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Research Article

Association of Sperm DNA Fragmentation Level with Body Mass Index of Male and In Vitro Fertilization Outcomes: A Large-Scaled Retrospective Study

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Keywords

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- Pregnancy outcome

Abstract

Objective: To investigate the effect of male's BMI on semen quality, embryo formation, development and pregnancy outcomes in couples undergoing *in vitro* fertilization-embryo transfer (IVF-ET) fresh cycle treatment.

Methods: The clinical data of 5,997 IVF-ET cycles that were performed between January 2018 and May 2020 were retrospectively analyzed. According to the BMI, these cycles were divided into Lean group (BMI < 18.5 Kg/m^2), Normal group (18.5 Kg/m^2 ≤ BMI < 24 Kg/m^2), Overweight group (24 Kg/m^2 ≤ BMI < 28 Kg/m^2) and Obese group (BMI ≥ 28 Kg/m^2). At the same time, the cycles were divided into the Low DFI group (DFI < 20%) and High DFI group (DFI ≥ 20%) according to sperm DNA fragmentation index (DFI). Then, the indicators of fertilization and (or) embryo development, embryo quality, pregnancy outcome were compared among the groups. Finally, logistic regression analysis was used to exclude the influence of female factor and other confounding factors in order to better study the effect of BMI of male on clinical pregnancy outcome.

Results: Progressive motility sperm percentage in Normal group was higher compared to Obese group, and the DFI in Overweight and Obese group was higher than that in Normal group (P<0.05). The rate of blastocyst formation in Lean group was higher than that in Obese group, while compared with Normal group, blastocyst formation rate in Overweight and Obese group was lower (P<0.05). Compared with Low DFI group, the blastocyst formation rate and high scoring blastocyst rate in High DFI group were lower(P<0.05). Abortion rates in Normal group was lower than that in Overweight group, the live birth rates in Normal group were significantly higher than that in Overweight and Obese group (P<0.05).

Conclusion: BMI of male did not affect the clinical pregnancy outcome. While, DFI had an influence on clinical pregnancy and live birth, but had no impact on abortion. High BMI of male leads high DFI, high BMI of male indirectly has harmful effects on the outcome of IVF cycles by affecting DFI. In assisted reproductive technology (ART) process, men also should control their BMI in order to have a good pregnancy outcome.

INTRODUCTION

Social development has changed many aspects of human living, including people's lifestyles and diets. Obesity, which has already reached epidemic levels in some countries, has become one of the topics of urgent importance. According to the World Health Organization, as of 2016, 13 % of the world's adults were obese, with the prevalence of obesity being 15% in women and 11% in men. Obesity has become an important risk factor for a number of non-communicable diseases, such as hypertension, cardiovascular disease, type 2 diabetes and other metabolic diseases [1], osteoarthritis [2], gallstone disease [3], asthma, and other chronic respiratory diseases [4-6], and multiple malignancies [7].

Obesity is often accompanied by changes in the body's endocrine hormone levels and affects reproductive-related hormone levels. Women who are overweight are more likely to

suffer from polycystic ovary syndrome, menstrual disorders, infertility, miscarriage, poor pregnancy outcomes, and multiple pregnancy complications (including gestational diabetes, pre-eclampsia, and fetal macrosomia) [8-11]. Obesity in women leads to decreased fertility, poor pregnancy outcomes, and obstetric outcomes. The effect of male obesity on fertility mainly focused on sperm DNA fragmentation index (DFI), while the effect of male obesity on embryo development, pregnancy outcome, and obstetric outcome is less studied. Overweight and obese men may be at greater risk of infertility. However, according to existing studies, the relationship between increased body mass index (BMI) of male and clinical outcomes after assisted reproductive technology (ART) remains controversial.

