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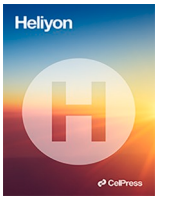

Influence of maternal obesity on embryonic vitrification injury and subsequent pregnancy outcomes: A retrospective cohort study☆

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ABSTRACT

Background: We previously reported that obese mice had significantly high lipid content in embryos, and excessive lipids are detrimental to embryonic development. However, whether maternal obesity has an effect on embryonic vitrification injury and subsequent pregnancy outcomes is still controversial. This study was conducted to clarify the influence of maternal obesity on embryonic vitrification injury and subsequent pregnancy outcomes by in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI).

Methods: We retrospectively collected medical record of IVF/ICSI patients from reproductive medicine centers in two tertiary hospitals. The patients were classified into a low-weight group (<18.5 kg/m2), normal-weight group (18.5–23.9 kg/m2), overweight group (24.0–27.9 kg/m2) and obese group (≥28.0 kg/m2) according to their body mass index (BMI). Multivariable logistic regression analysis was performed to compare pregnancy outcomes in fresh and frozen embryo transfer among different BMI groups to define the correlation between BMI and embryonic vitrification injury.

Results: A total of 44 773 women among 20–40 years old were recruited in this study, of which 27 797 underwent their first fresh embryo transfer and 16 976 underwent their first frozen embryo transfer. For fresh embryo transfer, there was no significant difference in the clinical pregnancy rate, live birth rate, and miscarriage rate of 4 BMI groups. For frozen-thawed embryo transfer, there was a significant increase in the clinical pregnancy rate of the overweight group (AOR = 1.14, 95% CI: 1.05–1.25) and the obese group (AOR = 1.24, 95% CI: 1.03–1.50), while the

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miscarriage rate (AOR = 1.42, 95% CI: 1.05–1.92) also showed a significant increase in the obese group compared to the normal-weight group.
Conclusion: This study provided a new understanding of the effect of maternal obesity on embryonic vitrification injury. Maternal obesity does not worsen the outcome of IVF/ICSI, particularly in the frozen-thawed group.

1. Introduction

Vitrification is increasingly popular employed in assisted reproductive technology [1], characterized by rapidly freezing of tissue or intracellular fluid to a glass-like structure to reduce freezing damage [2]. However, vitrification can also cause damage to the embryo, including cryoprotectant and low-temperature damage. Cryoprotectants [3], typically used to reduce ice crystal formation during the freezing process, have its own risks, including toxicity and osmotic damage. Additionally, Cavoretto Paoloin's study discovered that in assisted reproduction technique pregnancies from blastocyst transfer, particularly after cryopreservation, both the nuchal translucency measurement and free β-human chorionic gonadotrophin (β-hCG) concentration are significantly higher as compared to spontaneous conceptions, which might be another form of injury associated with the vitrification technique [4]. To mitigate these risks, researchers have explored several strategies, such as selecting cryoprotectants with low concentrations and toxicity levels, minimizing the volume of the embryo, and accelerating the freezing and thawing rate. Despite the effective implementation of these strategies, the problem of vitrification damage still remains. Consequently, further investigations into techniques for reducing freezing damage and improving the developmental potential of embryos are urgently needed.

Despite the fact that obesity is increasing among reproductive-age females, there is growing concern about its effects on pregnancy outcomes [5]. The World Health Organization defines obesity as a body mass index (BMI) > 30 kg/m^2. However, in China, obesity is defined as a BMI$\geq$28 kg/m^2 due to higher susceptibility to obesity-related complications at lower BMIs than Europeans [6]. Recent research has suggested that obesity is associated with an increased risk of severe diseases such as diabetes, hypertension, and fatty liver [7,8], and it also closely affects pregnancy outcomes.

During embryo freezing, the cell membrane and the internal structure are exposed to low temperatures and chemical stress due to ice crystals. Lipids are the primary macro-molecular substances in cells and the embryo's developmental potential is closely related to its metabolic rate, and hence adequate ATP is imperative for embryonic cells [9]. Fleming and Saacke [10] discovered a close spatial correlation between mitochondria and the endoplasmic reticulum in bovine eggs; they were distributed near lipids and formed "metabolic units".

