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# Body mass index impacts ectopic pregnancy during in vitro fertilization: an analysis of 42,362 clinical pregnancy cycles

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## Abstract

**Purpose** This large, single-center, retrospective cohort study was aimed to explore the effect of female body mass index (BMI) on ectopic pregnancy (EP) following fresh and frozen-thawed embryo transfers (ET).

**Methods** A total of 27,600 pregnancies after fresh ET and 14,762 pregnancies after frozen-thawed ET were included between January 2010 to June 2022. Women were divided into three groups based on BMI according to the Working Group on Obesity in China (WGO), International Life Sciences Institute (ILSI): underweight (BMI < 18.5 kg/m<sup>2</sup>), normal weight (BMI, 18.5–23.9 kg/m<sup>2</sup>), and overweight or obesity (≥ 24 kg/m<sup>2</sup>). Compare EP rates among BMI categories in fresh and frozen-thawed ET cycles respectively. Multivariate logistic regression analyses were used to investigate the association between female BMI and EP.

**Results** The overall EP rates in fresh, and frozen thawed transfer cycles were 2.43% (672/27,600) and 2.82% (417/14,762), respectively. In fresh ET cycles, underweight women yielded a significantly higher EP rate than those with normal and excess weight (3.29% vs. 2.29% vs. 2.54%,  $P=0.029$ ). But EP rates did not differ among the three BMI groups (2.72% vs. 2.76% vs. 2.96%,  $P=0.782$ ) in frozen-thawed ET cycles. In fresh ET cycles, after adjusting for potential confounding factors, no significant association was found between female BMI and EP occurrence (adjusted OR: 0.98, 95% CI 0.70–1.37,  $P=0.894$ , for BMI 18.5–23.9 kg/m<sup>2</sup>; adjusted OR: 0.89, 95% CI 0.75–1.06,  $P=0.205$ , for BMI ≥ 24 kg/m<sup>2</sup>. Reference=BMI < 18.5 kg/m<sup>2</sup>).

**Conclusion(s)** Female BMI did not affect the occurrence of ectopic pregnancy in either fresh or frozen-thawed embryo transfer cycles.

**Keywords** Female BMI, Ectopic pregnancy, IVF/ICSI, Embryo transfer

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## Introduction

Ectopic pregnancy (EP), which mainly includes fallopian tube pregnancy, cornual pregnancy, cervical pregnancy, and ovarian pregnancy, is one of the potentially life-threatening conditions [1, 2]. In pregnancies after natural conception, the incidence of this early pregnancy complication varies from 1 to 2% [3]. However, the rate of EP in assisted reproduction techniques (ART), especially in in vitro fertilization (IVF) and embryo transfer (ET) treatment cycles, reaches 1.4–8.6% [4]. During spontaneous pregnancy, it is known that EP is highly associated with tubal surgery history, pelvic inflammatory disease. However, tubal factor infertility is one of the most common reasons for women undergoing IVF-ET treatment. Therefore, it is reasonable that the incidence of EP in IVF-ET cycles is much higher than that in natural conception. Currently, several large cohort studies consistently indicated that other factors, including stage of embryos transferred (cleavage stage or blastocyst stage), high estrogen levels during ovarian stimulation, and endometrial thickness, had an impact on EP rate in IVF-ET cycles [5–7]. In addition, many other studies also demonstrated that other potential factors including ovarian stimulation protocols, number of embryos transferred, and type of embryos (fresh or frozen-thawed) were associated with EP rate [8–10].

Body mass index (BMI) is believed to be associated with both pregnancy outcomes and obstetrical complications during IVF treatment. Having a high BMI before IVF treatment increased the risk of embryo implantation failure and spontaneous miscarriage in infertile patients [11, 12]. Moreover, overweight and obesity were also risk factors for gestational diabetes, preterm birth, low birth weight, postpartum infection, and other obstetrical and prenatal complications [13, 14].

Recently, a few studies have examined the association of maternal BMI with EP during IVF. In 2016, a nationwide database study showed that, in both luteal Gonadotropin-releasing hormone (GnRH) agonist and GnRH antagonist protocols, there was a significant increase of EP in patients with  $\text{BMI} \geq 30 \text{ kg/m}^2$  in comparison with non-obese patients ( $\text{BMI} < 30 \text{ kg/m}^2$ ) [8]. However, another large sample study reported that the rate of EP was significantly increased in the low BMI group ( $< 18.5 \text{ kg/m}^2$ ), but not in the high BMI group ( $\geq 25 \text{ kg/m}^2$ ), indicating that low BMI was associated with an increased risk of EP [15].

