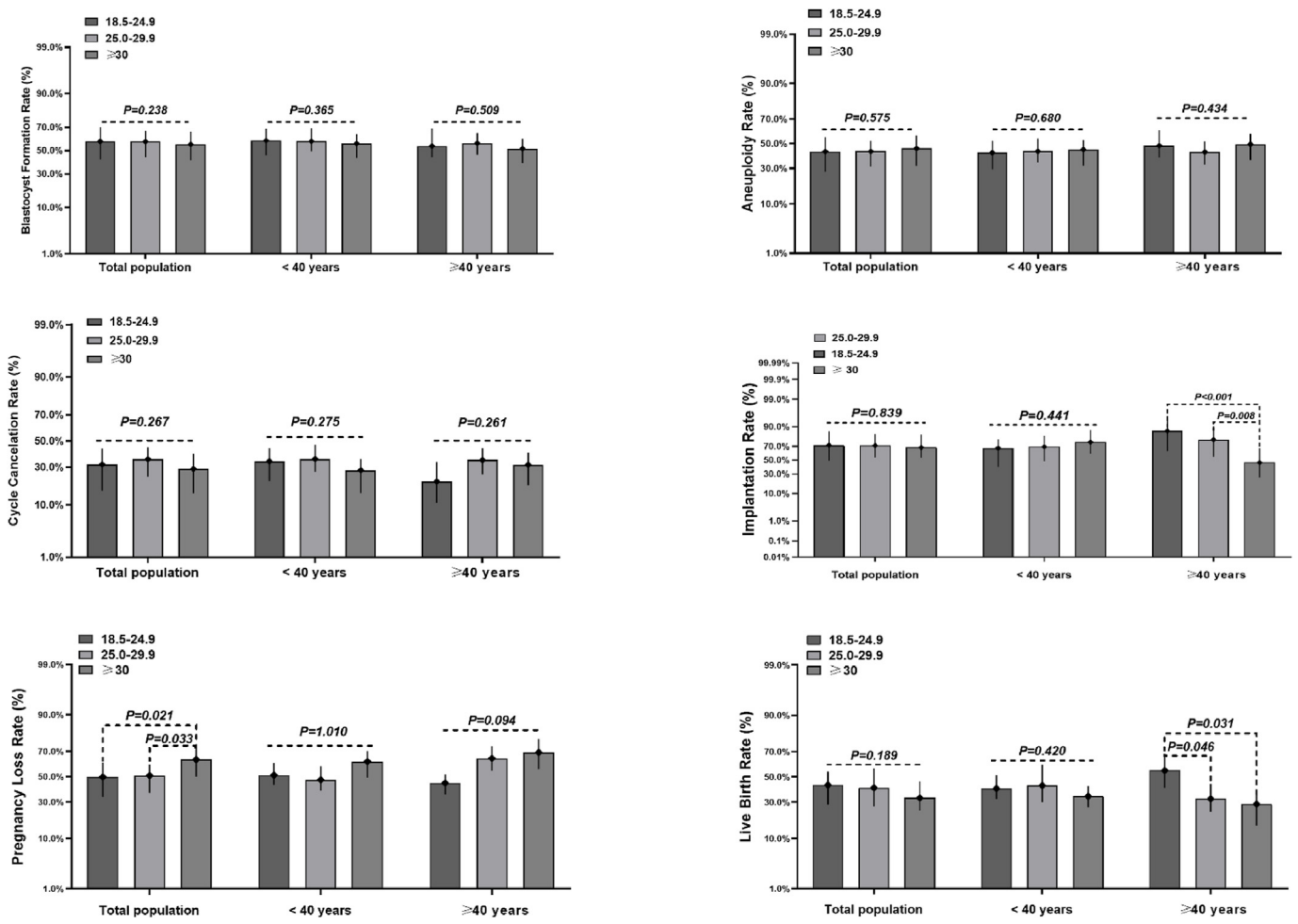

Fig. 3. Ratios of blastocyst formation, aneuploidy, cycle cancellation, embryo implantation, pregnancy loss, and live birth among the total population, younger men, and men with advanced age. Only pairwise comparisons of implantation rate in advanced age group showed statistical differences, according to Bonferroni correction level.

implantation failure increased by 12.6% for their spouses (aOR=1.126, 95%CI=1.016–4.097, P=0.039), Table 2. As stratified by paternal BMI, if obese men were also older than 40, implantation failure risks of their spouses were 1.143 times greater than that of counterparts (aOR= 1.143, 95%CI= 1.091–2.197, P= 0.024), Table 3.