Roberta et al., reported that BMI of male did not affect ejaculate volume, sperm morphological vitality, and sperm morphology [12]. While, Taha et al., found that an increase in BMI had a negative effect on semen parameters in fertile men. They revealed that the sperm concentration, the percentage of forward-moving sperm, and the rate of normal sperm morphology were significantly lower in obese men compared with normal-weight and overweight men ($p < 0.05$) [13]. Previous studies have shown that obese men had significantly higher DFI levels than men of normal weight [12-15]. Yet, it remains unclear whether being overweight implies higher DFI. Some researchers suggested that higher BMI leaded higher DFI in men [14], while some researchers argued that BMI did not affect DFI [15]. Recent studies have also shown that male obesity is associated with decreased fertility and sperm DNA breakage and that a high BMI seems to mean a higher DFI [16-20]. However, there is not enough data to show a positive association between BMI and DFI. Furthermore, high and low, DFI are independent of BMI, and high BMI seems to be independent of impaired sperm DNA integrity [21-24].

The effect of male obesity on fetal development, pregnancy, and obstetric outcomes is a debatable issue. Researchers have found that male BMI has different effects on embryo development and pregnancy and obstetric outcomes in the in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles [25,26]. Studies have shown that BMI of male affected the quality of the embryo, which in turn affected pregnancy outcomes [27,28]. While Merh et al., considered that male obesity was related to the outcomes of IVF pregnancy, but the quality of embryos was not affected [29], other studies suggested that BMI of male did not affect in vitro fertilization, embryo development and quality, pregnancy outcomes, and obstetric outcomes [30-38].

In the current study, we first analyzed the effect of BMI of male on DFI and then analyzed the effect of BMI of male and/or DFI on embryo development and clinical pregnancy outcomes in IVF cycles. We found that higher BMI of male was associated with had higher DFI, lower blastocyst formation, higher abortion rate, and lower live birth rate. Higher DFI was harmful to blastocyst formation and the quality of blastocyst. In addition, we found that DFI was a factor that affected clinical pregnancy and live birth.

MATERIALS AND METHODS

Patients

The data of 18,326 IVF cycles performed in the Reproductive Medical Center, First Affiliated Hospital of Zhengzhou University, and the Henan Province Key Laboratory for Reproduction and Genetics between January 2018 and May 2020 were collected. Eventually, 5,997 cycles were included and analyzed in accordance with the inclusion criteria and exclusion criteria.

According to WHO standard, diet structure of Chinese and weight distribution in Chinese men, these cycles were divided into Lean group (BMI < 18.5 Kg/m2, N=134), Normal group (18.5 Kg/m2 ≤ BMI < 24 Kg/m2, N=2219), Overweight group (24 Kg/m2 ≤ BMI < 28 Kg/m2, N=2497) and Obese group (BMI ≥ 28 Kg/m2, N= 1147). After grouping, the indicators of semen parameters, fertilization, embryo development, embryo quality, and pregnancy outcomes were compared among the groups. Next, the cycles were divided into the Low DFI group (DFI < 20%, N=4969) and High DFI group (DFI ≥ 20%, N=1028) based on the sperm DNA fragmentation index (DFI). The indicators of fertilization, embryo development and embryo quality were compared among the groups. The route of our study is shown in Figure 1a, and the percentages of different groups are shown in Figure 1b.

Inclusion criteria and Exclusion criteria

Inclusion criteria were the following: 1) couples undergoing IVF cycles; 2) couples with successful egg retrieval, embryo formation, and fresh transplantation after controlled ovarian stimulation (COS); 3) the male partner's BMI, semen routine parameters, and DFI recorded completely. Exclusion criteria were: 1) donor cycles and frozen semen cycles; 2) incomplete cycle of follow-up data; 3) cycles of women with poor ovarian response (woman's age ≥40 years + anti-mullerian hormone (AMH) < 0.5-1.1 ng/ml; woman's age ≥40 years + Antral Follicle Counting (AFC) < 5-7); 4) cycles of woman or (and) man with the chromosome abnormality, the single-gene hereditary disease, and similar hereditary disease; 5) Couples, where men suffered from diseases affecting semen quality or causing infertility, such as Varicocele, genitourinary infections, medications, etc.

Analysis and treatment of semen and *in vitro* fertilization-embryo formation

Marker plate count and hematoxylin-eosin (HE) staining routinely analyzed semen. The volume of semen obtained, sperm concentration, percentage of progressive motility sperm, percentage of non-progressive motility sperm, percentage of in-motility sperm and DFI were measured. DFI was determined by sperm chromatin structure analysis (SCSA) Kit (CP0101-10T) purchased from Zhejiang CellPro Biotech Co., Ltd. (Ningbo, China). The test is conducted in strict accordance with the manufacturer's instructions.The oocytes were taken 37 hours after human chorionic gonadotropin (HCG) injection, the granulosa cells were removed after short-term fertilization, and the pro-karyocyte formation was observed 16-18 hours after fertilization. Vitrolife or COOK medium was used for in vitro culture.