BMI is a critical parameter for IVF treatment. The starting dosage of gonadotrophins for ovarian stimulation is usually based on female BMI before IVF. More importantly, BMI also influences our decision in embryo transfer strategy to reduce the risk of ovarian hyperstimulation syndrome (OHSS) in fresh cycles, as lean women are accompanied with higher estrogen level and will benefit

from selective single blastocyst transfer. As mentioned above, estrogen level and blastocyst transfer are both critical factors affecting EP. Thus, it is necessary to adjust for confounding factors when exploring the impact of BMI on EP.

Understanding the impact of BMI on EP during IVF treatment may be useful for predicting results and lead to the development of giving proper suggestions to patients. To answer the question of whether underweight and overweight/obesity predict EP, we performed this large cohort study using data from women undergoing fresh or frozen-thawed embryo transfers in our center from 2010 to 2022.

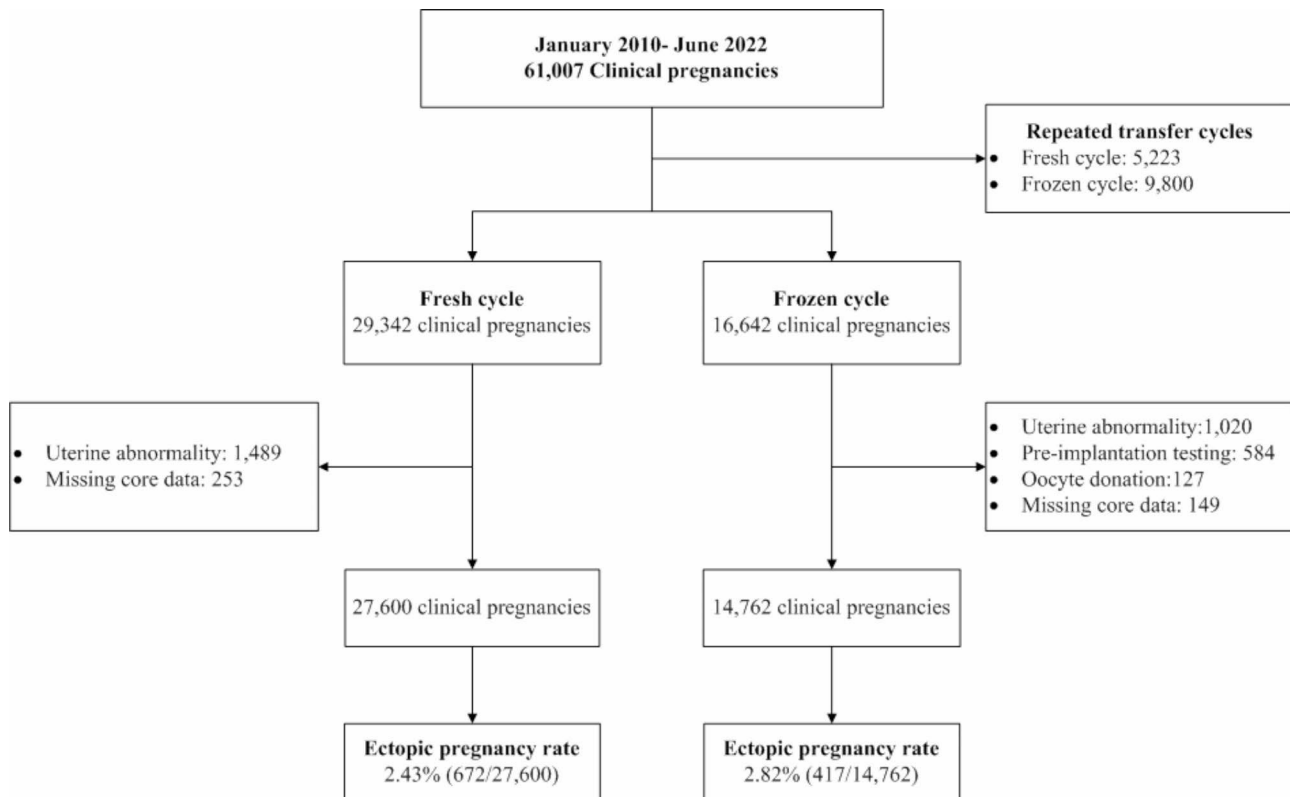
## Materials and methods

### Study design and population

This retrospective study included all IVF/intracytoplasmic sperm injection (ICSI)-ET cycles with outcomes reported as clinical pregnancy (including clinical intrauterine, ectopic, or heterotopic pregnancy) from January 2010 to June 2022 at the Reproductive Medicine Center of the First Affiliated Hospital of Zhengzhou University. Data collection was from the Clinical Reproductive Medicine Management System/Electronic Medical Record Cohort Database (CCRM/EMRCD) of our center. This study was approved by the Ethics Review Committee of the hospital and written informed consent was waived due to the retrospective nature of the study.

All patients underwent routine uterine ultrasound and hysteroscopy before ovarian stimulation. To avoid the interaction caused by repeated cycles, only patients between the ages of 20 and 45 years who had their first autologous fresh/frozen-thawed embryo transfer (FET) cycles were included. The exclusion criteria were as follows: preimplantation genetic diagnosis/screening cycles; patients with untreated hydrosalpinx; patients with uterine abnormalities (uterine malformation; uterine fibroids  $\geq 3 \text{ cm}$  in diameter or compressing the endometrium; endometrial polyps; intrauterine adhesion); cycles with incomplete core data. The definition of intrauterine pregnancy (IUP) and EP was elaborated in our previous study [7]. Heterotopic pregnancy was also classified as the EP. A detailed flow chart of sample selection is shown in Fig. 1.