However, further studies investigating the effects of vitrification on embryonic lipids are lacking. Jung et al. [11] found that lipids in mouse eggs and cell membranes were redistributed and rearranged after vitrification, although the overall lipid content remained unchanged. Upon vitrification in liquid nitrogen for two weeks, the eggs had a significant decrease in their lipid content, a key source of energy for embryonic development. Supporting this, Aono et al. [12] claimed that vitrification of bovine eggs led to a reorganization of lipids and a destruction of the integrity of the egg's plasma and inner membrane.

Excessive lipids have been shown to detrimentally affect embryonic development. Wu et al. [13] found a significant increase of lipids in ova in high-fat diet-induced obese mice, accompanied by a decrease in mitochondrial membrane potential and an increase in endoplasmic reticulum stress genes, subsequently resulting in a decreased fertilization rate and decreased embryonic developmental rate. Studies on porcine and bovine embryos have also shown that elevated lipid accumulation can worsen vitrification injury [14,15]. While the lipids of bovine (63 ng/oocyte) and porcine oocytes (161 ng/oocyte) are relatively high, human oocytes have a much lower lipid content of 4 ng/oocyte [16,17]. In 2012, we investigated the effects of obesity on mouse embryonic vitrification injury and found

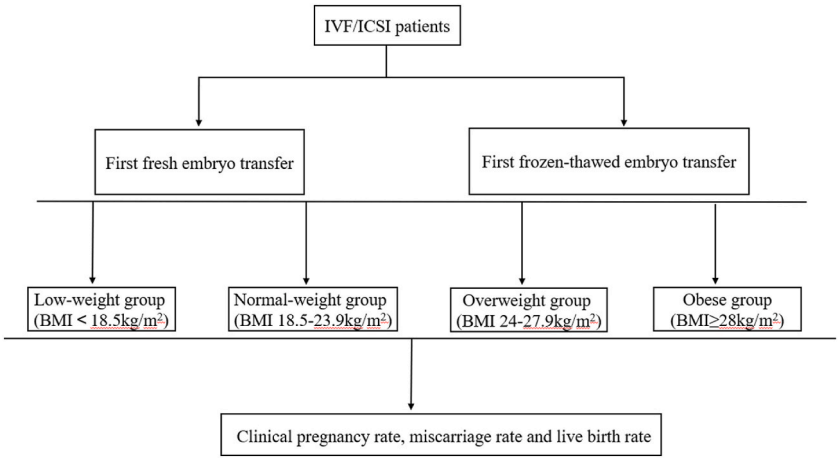

Fig. 1. Flow chart of this study.

that high-fat diet-induced obese mice were significantly different in lipid content (0.044 vs. 0.030, $P < 0.01$), apoptosis rate (15.1% vs. 9.3%, $P < 0.05$), blastocyst survival rate (83.1% vs. 93.1%, $P < 0.01$) and developmental competence than their normal-weight counterparts [18]. However, no significant difference was found in thawed embryos between the two groups. This study concluded that high lipid levels had a lipotoxic effect on fresh mouse embryos, and could potentially act as a protective factor for frozen-thawed mouse embryos. In addition, we further explored the effects of weight on embryonic vitrification injury in Chinese women and the data suggested that being overweight or obese did not exacerbate vitrification injuries [19]. This was a significant finding, but due to the limited size of the study, more studies are needed to provide high-quality evidence to support this conclusion.

2. Methods

2.1. Study design and study population

This was a retrospective cohort study from two tertiary hospitals in China. Patients were recruited from the Reproductive Medical Center of the First Affiliated Hospital of Guangxi Medical University from January 2014 to January 2021 and Reproductive Medical Center of Liuzhou Municipal Maternity and Child Health Care Hospital from January 2010 to January 2020. The flow of this study was shown in Fig. 1. A total of 44 773 in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles were collected in this study, of which 27 797 underwent fresh embryo transfer and 16 976 underwent frozen embryo transfer.

The inclusion criteria for the study were age of 20–40 years, the first fresh/frozen embryo transfer cycle, and complete medical records. The exclusion criteria were couples with genital tract malformation, intrauterine adhesion, hydrosalpinx, male azoospermia, recurrent miscarriage, adenomyosis, or chromosomal abnormalities (in one or both of the couple). Multivariable logistic regression was used to adjust for confounding factors for pregnancy outcomes.