Embryo transfer and luteal support

On the 3rd or 5th day after oocyte retrieval, high-quality D3 cleavage stage or D5 blastocyst stage embryos were selected for transplantation according to the embryo morphological score and the woman's physical condition. Grading criteria for high-quality embryos and high scoring blastocyst were according to

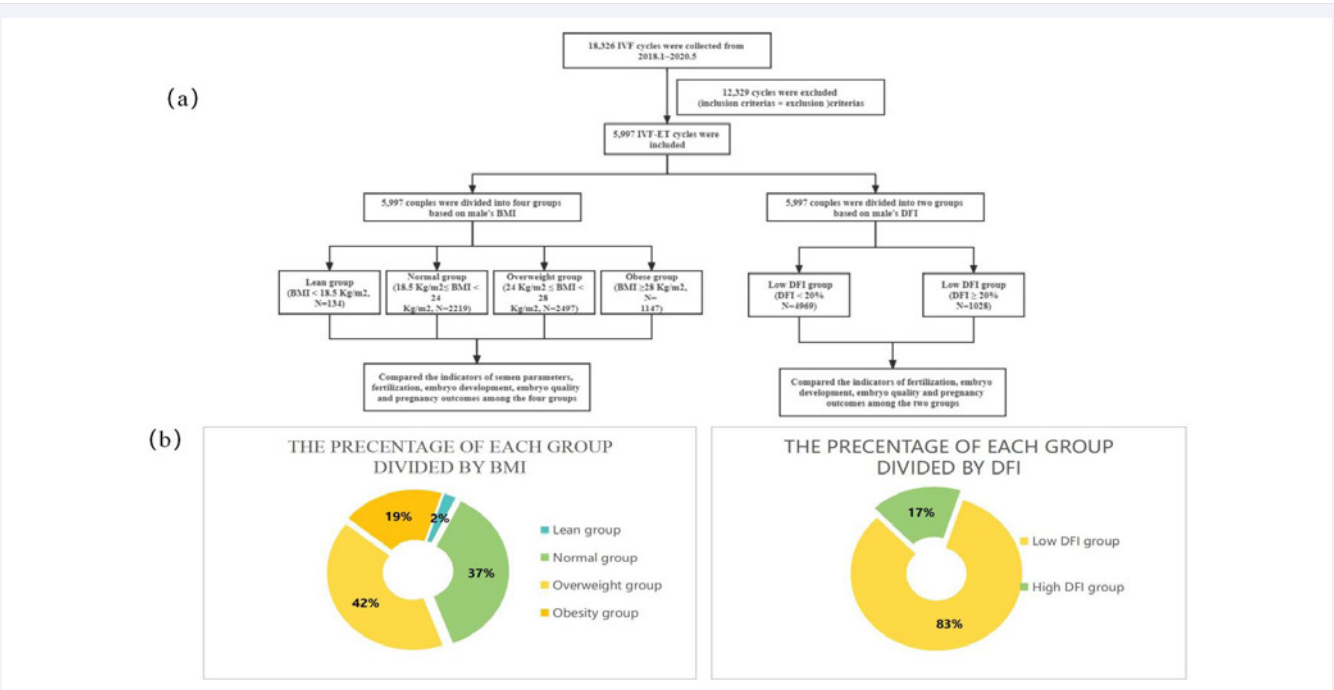

Figure 1 (a): Flow charts representing the populations included in the current study. (b) Pie charts representing the percentages of different groups.

the previously published article by Our Center [39]. We usually chose 1-2 cleavage stage embryos or 1 blastocyst stage embryo for transfer. The remaining embryos or blastocysts were selected according to certain quality standards, after which the embryos or blastocysts were vitrified. Luteal phase support was given from the day of oocytes retrieval.