Maternal BMI was derived from measured height and weight recorded at the beginning of ovarian stimulation or endometrial preparation. BMI was categorized into three groups: underweight ( $< 18.5 \text{ kg/m}^2$ ), normal weight ( $18.5\text{--}23.9 \text{ kg/m}^2$ ), overweight and obesity ( $\geq 24 \text{ kg/m}^2$ ). These criteria were from Working Group on Obesity in China (WGOC), International Life Sciences Institute (ILSI) [16]. The diagnosis of tubal infertility has also been described previously [7]. Cleavage stage embryo refers to embryo at day 3. Blastocyst stage includes embryos



**Fig. 1** Flowchart of the retrospective cohort study

at day 5 or day 6. Endometrial thickness was measured on trigger day in fresh ET cycles, or on day of ovulation/progesterone administration in frozen thawed ET (FET) cycles. Patients were divided into thin (<7 mm), medium (8–13 mm), and thick ( $\geq 14$  mm) groups according to endometrial thickness. Peak estrogen level was measured on trigger day in fresh embryo transfer cycles.

#### ART protocols

In fresh cycles, ovarian stimulation protocols were carried out depending on female age and ovarian reserve. During the cycle, follicle growth was regularly monitored by transvaginal ultrasound and serum sex hormone levels, and the gonadotropin (Gn) dose was adjusted accordingly. When at least two follicles reached a diameter of 16 mm or wider, oocyte maturation was triggered by human chorionic gonadotropin (hCG). Transvaginal ultrasound-guided oocyte retrieval, fertilization, and embryo culture were performed as described elsewhere [17]. Fresh cleavage embryos or blastocysts were selected for transfer according to the patient's condition and embryo quality. Luteal support was started on the day of oocyte retrieval by using oral progesterone and daily transvaginal progesterone.

In FET cycles, the protocols for endometrial preparation included natural cycles and artificial cycles, which were mainly selected based on patients' menstruation

and their doctor's experience. After vitrified embryos being warmed, ET was conducted under ultrasound guidance. The details of protocols were published previously [18].

All patients were followed up and luteal support was continued if the serum hCG concentration was >50 IU/L 14 days after ET. Transvaginal ultrasound was performed 5 weeks after ET and the definition of IUP and EP was described in detail previously [7].

#### Statistical analysis

Data were examined for normal distribution, and appropriate tests were applied. The data were presented as mean  $\pm$  standard deviation (SD) for normal distribution, and analyzed by Student's *t* test or one-way analysis of variance. For non-normal distribution data, they were shown as median (25th, 75th), and nonparametric test (Kruskal-Wallis test) was performed for comparison. Chi-square test was used to detect difference between categorical variables.