4. Discussion

By analyzing the large-scale dataset of a uRPL population undergoing the first PGT-A in our center, we demonstrated that male patients possessing both advanced age and obesity had a low implantation rate than those younger and normal-weight males, while neither paternal age nor paternal BMI has significant effects on embryo quality and pregnancy outcomes. We inferred that the adverse paternal effects on pregnancy outcome are more substantial when two or more paternal reproductive risk factors are coincident.

The unique situations around uRPL make our study population differ from those in oocyte donation cycle studies, a which model can better eliminate the interferences of maternal factors. Interestingly, in our study there were no statistically significant differences in blastocyst aneuploidy rate among three BMI groups at two age layers, highlighting that the maternal effects play more important roles in the formation of aneuploidies. There are two likely reasons for this phenomenon. First, only women under 38 years old were included in our study. It is probable that youthful oocytes benefit pregnancy outcomes and may even reverse the adverse effects of imperfect sperm. Second, all oocytes were inseminated by ICSI, clearing physiological hurdles which may be the main obstacles for older sperm. During natural conception or routine IVF, oxidative damage to the sperm membrane will normally block fertilization, preventing the damaged paternal DNA from creating an embryo. However, during ICSI-PGT this natural barrier to fertilization is lost and sperm containing significantly damaged DNA can still achieve fertilization following microinjection. While many of these embryos will ultimately fail at the blastocyst or early fetal stage, there is the potential for a child to be born with damaged paternal derived DNA. The consequences of which are yet unknown.[41]

Broadly speaking, our conclusions are most applicable to uRPL population undergoing PGT and highlight several paternal factors warranting consideration that may lead to repeated miscarriages or poor blastocyst development, such as abnormality of karyotype or DFI>30%. Semen parameters are often utilized as indicators for paternal fertility evaluation in the clinic, but these superficial morphological tests are imperfect to elucidate the true quality of sperm. While DFI can provide physicians with information about sperms' chromatin integrity on the molecular level of large fragments, some special male patients with a DFI greater than 30 still experience miscarriages after treatment. Excepting both the older stratification or younger stratification, there were no statistical differences in pregnancy loss rate and live birth rate among three BMI groups; although, there had the implantation rate. These results indicate that the adverse paternal factors may be more likely to affect early pregnancy via involvement in embryogenesis and that as the number of gestational weeks increase, the maternal factors increase in dominance over determining final pregnancy outcomes.

Table 1
The demographic characteristics and cycle indicators of male patients stratified by BMI at two ages.