2.2. Measures

According to BMI, patients were divided into a low-weight group (<18.5 kg/m^2), normal-weight group (18.5–23.9 kg/m^2), overweight group (24.0–27.9 kg/m^2), and obese group (≥ 28.0 kg/m^2).

The primary outcomes included the live birth rate, defined as the ratio of live births to transferred cycles; the clinical pregnancy rate, defined as the ratio of the sum of intrauterine gestation and ectopic pregnancy to transferred cycles; and the miscarriage rate, defined as the ratio of miscarriage to clinical pregnancy.

Patients underwent the IVF/ICSI technique according to the patient's general characterizations. The fertilized oocytes were cultured with G1.5 and G2.5 sequential media (Vitrolife, Kungsbacka, Sweden) at 37 °C with 6% CO2 and 5% O2. On the 3rd day after fertilization, embryos were graded according to the number and regularity of blastomeres and the degree of embryonic fragmentation from Cummins's criteria. Grades 1 and 2 were considered high-quality embryos. On day 5 or 6, the quality of blastocysts was scored according to the Gardner blastocyst grading system. At this stage, the blastocysts with >3BB grade were considered high-quality, whereas the blastocysts with >3CC grade were transferred or frozen. We transferred blastomere or blastocyst embryos on the 3rd or 5th day after oocyte collection based on the patient's wishes and the quality and quantity of embryos. If the number of high-quality D3 embryos is more than 4, blastocyst culturing is recommended. If the patient was not suitable for fresh embryo transfer, two of D3 cleavage-stage embryos were collected to freeze, and the rest were collected to culture to blastocyst to freeze and thaw when needed.

Peripheral blood β-HCG was measured 12–14 days after transplantation to determine pregnancy, and intrauterine gestational sacs were examined by ultrasonography 28–35 days after transplantation to determine clinical pregnancy. If pregnancy was established, luteal support was used until 10–12 weeks of gestation.

2.3. Vitrification and warming procedure

The procedure of vitrification was according to the manufacturer's instruction (Kitazato BioPharma, Shizuoka, Japan). In brief, embryos were carefully transferred to the top centre of a 300 μl droplet of equilibration solution (ES) at room temperature (RT) and allowed to remain there for a maximum of 15 min. During this time, a cycle of shrinkage (dehydration) and re-expansion (ES infiltration) was observed. Once the embryos were adequately equilibrated and had reached the minimum volume of ES, they were transferred to the surface of a 300 μl droplet of vitrification solution 1 (VS1) at RT for a duration of 30 s. Within this period, the embryos were gently displaced three times within the VS1 droplet to ensure complete removal of ES. Subsequently, the embryos, now with the minimum volume of VS1, were transferred to a 300 μl droplet of vitrification solution 2 (VS2) for an additional 30 s. During this time, the embryos were stirred and displaced twice within the VS2 droplet, following the same procedure as before, until a noticeable flat shrinkage occurred, indicating complete dehydration. As soon as the embryos were placed onto the thin polypropylene strip of the cryotop, the excess VS2 around the embryos was removed and immediately submerged vertically into liquid nitrogen.

For warming, the cryotop was immersed directly into 1 ml of pre-warmed (37 °C) thawing solution (TS) for 1 min. Subsequently, the thawed embryos in TS were gently deposited at the bottom of a 300 μl droplet of dilution solution (DS) for a gradual displacement from TS to DS for 3 min at RT. After that, embryos with a 2 mm column of DS (from the tip of the pipette) were gently deposited at the bottom of a 300 μl droplet of WS1 for gradual displacement from DS to WS1 for 5 min at RT. Once the embryos reached the minimal volume of WS1, they were subjected to two additional submersions in washing solution 2 (WS2) before being prepared for embryo culture.