Firstly, possible factors affecting EP were screened in both fresh and frozen-thawed ET cycles. Then, multivariate logistic regression analysis was conducted to investigate the association between female BMI and EP after adjusting potential confounding risk factors. In fresh ET cycles, adjusted factors were BMI, infertility type, tubal factor, peak estrogen level, endometrial thickness, type

of embryos, and number of embryos transferred. In FET cycles, adjusted factors were BMI, tubal factor, endometrial thickness, type of embryos, and number of embryos transferred. Statistical analysis was performed with SPSS (Statistical Package for Social Science, SPSS Inc, Chicago, IL, USA) version 21.0. A  $P < 0.05$  was considered statistically significant.

## Results

### Characteristics of the study cohort

From January 2010 to June 2022, a total of 61,007 cycles were reported as clinical pregnancy after fresh, or frozen-thawed ET. After excluding repeat cycles and patients with exclusion criteria, 27,600 pregnant cycles following fresh ET and 14,762 pregnant cycles after FET were included in the final analysis. In total, there were 672 and 471 ectopic pregnancies in fresh, and frozen-thawed ET cycles, respectively. The overall EP rate was 2.57% (1,089/42,362). EP rate was 2.43% (672/27,600) and 2.82% (417/14,762) in fresh, and frozen-thawed ET cycles, respectively (Fig. 1).

### Comparison of differences among three BMI groups

Table 1 showed the EP rates in different groups. In fresh ET cycles, EP rates were comparable among patients with different age ( $< 35$  years old, or  $\geq 35$  years old), and different ovarian stimulation protocols (agonist, antagonist, or others). However, underweight patients yielded a significantly higher EP rate compared with normal and overweight/obesity women (3.29% vs. 2.29% vs. 2.54%;  $P = 0.029$ ). Moreover, the EP rate was also higher in patients with secondary infertility (compared with primary infertility), with tubal infertility (compared with non-tubal infertility), with cleavage stage embryo transfer (compared with blastocyst transfer).

In addition, the EP rates increased with number of embryos transferred, while decreased with endometrial thickness. Interestingly, there seemed to be a positive correlation between EP rates and peak estrogen levels. It was  $< 2.5\%$  in patients with estrogen  $< 5000$  pg/ml, and 3.38% in patients with high estrogen level ( $\geq 7500$  pg/ml). In frozen-thawed embryo transfer cycles, the situation was similar with that in fresh embryo transfer cycles. However, the EP rates did not differ among different BMI categories (2.72% vs. 2.76% vs. 2.96%;  $P = 0.782$ ).

Basic parameters and EP rates in fresh ET cycles with different BMI were compared in Table 2. Besides EP rate, patients' basic demographic characteristics were different among underweight, normal weight, and overweight/obesity groups. Importantly, peak estrogen level was significantly higher in underweight patients when compared with that in the other two groups (3787 pg/ml vs. 3324 pg/ml vs. 2676 pg/ml;  $P < 0.001$ ).

In Table 3, it was shown that baseline was not comparable among these three BMI groups, either. However, the ectopic pregnancy rate was similar. The ectopic pregnancy rate was also further compared in cleavage embryo and blastocyst embryo transfer cycles. As shown in Fig. 2, in fresh embryo transfer cycles, the ectopic pregnancy rate was comparable among three BMI groups in both cleavage and blastocyst transfers (Cleavage stage: 3.37% vs. 2.48% vs. 2.75%,  $P = 0.096$ ; Blastocyst stage: 2.87% vs. 1.45% vs. 1.84%;  $P = 0.159$ ). In frozen thawed embryo transfer cycles, the ectopic pregnancy rate was also similar among three BMI groups irrespective of embryo stage (Cleavage stage: 3.85% vs. 3.46% vs. 4.16%,  $P = 0.393$ ; Blastocyst stage: 1.58% vs. 2.17% vs. 2.15%;  $P = 0.714$ ).

### Relationship between female BMI and EP

In Table 4, multivariate logistic regression analysis was performed to explore risk factors for ectopic pregnancy. In fresh embryo transfer cycles, secondary infertility, tubal infertility, elevated estrogen level, thin endometrial thickness, and cleavage stage embryo transfer were risk factors for ectopic pregnancy. However, female BMI was not associated with ectopic pregnancy (adjusted OR: 0.98,  $P = 0.894$ , for BMI 18.5–23.9 kg/m<sup>2</sup>; adjusted OR: 0.89,  $P = 0.205$ , for BMI  $\geq 24$  kg/m<sup>2</sup>. Reference = BMI  $< 18.5$  kg/m<sup>2</sup>). In FET cycles, only thin endometrial thickness, and cleavage stage embryo transfer were risk factors for ectopic pregnancy. Female BMI was not predictable for ectopic pregnancy, either (adjusted OR: 1.15,  $P = 0.513$ , for BMI 18.5–23.9 kg/m<sup>2</sup>; adjusted OR: 1.24,  $P = 0.367$ , for BMI  $\geq 24$  kg/m<sup>2</sup>. Reference = BMI  $< 18.5$  kg/m<sup>2</sup>).