Periodic follow-up

The concentration of β-HCG in blood was measured 14/18 days after embryo transfer, and the concentration of β-HCG > 50 IU/L indicated biochemical pregnancy. Follow-up was continued until 35 days after embryo transfer. Abdominal color Doppler sonography was performed to confirm the presence or absence of a gestational sac. If an ultrasound showed a gestational sac, it was recorded as a clinical pregnancy [40]. Subsequently, in patients with regular prenatal check-ups, periodic perinatal insurance, pregnant and obstetric outcomes such as live birth and abortion in the third trimester were followed up and recorded by telephone by the full-time teacher in the medical record room of the center. Birth of a neonate on or after 24 weeks of gestation was defined as a live birth.

Statistical analysis

The data were analyzed with SPSS 25.0 software. The data of continuous numerical variables were expressed by means of ± SD. The intergroup differences were analyzed by one-way ANOVA and the Mann-Whitney U test. The categorical variables were expressed as frequency and percentage (%), and the differences between groups were analyzed by chi-square test (2-test). P < 0.05 was considered statistically significant.

RESULTS

We collected the clinical data of 5,997 infertile couples who underwent IVF treatment and fresh transplants at our center between January 2018 and May 2020. They were divided into four groups: Lean group (BMI < 18.5 Kg/m2, N = 134), Normal group (18.5 Kg/m2≤ BMI < 24 Kg/m2, N = 2219), Overweight group (24 Kg/m2 ≤ BMI < 28Kg/m2, N = 2497) and Obese group (BMI ≥ 28 Kg/m2, N = 1147). The baseline data for male patients in different BMI subgroups were expressed as mean ± standard deviation, as shown in Table 1.

Compared with the Overweight group (32.41 ± 5.48) and the Obese group (32.07 ± 4.88), the age of the participants in the Lean group (29.49 ± 3.92) and the Normal group (30.98 ± 5.03) was significantly lower (P = 0.000). In addition, men in the Lean group were younger than those in Normal group (29.49 ± 3.92 vs 30.98 ± 5.03, P = 0.000), while there was no significant difference in the age distribution between the Overweight group and the Obese group (P > 0.05) (Table 1). The progressive motility sperm percentage of the Normal group was higher than that of the Obese group (42.86 ± 10.11 vs. 42.11 ± 10.54, P = 0.044). DFI in Overweight and Obese group was significantly higher than in Normal group (14.12 ± 8.34 vs 12.76 ± 6.77, P = 0.000; 13.79 ± 8.14 vs 12.76 ± 6.77, P = 0.002). There was no significant difference between other groups (P > 0.05) (Table 1). There were no significant differences in semen volume, concentration, percentage of non-progressive motility, and in-motility sperm among the four groups (P > 0.05) (Table 1).

As shown in Table 1, BMI of male influenced the percentage of progressive motility sperm and DFI. Consequently, we further analyzed the effect of BMI of male and DFI on fertilization, embryo development, and embryo quality by comparing fertilization rate, normal fertilization cleavage rate, high-quality embryo rate, blastocyst formation rate, and high scoring blastocyst rate among the groups (Table 2). The rate of blastocyst formation in the Lean

Table 1. Baseline data of men in different BMI groups.

Item	Lean group (BMI < 18.5 Kg/m², N=134)	Normal group (18.5 Kg/m²≤ BMI < 24 Kg/m², N=2219)	Overweight group (24 Kg/m² ≤ BMI < 28 Kg/m², N=2497)	Obese group (BMI ≥28 Kg/m², N= 1147)	P value
Age (year)	29.49±3.92	30.98±5.03	32.41±5.48	32.07±4.88	$0.000^{abc\alpha\beta}$ 0.294^{γ}
Semen volume (ml)	2.93±0.95	3.08±1.04	3.01±1.03	3.01±1.06	$> 0.05^{abc\alpha\beta\gamma}$
Concentration (10^6/ml)	57.03±48.53	57.99±85.99	57.01±50.25	53.11±46.05	$> 0.05^{abc\alpha\beta\gamma}$
PR (%)	43.11±9.45	42.86±10.11	42.35±10.35	42.11±10.54	$> 0.05^{abc\alpha\gamma}$ 0.044^{β}
NP (%)	10.08±2.71	10.10±2.88	10.19±2.91	10.12±2.80	$> 0.05^{abc\alpha\beta\gamma}$
IM (%)	46.83±9.70	47.04±10.38	47.45±10.74	47.77±10.77	$> 0.05^{abc\alpha\beta\gamma}$
DFI (%)	13.52±7.32	12.76±6.77	14.12±8.34	13.79±8.14	$> 0.05^{abc\gamma}$ 0.000^{α} 0.002^{β}