2.4. Statistical analysis

SPSS Statistics version 26.0 was used to analyze the data. Continuous variables are described as the mean ± standard deviation (mean ± SD) and differences between groups were compared by independent-sample *t*-test; categorical variables are described as the frequency and percentage (%), and the chi-square test or continuous adjusted chi-square test compared proportions between groups. Multivariable logistic regression was performed to calculate the crude odds ratio (COR), adjusted odds ratio (AOR), and corresponding 95% confidence interval (CI). $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Basic characteristics of patients undergoing fresh/frozen-thawed embryo transfer

A total of 27 797 fresh embryo transfer cycles were recruited, including 3342 low-weight, 18 884 normal-weight, 4638 overweight and 933 obese patients (Table 1). A total of 16 976 frozen-thawed embryo transfer cycles were enrolled, including 2136 low-weight, 11 723 normal-weight, 2622 overweight, and 495 obese patients (Table 2). The numbers of high-quality embryos and embryo stage were significantly different among the fresh/frozen-thawed cycles of the 4 BMI groups, showing a decrease in the number of high-quality embryos and transferred embryos in obese patients.

3.2. Pregnancy outcomes of fresh cycles among different maternal BMIs

Pregnancy outcomes for the fresh cycle were compared between different BMI groups after adjustment for maternal age, paternal age, causes of infertility, ovarian hyperstimulation protocol, number of high-quality embryos, and D3/D5 transferred embryos. The clinical pregnancy rate (AOR = 1.00, 95% CI: 0.92–1.08), live birth rate (AOR = 1.02, 95% CI: 0.93–1.11), and miscarriage rate (AOR = 0.83, 95% CI: 0.69–1.00) of the low-weight group were not significantly different from those of the normal BMI group. For the overweight group, there was no significant difference in the clinical pregnancy rate (AOR = 1.01, 95% CI: 0.94–1.08), live birth rate (AOR = 0.99, 95% CI: 0.92–1.07), or miscarriage rate (AOR = 1.00, 95% CI: 0.86–1.15). The obese group also showed no significant

Table 1
Basic characteristics and pregnancy outcomes of 4 groups patients in their first fresh embryo transfer cycle.

Parameter		BMI(kg/m^2)				P value
	total	<18.5	18.5–23.9	24.0–27.9	≥28.0	
Cases, n (%)	27797	3342 (12.02%)	18884 (67.94%)	4638 (16.69%)	933 (3.36%)	
Maternal age, n (%)						
<30	8008	1343 (40.19%)	5430 (28.75%)	1010 (21.78%)	225 (24.12%)	<0.001
30–34	11309	1389 (41.56%)	7678 (40.66%)	1866 (40.23%)	376 (40.30%)	
35–39	7466	559 (16.73%)	5065 (26.82%)	1553 (33.48%)	289 (30.98%)	
≥40	1014	51 (1.53%)	711 (3.77%)	209 (4.51%)	43 (4.61%)	
Paternal age, n (%)						
<40	23191	3048 (91.20%)	15766 (83.49%)	3634 (78.35%)	743 (79.64%)	<0.001
40–49	4379	289 (8.65%)	2961 (15.68%)	947 (20.42%)	182 (19.51%)	
≥50	227	5 (0.15%)	157 (0.83%)	57 (1.23%)	8 (0.86%)	
Causes of infertility, n (%)						
Paternal factors	2818	384 (11.49%)	1933 (10.24%)	409 (8.82%)	92 (9.86%)	<0.001
Fallopian factors	10783	1279 (38.27%)	7372 (39.04%)	1814 (39.11%)	318 (34.08%)	
PCOS	316	20 (0.60%)	195 (1.03%)	76 (1.64%)	25 (2.68%)	
Others	13880	1659 (49.64%)	9384 (49.69%)	2339 (50.43%)	498 (53.38%)	
Protocol, n (%)						
GnRH-agonist long protocol	21397	2683 (80.28%)	14704 (77.86%)	3373 (72.73%)	637 (68.27%)	<0.001
GnRH-antagonist protocol	2921	265 (7.93%)	1854 (9.82%)	651 (14.04%)	151 (16.18%)	
Minimal stimulation protocol	1393	171 (5.12%)	965 (5.11%)	213 (4.59%)	44 (4.72%)	
Others	2086	223 (6.67%)	1361 (7.21%)	401 (8.65%)	101 (10.83%)	
High-quality embryos, mean (SD)	3.46 ± 3.49	3.55 ± 3.51	3.46 ± 3.48	3.43 ± 3.53	3.14 ± 3.23	0.017
D3, n (%)	13263	1501 (78.67%)	8959 (80.71%)	2343 (82.27%)	460 (82.29%)	0.015
D5, n (%)	3152	407 (21.33%)	2141 (19.29%)	505 (17.73%)	99 (17.71%)	
D3 transfer, mean (SD)	1.90 ± 0.36	1.89 ± 0.34	1.90 ± 0.36	1.90 ± 0.37	1.84 ± 0.39	0.014
D5 transfer, mean (SD)	1.08 ± 0.27	1.08 ± 0.27	1.08 ± 0.27	1.09 ± 0.28	1.07 ± 0.26	0.962
Cycle cancellation, n (%)	9723	1170 (35.01%)	6617 (35.04%)	1581 (34.09%)	355 (38.05%)	0.138
Pregnancy outcomes, n (%)						
Clinical pregnancy	8959	1081 (32.35%)	6086 (32.23%)	1505 (32.45%)	287 (30.76%)	0.790
Miscarriage	1330	131 (12.12%)	923 (15.17%)	234 (15.55%)	42 (14.63%)	0.079
Live birth	7330	905 (27.08%)	4975 (26.35%)	1209 (26.07%)	241 (25.83%)	0.745