Age, years	BMI category	Normal (18.5–24.9)	Overweight (25.0–29.9)	Obese (≥30)	P value	P 1vs2	P 1vs3	P 2vs3
< 40	No.	240	264	129	— —			
	Age(years)	31.4 ± 4.0	32.3 ± 3.9	32.4 ± 3.5	***0.003***[a]	*0.007*	*NS*	*NS*
	BMI (kg/m^2)	22.8(3.1)	27.2(2.3)	31.9(3.3)	***<0.001***[b]	*<0.001*	*<0.001*	*<0.001*
	DFI>30% (%)	17.3(14/81)	29.2(21/72)	41.0(16/39)	***0.018***[c]	*NS*	*0.005*	*NS*
	Semen volume(ml)	3.8(1.6)	3.7(1.9)	3.2(1.6)	***0.043***[b]	*NS*	*NS*	*NS*
	Sperm concentration(×10^6/ml)	40.5(45.1)	44.5(50.0)	38.3(35.1)	*0.176*[b]			
	Total sperm number (×10^6)	152.4(170.9)	168.11(184.7)	134.55(143.6)	*0.138*[b]			
	Total motility (PR + NP, %)	51.8(31.9)	51.1(33.8)	50.3(26.4)	*0.225*[b]			
	Progressive motility (PR, %)	37.1(28.1)	39.5(25.2)	38.3(25.8)	***0.036***[b]	*0.012*	*NS*	*NS*
	Total motile sperm count (×10^6)	67.8(116.0)	73.5(125.1)	72.8(110.3)	*0.146*[b]			
	No. oocytes obtained	11(9)	9(8)	9(9)	*0.437*[b]			
	Blastocyst formation rate (%)	58.9(1185/2013)	58.3(1280/2195)	56.3(626/1112)	*0.365*[c]			
	Blastocyst aneuploidy rate (%)	42.5(374/879)	43.6(406/931)	45.1(193/428)	*0.680*[c]			
	Cycle cancelation rate[h] (%)	34.2(82/240)	36.0(95/264)	27.9(36/129)	*0.275*[c]			
	Implantation rate (%)	67.9(106/156[e])	69.6(117/168[f])	75.6(68/90[g])	*0.441*[c]			
	Pregnancy loss rate (%)	51.2(66/129)	47.4(65/137)	62.2(51/82)	*0.101*[c]			
	Live birth rate (%)	40.4(63/156)	42.9(72/168)	34.4(31/90)	*0.420*[c]			
≥40	No.	48	57	41	— —			
	Age(years)	42.4 ± 3.5	42.5 ± 2.8	42.5 ± 3.3	*0.831*[a]			
	BMI (kg/m^2)	23.4(1.8)	27.2(2.4)	32.4(1.2)	***<0.001***[b]	*<0.001*	*<0.001*	*<0.001*
	DFI>30% (%)	54.5(6/11)	52.6(10/19)	61.5(8/13)	*0.925*[d]			
	Semen volume(ml)	3.4(1.8)	3.3(2.2)	2.7(1.4)	*0.097*[b]			
	Sperm concentration(×10^6/ml)	40.0(40.0)	44.8(38.0)	45.8(45.4)	*0.726*[b]			
	Total sperm number (×10^6)	162.7(128.9)	147.1(145.0)	128.2(166.2)	***<0.001***[b]	*NS*	*<0.001*	*<0.001*
	Total motility (PR + NP, %)	46.7(36.3)	43.1(24.2)	39.5(21.0)	***0.010***[b]	*NS*	*<0.001*	*0.016*
	Progressive motility (PR, %)	43.8(36.6)	38.7(23.9)	34.3(17.8)	***0.021***[b]	*NS*	*0.013*	*NS*
	Total motile sperm count (×10^6)	64.2(137.5)	62.5(83.6)	49.3(62.2)	***<0.001***[b]	*0.011*	*<0.001*	*<0.001*
	No. oocytes obtained	9(8)	9(6)	8(4)	*0.267*[b]			
	Blastocyst formation rate (%)	54.1(173/320)	56.5(230/407)	51.8(116/224)	*0.509*[c]			
	Blastocyst aneuploidy rate (%)	48.4(78/161)	43.0(89/207)	49.5(54/109)	*0.434*[c]			
	Cycle cancelation rate[h] (%)	20.8(10/48)	35.1(20/57)	31.7(13/41)	*0.261*[c]			
	Implantation rate (%)	86.8(33/38)	78.4(29/37)	46.4(13/28)	***0.001***[c]	*NS*	*<0.001*	*0.008*
	Pregnancy loss rate (%)	44.7(17/38)	64.7(22/34)	69.2(18/26)	*0.094*[c]			
	Live birth rate (%)	55.3(21/38)	32.4(12/37)	28.6(8/28)	***0.047***[c]	*NS*	*NS*	*NS*

Data are shown as median (IQR), mean ± SD and (%) (no./total no.).
[a] Analysis of Variance (ANOVA).
[b] Kruskal-Wallis test.
[c] Chi-Square Goodness-of-Fit Test.
[d] Fisher's Exact Test.
[e] One ectopic pregnancy and one mosaic embryo transfer not included.
[f] One break the cycle for economic reason not included.
[g] Three mosaic embryo transfer not included.
[h] Transplantable euploid embryos were not obtained.

Table 2
Interaction test between implantation status and paternal BMI following paternal age stratification.

		aOR	95% CI	*P*-value
Age: <40	**BMI: ≥30**	0.873	0.767–1.269	0.123
	BMI: 25.0–29.9	0.996	0.946–1.593	0.275
	BMI: 18.5–24.9	–	–	–
Age:≥40	**BMI: ≥30**	1.126	1.016–4.097	**0.039***
	BMI: 25.0–29.9	1.014	0.904–2.201	0.103
	BMI: 18.5–24.9	–	–	–

* Statistically significant.
Adjusted for the men with DFI>30%, maternal age, maternal BMI, times of previous miscarriages, maternal FSH; maternal AMH; maternal AFC; women with PCOS, protocols of endometrial preparation, serum E2 of transformation day, the endometrium thickness of transfer day, history of endometrium scratching.
Embryo transfer success is the reference group.