BMI: Body Mass Index; PR: Progressive motility; NP: Non-progressive motility; IM: In-motility; DFI: Sperm DNA fragmentation index; P < 0.05 was considered to be statistically significant.
a: Lean group vs. Normal group; b: Lean group vs. Overweight group; c: Lean group vs. Obese group; α: Normal group vs. Overweight group; β: Normal group vs. Obese group; γ: Overweight group vs. Obese group

group was significantly higher than that in the Obese group (0.54 ± 0.28 VS 0.44 ± 0.29, P = 0.003), the blastocyst formation rates in Overweight and Obese groups were significantly lower (0.48 ± 0.32 VS 0.56 ± 0.31, P = 0.000; 0.44 ± 0.29 VS 0.56 ± 0.31, P = 0.000) (Table 2a). However, there was no significant difference in normal fertilization rate, normal fertilization cleavage rate, and high-quality embryo rate between the two groups (P > 0.05) (Table 2b).

The comprehensive analysis of Table 1 and 2 showed that BMI of male affects DFI, and BMI and DFI had adverse effects on blastocyst formation and blastocyst quality. To further explore the effect of BMI of male and DFI on pregnancy outcome, we further analyzed the effects of BMI on clinical pregnancy rate, miscarriage rate, and live birth rate, as shown in Table 3. The results revealed that the abortion rates in Normal group were significantly lower than that in Overweight group (9.23% VS 11.29%, P= 0.021), but the live birth rates in Normal group were significantly higher than that in Overweight and Obese group (57.73% VS 53.82%, P= 0.008; 57.73% VS 53.53%, P= 0.021). There was no significant difference in clinical pregnancy rates among groups (P > 0.05) (Table 3).

From the Table 3, we found that high BMI of male had a bad influence on the abortion rate and the live birth rate, but did not affect the clinical pregnancy rate. In the analysis in Table 3 we did not take into account factors such as the age and BMI of female. In order to better study the effect of BMI of male on clinical pregnancy outcome, we conducted a further logistic regression analysis, the results shown in the Table 4.

From the results of Table 4, we could find that after removing some of the female factors (such as age and BMI of female), the BMI of male did not affect the clinical pregnancy outcome, whether it's clinical pregnancy, abortion, or live birth. While, DFI had an influence on clinical pregnancy and live birth, but had no impact on abortion.

DISCUSSION

Following the social development, the living standard of human beings has been substantially improved, and the lifestyle changed. The proportion of obese people worldwide has been steadily increasing. Over recent years, the effect of obesity on egg and sperm quality and the effect of obesity on embryo development, pregnancy, and obstetric outcomes have become the focal topic in the field of assisted reproduction.

Male obesity affects semen quality and spermatogenesis by affecting reproductive endocrinology and changing spermatogenic environment. An increase in the volume and number of fat cells in obese men is associated with lower levels of free and total testosterone in obese men [41]. The majority of obese infertile men are characterized by low Gonadotropin, high estrogen, and low androgen, the secondary hypogonadal function (MOSH) associated with male obesity [41,42]. The results showed that 40% of men with BMI > 30Kg/m2 had MOSH [43], which may be related to the increased expression and activity of aromatase cytochrome P450 in adipose tissue of obese men, adipose tissue large amounts of androgens into estrogen related [44]. In the human body, the hypothalamus produces pulses of Progonadoliberin-1, which act on the pituitary to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH), FSH and LH act on testicular tissue to make it secrete steroid hormone. There is ESR1 in the male hypothalamus, and excess estrogen in obese men can reduce testosterone through a negative feedback mechanism [45]. The obesity male body fat tissue content increases, the fat tissue excessively expands and the blood vessel produces the imbalance to cause the fat tissue low oxygen condition, promotes the fat tissue inflammation the occurrence, causes the secretion the cellular factor ingredient to have the change, secretes the inflammation medium to promote the chronic inflammation. These inflammatory cytokines cause a decrease in pituitary Gonadotropin secretion, which in turn leads to a decrease in testosterone secretion [46]. Testosterone acts on Sertoli cells and promotes spermatogenesis by regulating their function. Studies have found that normal spermatogenesis is maintained when testosterone levels in the testis are above 70 nmol/L [47]. Therefore, the decrease of testosterone level affects spermatogenesis. Hormone deficiency, inflammation and

Table 2a. Effect of BMI of male on fertilization, embryo development, and embryo quality.