Clinical pregnancy rate: the ratio of the sum of intrauterine gestation and ectopic pregnancy to transferred cycles; miscarriage rate: the ratio of miscarriages to clinical pregnancy; live birth rate: the ratio of live births to transferred cycles. $P < 0.05$ indicated that the difference was statistically significant.

Table 2
Basic characteristics and pregnancy outcomes of 4 groups patients in their first thawed embryo transfer cycle.

Parameter	total	BMI(kg/m^2)				P value
		<18.5	18.5–23.9	24.0–27.9	≥28.0	
Cases, n (%)	16976	2136 (12.58%)	11723 (69.06%)	2622 (15.45%)	495 (2.92%)	
Maternal age, n (%)						
<30	4377	779 (36.47%)	2982 (25.44%)	515 (19.64%)	101 (20.40%)	<0.001
30–34	7081	907 (42.46%)	4901 (41.81%)	1065 (40.62%)	208 (42.02%)	
35–39	4859	419 (19.62%)	3365 (28.70%)	910 (34.71%)	165 (33.33%)	
≥40	659	31 (1.45%)	475 (4.05%)	132 (5.03%)	21 (4.24%)	
Paternal age, n (%)						
<40	13957	1924 (90.07%)	9636 (82.20%)	1995 (76.09%)	402 (81.21%)	<0.001
40–49	2868	208 (9.74%)	1980 (16.89%)	587 (22.39%)	93 (18.79%)	
≥50	151	4 (0.19%)	107 (0.91%)	40 (1.53%)	0 (0%)	
Protocol, n (%)						
Natural cycle	6398	857 (40.12%)	4588 (39.14%)	817 (31.16%)	136 (27.47%)	<0.001
HRT cycle	5682	666 (31.18%)	3808 (32.48%)	1010 (38.52%)	198 (40.00%)	
Ovulation induction cycle	4565	567 (26.54%)	3094 (26.39%)	748 (28.53%)	156 (31.52%)	
HRT cycle with GnRH agonists	331	46 (2.15%)	233 (1.99%)	47 (1.79%)	5 (1.01%)	
High-quality embryos, mean (SD)	0.96 ± 0.84	0.97 ± 0.89	0.96 ± 0.83	0.97 ± 0.83	0.86 ± 0.83	0.049
Transferred embryos, mean (SD)	1.64 ± 0.58	1.63 ± 0.58	1.65 ± 0.58	1.62 ± 0.59	1.57 ± 0.58	0.001
D3, n (%)	9304	1107 (51.90%)	6475 (55.39%)	1470 (56.28%)	252 (50.91%)	0.003
D5, n (%)	7625	1026 (48.10%)	5214 (44.61%)	1142 (43.72%)	243 (49.09%)	
D3 transfer, mean (SD)	1.91 ± 0.52	1.93 ± 0.50	1.92 ± 0.51	1.88 ± 0.53	1.80 ± 0.56	0.001
D5 transfer, mean (SD)	1.33 ± 0.48	1.31 ± 0.47	1.33 ± 0.48	1.31 ± 0.47	1.33 ± 0.49	0.206
Cycle cancellation, n (%)	134	15 (0.70%)	89 (0.76%)	23 (0.88%)	7 (1.41%)	0.381
Pregnancy outcomes, n (%)						
Clinical pregnancy	7854	100 (47.10%)	5350 (45.64%)	1251 (47.71%)	247 (49.90%)	0.063
Miscarriage	1213	136 (13.52%)	818 (15.29%)	209 (16.71%)	50 (20.24%)	0.009
Live birth	6390	841 (39.37%)	4361 (37.20%)	997 (38.02%)	191 (38.59%)	0.256