Table 3
Interaction test between implantation status and paternal age following paternal BMI stratification.

		aOR	95% CI	*P*-value
BMI: ≥30	**Age:≥40**	1.143	1.091–2.197	**0.024***
	Age: <40	–	–	–
BMI: 25.0–29.9	**Age:≥40**	0.839	0.703–1.414	0.213
	Age: <40	–	–	–
BMI: 18.5–24.9	**Age:≥40**	0.537	0.401–1.137	0.311
	Age: <40	–	–	–

* Statistically significant.
Adjusted for the men with DFI>30%, maternal age, maternal BMI, times of previous miscarriages, maternal FSH; maternal AMH; maternal AFC; women with PCOS, protocols of endometrial preparation, serum E2 of transformation day, the endometrium thickness of transfer day, history of endometrium scratching.
Embryo transfer success is the reference group.

It has been well documented that women who transfer euploid embryos tend to have better pregnancy outcomes and lower pregnancy loss rates. However, the pregnancy loss rate of women in our study who received euploid embryos was still as high as ~50%. Several possible reasons are as follows: First, these women had suffered three or more spontaneous miscarriages before starting this PGT cycle, either medicinal or curettage, and these previous experiences are potentially damaging to their uteri. Times of previous miscarriages have been confirmed as a predictive variable for the next gestation. Second, we ought to differentiate between RPL population with known causes and the uRPL population. For RPL population, PGT is an appropriate and necessary remedy to infertility, while for uRPL population, PGT is a desperate attempt to eliminate the embryo factors as much as

possible. This distinction is consistent with previous evaluations of PGT.[42] Lastly, because the RPL causes of these couples were not confirmed, confounding interferences—unknown to us—might be other drivers of the elevated pregnancy loss rate.

Obesity has been shown to not only causes male endocrine disorders by affecting the hypothalamus-pituitary-gonadal axis but also increases the levels of cytokines from white adipose tissues in serum, testicular tissues, and spermatic plasma. Concurrently, Male obesity is associated with male subfertility, impaired sex hormones, reduced sperm counts, increased oxidative sperm DNA damage, and alterations to the epigenetic status of sperm. It is notable that the adverse effects of paternal obesity on pregnancy outcomes do not appear significant in younger men in our study. Such a result highlights that the poor implantation associated with the advanced age male population is likely not solely attributable to obesity, but aging or other age-related cofactors as well. Some of the more microscopic epigenetic changes brought by obesity and advanced age may be undetectable and thus unintentionally ignored. This hypothesis is supported by our results that the blastocyst formation rate was lower in the advanced age group than the younger group (P=0.043), but there was no significant difference in blastocyst aneuploidy rate. These changes will gradually manifest later in the process of embryo implantation, cell reprogramming, and the development of progeny.

This is a study of a large sample size of uRPL patients, and the strict inclusion criteria ensured the strong heterogeneity of our study population. Also, we excluded the interference from AMA, high DFI, and aneuploidy. GLM was used to correct the data and test the interactions, assuring the accuracy and reliability of our conclusions. A shortcoming of our study is the usage of BMI. Although BMI is a widely used indicator for overweight and obesity,[43] it cannot reflect individual differences in body composition. Further study investigating the relationship between body fat percentage and infertility is required. Moreover, mosaicism might be missed, due to the technical limitation of array-CGH. Furthermore, the euploid was defined to be any multiple of the haploid chromosome number (n=23) and therefore is not always normal (diploid). It is worth noting that some abnormal euploid embryos (e.g. 3n=69, 4n=92) may not be differentiated from normal diploid embryos (2n=46). Our results are limited to reproductive outcomes of PGT-A treatment and cannot be extended to guide couples on potential risks for born children. In the future, research is needed to see if weight control improves fertility for overweight and obese men.

Authors' roles

J.Y. conceived and designed the study. S.L. conducted data collection, analyzed, and wrote the manuscript. Y. S. revised the paper. All authors were involved in interpreting the data and approved the final version.

Consent for publication

All authors have consented for the publication of the article.

Funding

This study was supported by the National Key Research and Development Program of China (2018YFC1002804).

Disclosure summary

The authors have nothing to disclose.

Conflict of interest

None declared.

Acknowledgements

We thank Jingfu Yang for his invaluable help during data collection.

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