Item	Lean group (BMI < 18.5 Kg/m², N=134)	Normal group (18.5 Kg/m²≤ BMI < 24 Kg/m², N=2219)	Overweight group (24 Kg/m² ≤ BMI < 28 Kg/m², N=2497)	Obese group (BMI ≥28 Kg/m², N= 1147)	P value
2PN fertilization rate (%)	0.63±0.18	0.64±0.17	0.64±0.18	0.65±0.18	> $0.05^{abc\alpha\beta\gamma}$
Normal fertilization cleavage rate (%)	0.99±0.04	0.99±0.05	0.99±0.05	0.99±0.05	> $0.05^{abc\alpha\beta\gamma}$
High quality embryo rate (%)	0.71±0.26	0.70±0.26	0.70±0.25	0.70±0.25	> $0.05^{abc\alpha\beta\gamma}$
Blastocyst formation rate (%)	0.54±0.28	0.56±0.31	0.48±0.32	0.44±0.29	> $0.05^{ab\gamma}$ 0.003^{c} 0.000$^{\alpha\beta}$
High scoring blastocyst rate (%)	0.15±0.20	0.14±0.20	0.14±0.21	0.15±0.21	> $0.05^{abc\alpha\beta\gamma}$

BMI: Body Mass Index; 2PN: two Pronuclear; P < 0.05 was considered to be statistically significant.
a: Lean group vs. Normal group; b: Lean group vs. Overweight group; c: Lean group vs. Obese group. a: Normal group vs. Overweight group; β: Normal group vs. Obese group; γ: Overweight group vs. Obese group
DFI: Sperm DNA fragmentation index; P < 0.05 was considered to be statistically significant.

Table 2b. Effect of Male's DFI on fertilization, embryo development, and embryo quality.

Item	Low DFI group (DFI < 20% N=4969)	High DFI group (DFI ≥ 20% N=1028)	P value
2PN fertilization rate (%)	0.64±0.17	0.65±0.18	0.059
Normal fertilization cleavage rate (%)	0.99±0.05	0.99±0.04	0.962
High quality embryo rate (%)	0.70±0.26	0.68±0.25	0.088
Blastocyst formation rate (%)	0.52±0.31	0.43±0.32	0.000
High scoring blastocyst rate (%)	0.15±0.21	0.12±0.20	0.000

DFI: Sperm DNA fragmentation index; P < 0.05 was considered to be statistically significant.

Table 3: Effect of BMI of male on Pregnancy Outcome.

Item	Lean group (BMI < 18.5 Kg/m², N=134)	Normal group (18.5 Kg/m²≤ BMI < 24 Kg/m², N=2219)	Overweight group (24 Kg/m² ≤ BMI < 28 Kg/m², N=2497)	Obese group (BMI ≥28 Kg/m², N= 1147)	P value
Clinical pregnancy rate (%)	68.66% (92/134)	67.15% (1490/2219)	65.36% (1632/2497)	64.78% (743/1147)	> $0.05^{abc\alpha\beta\gamma}$
Abortion rate (%)	13.43% (18/134)	9.23 % (205/2219)	11.29% (282/2497)	11.16% (128/1147)	> $0.05^{abc\beta\gamma}$ 0.021$^{\alpha}$
Live birth rate (%)	55.22% (74/134)	57.73% (1281/2219)	53.82% (1344/2497)	53.53% (614/1147)	> $0.05^{abc\gamma}$ 0.008$^{\alpha}$ 0.021$^{\beta}$

BMI: Body Mass Index; P < 0.05 was considered to be statistically significant.
a: Slimming group vs. Normal group; b: Slimming group vs. Overweight group; c: Slimming group vs. Obesity group; α: Normal group vs. Overweight group; β: Normal group vs. Obesity group; γ: Overweight group vs. Obesity group

oxidative stress gradually impair the environment necessary for testicular spermatogenesis and epididymal sperm maturation. The adverse effects of male obesity on spermatogenesis and sperm maturation lead to a decrease in sperm quality, an increase in DNA damage, abnormal Epigenetics, resulting in a decline in male health and fertility, impaired embryo quality, and stunted development, high rate of miscarriage. The effect of male obesity on the outcome of in IVF-ET has been controversial.