HRT: hormone replacement therapy. Clinical pregnancy rate: the ratio of the sum of intrauterine gestation and ectopic pregnancy to transferred cycles; miscarriage rate: the ratio of miscarriages to clinical pregnancy; live birth rate: the ratio of live births to transferred cycles. $P < 0.05$ indicated that the difference was statistically significant.

difference in the clinical pregnancy rate (AOR = 0.97, 95% CI: 0.84–1.12), live birth rate (AOR = 1.02, 95% CI: 0.88–1.19), or miscarriage rate (AOR = 0.90, 95% CI: 0.66–1.24) (Tables 3–5).

3.3. Pregnancy outcomes of frozen-thawed cycles among different maternal BMIs

When analyzing the effect of different BMIs on frozen-thawed embryo transfer after adjustment for maternal age, paternal age, endometrial preparation protocol, and the number of high-quality embryos and D3/D5 transferred embryos, the low-weight group had no significant difference in the clinical pregnancy rate (AOR = 0.97, 95% CI: 0.88–1.07), live birth rate (AOR = 0.99, 95% CI: 0.90–1.10) or miscarriage rate (AOR = 0.93, 95% CI: 0.77–1.13) compared to the normal BMI group. The overweight group had no significant difference in the live birth rate (AOR = 1.10, 95% CI: 1.00–1.21) or miscarriage rate (AOR = 1.12, 95% CI: 0.95–1.31). However, the clinical pregnancy rate (AOR = 1.14, 95% CI: 1.05–1.25) was significantly higher than that in the normal BMI group. For the obese group, the clinical pregnancy rate (AOR = 1.24, 95% CI: 1.03–1.50) and miscarriage rate (AOR = 1.42, 95% CI: 1.05–1.92) were statistically higher, while the live birth rate (AOR = 1.12, 95% CI: 0.92–1.35) showed no significant difference (Tables 3–5).

4. Discussion

In this large-scale retrospective study, we evaluated the influence of female obesity and overweight on pregnancy outcomes after

Table 3
The effect of BMI on the clinical pregnancy rate of vitrified embryo transfer.

Parameter	Fresh cycle				Thawed cycle (vitrification)			
	clinical pregnancy rate (%)	COR	AOR	95% CI	clinical pregnancy rate (%)	COR	AOR	95% CI
Number of cases	8959				7854			
<18.5 kg/m^2	32.35%	1.01	1.00	[0.92,1.08]	47.10%	1.06	0.97	[0.88,1.07]
18.5–23.9 kg/m^2	32.23%	1.00	1.00	Reference	45.64%	1.00	1.00	Reference
24–27.9 kg/m^2	32.45%	1.01	1.01	[0.94,1.08]	47.71%	1.09	1.14	**[1.05,1.25]**
≥28 kg/m^2	30.76%	0.93	0.97	[0.84,1.12]	49.90%	1.19	1.24	**[1.03,1.50]**

COR: crude odds ratio; AOR: adjusted odds ratio; CI: confidence interval. The OR value was adjusted by maternal age, paternal age, causes of infertility, protocol, number of high-quality embryos and D3/D5 transferred embryos.

Table 4
The effect of BMI on the live birth rate of vitrified embryo transfer.

Parameter	Fresh cycle				Thawed cycle (vitrification)			
	Live birth rate (%)	COR	AOR	95% CI	Live birth rate (%)	COR	AOR	95% CI
Number of cases	7330				6390			
<18.5 kg/m^2	27.08%	1.04	1.02	[0.93,1.11]	39.37%	1.10	0.99	[0.90,1.10]
18.5–23.9 kg/m^2	26.35%	1.00	1.00	Reference	37.20%	1.00	1.00	Reference
24–27.9 kg/m^2	26.07%	0.99	0.99	[0.92,1.07]	38.02%	1.04	1.10	**[1.00,1.21]**
≥28 kg/m^2	25.83%	0.97	1.02	[0.88,1.19]	38.59%	1.06	1.12	[0.92,1.35]

COR: crude odds ratio; AOR: adjusted odds ratio; CI: confidence interval. The OR value was adjusted by maternal age, paternal age, causes of infertility, protocol, number of high-quality embryos and D3/D5 transferred embryos.

Table 5
The effect of BMI on miscarriage rate of vitrified embryo transfer.

Parameter	Fresh cycle				Thawed cycle (vitrification)			
	Miscarriage rate (%)	COR	AOR	95% CI	Miscarriage rate (%)	COR	AOR	95% CI
Number of cases	1330				1213			
<18.5 kg/m^2	12.12%	0.79	0.83	[0.69,1.00]	13.52%	0.91	0.93	[0.77,1.13]
18.5–23.9 kg/m^2	15.17%	1.00	1.00	Reference	15.29%	1.00	1.00	Reference
24–27.9 kg/m^2	15.55%	1.03	1.00	[0.86,1.15]	16.71%	1.16	1.12	[0.95,1.31]
≥28 kg/m^2	14.63%	0.92	0.90	[0.66,1.24]	20.24%	1.50	1.42	**[1.05,1.92]**

COR: crude odds ratio; AOR: adjusted odds ratio; CI: confidence interval. The OR value was adjusted by maternal age, paternal age, causes of infertility, protocol, number of high-quality embryos and D3/D5 transferred embryos.

the vitrification of embryo transfer. It was found that, the clinical pregnancy rate of embryo transfer after vitrification and thawed in overweight and obese women was significantly higher compared to that in the normal weight group. However, it was also found that the miscarriage rate in obese women was significantly higher. It was suggested that maternal overweight does not seem to aggravate vitrification injury of embryos, but obesity may have an impact.

4.1. The effect of obesity on embryo quality

Patients who underwent their first fresh/frozen embryo transfer cycle were represented in this analysis cohort. Numerous studies have suggested that obesity may be associated with insulin resistance [20], leptin resistance [21], and hyperandrogenism [22]. These conditions may affect ovarian response and reduce reproductive success. Our previous study suggested that obesity in mice induced ER stress and promoted the expression of stress-related genes, which adversely impacted embryo quality [18]. In this study, we found that the numbers of high-quality embryos and transferable embryos in the obese group were significantly lower in the fresh and thawed cycles, which was consistent with previous studies [23,24].

4.2. The effect of obesity on pregnancy outcomes undergoing fresh embryo transfer

Existing literature had extensively investigated the correlation between obesity and pregnancy outcomes. However, to the best of our knowledge, this is the first large-sample study to specifically study the influence of maternal obesity on embryonic vitrification and subsequent pregnancy outcomes. Previous studies have suggested that maternal obesity is associated with poorer pregnancy outcomes, such as a lower clinical pregnancy rate, lower live birth rate, and higher miscarriage rate [25,26]. Nevertheless, the impact of maternal obesity on assisted reproductive technology outcomes remains a topic of debate [27,28]. A study reported by Robker et al. [29] found that maternal BMI is positively correlated with the content of triglycerides in the follicular fluid. This increased content in obese women was found to be significantly higher than that of their normal-weight counterparts. Despite this, the clinical pregnancy rate, miscarriage rate, and live birth rate of overweight patients were not found to be significantly different from the normal-weight group in the fresh embryo transfer cycle. In our study, we also observed that the clinical pregnancy rate (30.76% vs. 32.23%) and live birth rate (25.83% vs. 26.35%) were similar in obese patients who underwent a fresh cycle compared with the normal weight group. Therefore, our study did not find any association between obesity and pregnancy outcomes undergoing fresh embryo transfer.

4.3. The effect of obesity on pregnancy outcomes undergoing frozen-thawed embryo transfer

Recent studies have suggested a link between maternal obesity and the risk of vitrification-induced embryo damage [13–15]. However, our data suggested a contrary outcome, revealing a significantly higher clinical pregnancy rate among overweight patients undergoing frozen-thawed embryo transfer, as compared to the normal weight group. And for obese patients underwent frozen-thawed embryo transfer cycle, the clinical pregnancy rate was statistically increased. In conclusion, these results suggested that maternal

overweight and obesity might play a protective role on the embryonic vitrification injury. This finding is in contrast to previous research, which suggested that high lipid content in pig/bovine embryos could exacerbate vitrification injury.