In males with an intact HPG axis, testosterone and inhibin B levels decrease as BMI increases, possibly leading to increases in LH and FSH, respectively. Chavarro et al., have shown that a high BMI could lead to impaired feedback regulation of the HPT axis, especially in men who eventually develop abnormal semen quality [48]. A strong correlation was found between decreased sperm motility and oxidative stress [12,13,15-29,49]. Sperm movement is produced by adenosine triphosphate (ATP) continuously produced by mitochondria located in the middle of the sperm. In order to produce ATP through oxidative phosphorylation, the mitochondrial membrane must be selectively permeable, thus maintaining an electrolyte gradient between the inside and outside of the mitochondria. An excess of reactive oxygen species (ROS) alters the phospholipid membrane, thereby destroying its selectivity, and also inhibits oxidative phosphorylation, ultimately resulting in reduced ATP

Table 4: Logistic regression analysis on influencing factors of clinical pregnancy outcome.

Factors	clinical pregnancy				abortion				live birth			
	β value	P value	OR	95%CI	β value	P value	OR	95%CI	β value	P value	OR	95%CI
Age of female	-0.089	0.000	0.915	0.898-0.933	0.083	0.000	1.087	1.054-1.020	-0.067	0.010	0.935	0.889-0.984
BMI of female	0.009	0.299	1.009	0.992-1.027	0.028	0.040	1.028	1.001-1.056	-0.001	0.972	0.999	0.955-1.045
Age of male	-0.018	0.027	0.982	0.966-0.998	-0.005	0.681	0.995	0.969-1.021	-0.008	0.728	0.993	0.951-1.035
BMI of male	-0.003	0.661	0.997	0.981-1.012	0.007	0.559	1.007	0.983-1.032	-0.011	0.597	0.989	0.951-1.030
PR	0.001	0.634	1.001	0.996-1.007	-0.004	0.363	0.996	0.987-1.005	0.025	0.001	1.026	1.010-1.041
DFI (%)	0.009	0.024	1.009	1.001-1.017	-0.008	0.231	0.992	0.980-1.005	0.022	0.045	1.022	1.001-1.044
Intercept	3.703	0.000	50.566	—	-4.564	0.000	0.010	—	4.008	0.000	55.028	—

BMI: Body Mass Index; PR: Progressive motility; DFI: Sperm DNA fragmentation index; P < 0.05 was considered to be statistically significant.

production [12,13,15-30,49,50]. Previous studies have found that high BMI leads to a decrease in the mitochondrial activity of men's sperm, which affects sperm motility and motility [12]. Our results showed that male BMI did not affect ejaculate volume and sperm concentration, which was similar to Roberta et al and Campbell et al. [12,16]. In contrast, we found that the Normal group had a higher percentage of progressive motility sperm than the Obesity group, while Roberta et al and Campbell et al suggested that male BMI did not affect sperm morphology and motility [12, 16]. Moreover, Taha and colleagues showed that the sperm concentration, the percentage of progressive motility sperm, and the percentage of normal morphology of sperm in fertile obese men were significantly lower than those in fertile men and overweight men (all P < 0.05) [13]. They argued that even among fertile men, an increase in BMI had a negative effect on semen parameters, which was consistent with put results.