It is postulated that cells undergoing embryonic vitrification injury may require fatty acids to facilitate the repair of membrane integrity, with the reduced lipid content during vitrification resulting in a relative deficiency of these essential components [12]. For the fresh embryo transfer cycle, high content of lipids leads to a decrease in mitochondrial membrane potential due to lipid peroxidation, ultimately inducing endoplasmic reticulum stress and diminishing embryonic development rates [13,30]. Conversely, during the frozen-thawed embryo transfer cycle, high lipid levels may accelerate cell membrane repair, enhance embryonic energy metabolism and foster embryonic development [31]. This proposed mechanism offers a plausible explanation for how maternal obesity may reduce embryonic vitrification injury. Besides, recent research has demonstrated that the uterine artery pulsatility index is lower in IVF/ICSI pregnancies conceived after frozen blastocyst transfer as compared to fresh blastocyst transfer [32]. This mechanism might be further modulated in obese patients undergoing frozen-thawed transfers, ultimately resulting in improved pregnancy outcomes.

On the other hand, we also found that the frozen-thawed embryo transfer cycle, obese women showed an increased miscarriage rate compared to the normal weight group. However, no significant difference was found between the overweight and normal weight groups, which may be attributed to potential sample bias. Furthermore, it remains unclear whether the lipid content has a protective effect on the embryonic vitrification injury within a specific range (threshold effect). It is hypothesized that a particular range of lipids may facilitate cell membrane repair, enhance embryonic energy metabolism and foster embryonic development, thereby potentially increasing the incidence of clinical pregnancy. Conversely, lipids exceeding this specific range could have detrimental effects on the embryo's developmental capacity, consequently elevating the risk of miscarriage. To explore this hypothesis, further in-depth studies are needed.

Several limitations of this study should be acknowledged. First, this study collected large amounts of data, but it was a retrospective study. Prospective cohort studies controlling BMI over the treatment period would strengthen our conclusions. Second, we only obtained the BMI of patients from medical records but it might change over the course of their treatment. However, we only included patients with the first fresh embryo transfer and the first thawed embryo transfer, so the interval between BMI recording and embryo transfer time should not be too long. Actually, as we mentioned that obesity might be associated with insulin resistance, leptin resistance, and hyperandrogenism, whether women had obesity related-symptoms need to be considered in baseline characteristics. However, we noted that not all patients provided insulin, leptin, and androgen results, thereby limiting our analysis. Finally, in the frozen embryo transfer group, some patients have experienced fresh embryo transfer on previous oocyte-pick-up cycle, which makes the difference between the two groups in terms of the number of embryo transfer. Thus, the conclusion should be further evaluated prospectively in a larger sample to provide better evidence.

In conclusion, this study provided a new understanding of the effect of maternal obesity on embryonic vitrification injury. Overweight and obese women achieved a significantly higher clinical pregnancy rate than the normal weight group in the frozen-thawed cycle, indicating that maternal obesity does not worsen the outcome of IVF/ICSI, particularly in the frozen-thawed group.

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Patient consent

All data were acquired with informed consent of patients after approval from the hospital committee. The patients' private information was highly protected.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University on June 13, 2022, approval number 2022-KY-E−(189), and the Liuzhou Municipal Maternity and Child Health Care Hospital on June 6, 2022, approval number 2022-007.

Author contribution statement

Zhonghong Zeng: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. </p>
Wenhong Ma, Yihua Yang, Bo Liu, Xiaoqian Fu: Conceived and designed the experiments. </p>
Xi Wang, Shanjia Yi, Yin Bi, Dan Mo: Analyzed and interpreted the data. </p>
Wenhong Ma, Yihua Yang, Jingjing Li: Contributed reagents, materials, analysis tools or data. </p>

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection; BMI: body mass index; GnRH: gonadotropin-releasing hormone; HRT: hormone replacement therapy; β-HCG: β-human chorionic gonadotrophin; COR: crude odds ratio; AOR: adjusted odds ratio; CI: confidence interval.

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