High BMI leads to ROS overproduction, which oxidizes DNA bases (mainly guanosine) by producing by-products of lipid degradation that bind to DNA or come in direct interaction with DNA strands, resulting in nonspecific C single and double-strand breaks and increased DNA damage [12,13,15-29]. Compared with normal-weight group (19.9% ± 1.96%), overweight group (25.8% ± 2.23%) and obese group (27.0% ± 3.16%) had higher DFI (P < 0.05); however, there was no significant difference in DFI between overweight group and obese group (25.8% ± 2.23% VS 27.0% ± 3.16%) (P > 0.05) [14]. Our results are consistent with those of Kort et al., [14]. In contrast, some studies showed that compared with the overweight group, the mitochondrial activity was lower, and the DFI was higher in the obese group (P< 0.05) [12,13]. A study from 2013 showed that DFI was significantly higher in obese men compared to men with normal BMI (P < 0.05) but not with overweight men (P > 0.05) [15]. Recent studies have shown that male obesity was associated with DNA breakage in sperm, and a higher BMI seemed to mean higher DFI [16-20]. Still, there was no consensus on the relationship between BMI and DFI in men. Some researchers argued that there was not enough data to show a positive association between BMI and DFI. High or low DFI was not associated with BMI, and high BMI did not seem to be associated with impaired sperm DNA integrity [21-24].

As the early development of embryos is mainly driven by oocytes, most of the existing studies have focused on the effect of obesity on embryo development in women, while there are few studies on the effect of male obesity on embryonic development [25,26,29-38,50]. Our analysis revealed that male BMI did not affect in vitro fertilization rates, cleavage rates, and high-quality embryo rates, which is consistent with previous studies [30-38,50]. We also found that high BMI led to a significant decrease in blastocyst formation rate, and high DFI led to a significant decrease in blastocyst formation rate and high scoring blastocyst rate. Our results showed that high BMI and high DFI were disadvantageous to blastocyst formation, and high DFI was mean a lower high scoring blastocyst rate. There has also been debate about the effect of male BMI on pregnancy outcomes. Many studies have shown that a man's BMI did not affect pregnancy and obstetric outcomes [30-38,50]. A prospective cohort study assessing the relationship between BMI and clinical outcomes in men showed no significant association between BMI and clinical pregnancy and live birth rates during IVF cycles (P > 0.05). Nevertheless, male obesity may deleteriously affect live birth rates during the ICSI cycle [25]. Likewise, Umul et al., and Mushtaq et al., [26,49] suggested that an increase in male BMI during the ICSI cycle results in a significant decrease in clinical pregnancy and live birth rates (P < 0.05) [26,49]. In addition, Yang et al also showed that the clinical pregnancy rate was significantly lower in men with a BMI of more than 28 Kg/m2 than in those with a normal BMI (P < 0.05) [28]. Our results showed that the too high BMI of male resulted in a significant increase in the abortion rate and a significant decrease in live birth rate (p < 0.05) but did not affect the clinical pregnancy rates (p > 0.05).

In our study, we investigated the effect of male obesity on sperm quality, after which we explored the effects of BMI and DFI on embryo development, embryo quality, and pregnancy outcomes. We found that male obesity leads to increased DFI, which affected blastocyst formation and clinical pregnancy outcomes. This suggested that we should not only focus on the BMI of women in clinical practice but also pay attention to the BMI of men to achieve better pregnancy outcomes. At present,

there are many studies on the mechanism of male obesity on semen quality, but few on the mechanism of male obesity on embryo development and embryo quality. To the best of our knowledge, we evaluated the effects of male BMI on semen quality, embryo development and embryo quality, and ultimately pregnancy outcome. Nonetheless, its specific impact mechanism needs to be further studied and validated.

CONCLUSION

High BMI in men leads to high DFI, low blastocyst formation, high miscarriage rate, and low live birth rate. High DFI is harmful to blastocyst formation and high scoring blastocyst rate, and DFI had an influence on clinical pregnancy and live birth. In a word, high BMI indirectly has harmful effects on the outcome of IVF cycles by affecting DFI. In ART process, men also should control their BMI in order to have a good pregnancy outcome.

ETHICAL APPROVAL

Our study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (No.2022-KY-0339-002)

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Lin Qi developed the original concept of this study. Lin Qi, Ya-ping Liu and Ying-Chun Su participated in the study design. Ya-Ping Liu, Shi-Ming Wang and Xiao-li Chen participated in data collection, Ya-Ping Liu conducted data analysis and interpretation and the writing of the original version of the manuscript. Hao Shi guided the data analysis and gave guidance. All authors participated in the manuscript revision. All authors have contributed to critical discussion and reviewed the final version.

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AVAILABILITY OF DATA AND MATERIALS

The data used in current study is available from the corresponding author on reasonable request.

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