## Discussion

In this retrospective study with a large sample size, no significant association was found between female BMI and the risk of EP in fresh ET cycles after controlled ovarian hyperstimulation (COH) after adjusting for potential confounding factors. In addition, the EP rates among three BMI groups were also comparable in FET cycles.

To date, there has been numerous data identifying risk factors for EP during ART treatment [4, 7, 19]. Whether a correlation exists between female BMI and the risk of EP has been previously discussed. Several Chinese studies of natural conceptions and artificial insemination cycles with donor sperm observed higher EP rates in obese women than those with underweight and normal weight [20, 21], suggesting potential obesity-related tubal dysfunction. In the study including 136,605 clinical pregnancies about the effect of ovarian hyperstimulation protocols on the EP in the United States, Londra et al. also observed that female obesity was associated with an increased risk of EP (BMI  $\geq 30$  kg/m<sup>2</sup> vs.  $< 30$  kg/m<sup>2</sup>: adjusted OR 1.33; 95% CI, 1.19–1.47;  $P < 0.001$ ) in fresh

**Table 1** Ectopic pregnancy rate in fresh embryo transfer cycles and in frozen-thawed embryo transfer cycles

|                                  | Fresh embryo transfer |     |         |         | Frozen-thawed embryo transfer |     |         |         |
|----------------------------------|-----------------------|-----|---------|---------|-------------------------------|-----|---------|---------|
|                                  | CP                    | EP  | EP rate | P value | CP                            | EP  | EP rate | P value |
| Total No.                        | 27,600                | 672 | 2.43%   |         | 14,762                        | 417 | 2.82%   |         |
| Age (year)                       |                       |     |         |         |                               |     |         |         |
| < 35                             | 22,914                | 557 | 2.43%   | 0.925   | 12,064                        | 342 | 2.83%   | 0.876   |
| ≥ 35                             | 4686                  | 115 | 2.45%   |         | 2698                          | 75  | 2.78%   |         |
| BMI (kg/m <sup>2</sup> )         |                       |     |         |         |                               |     |         |         |
| < 18.5                           | 1703                  | 56  | 3.29%   |         | 883                           | 24  | 2.72%   |         |
| 18.5–23.9                        | 16,840                | 386 | 2.29%   | 0.029   | 8840                          | 244 | 2.76%   | 0.782   |
| ≥ 24                             | 9057                  | 230 | 2.54%   |         | 5039                          | 149 | 2.96%   |         |
| Infertility type                 |                       |     |         |         |                               |     |         |         |
| Primary                          | 15,265                | 343 | 2.25%   | 0.024   | 6779                          | 173 | 2.56%   | 0.065   |
| Secondary                        | 12,335                | 329 | 2.67%   |         | 7983                          | 244 | 3.06%   |         |
| Tubal factor existed             |                       |     |         |         |                               |     |         |         |
| Yes                              | 11,075                | 402 | 3.63%   | <0.001  | 5902                          | 207 | 3.51%   | <0.001  |
| No                               | 16,525                | 270 | 1.63%   |         | 8860                          | 210 | 2.37%   |         |
| Ovarian stimulation protocol     |                       |     |         |         |                               |     |         |         |
| Agonist                          | 27,045                | 658 | 2.43%   |         | /                             |     |         |         |
| Antagonist                       | 250                   | 5   | 2.00%   | 0.763   | /                             |     |         |         |
| Others*                          | 305                   | 9   | 2.95%   |         | /                             |     |         |         |
| Peak Estrogen level (pg/ml)      |                       |     |         |         |                               |     |         |         |
| < 2500                           | 9806                  | 216 | 2.20%   |         | /                             |     |         |         |
| [2500–5000)                      | 12,092                | 286 | 2.37%   | 0.011   | /                             |     |         |         |
| [5000–7500)                      | 4104                  | 116 | 2.83%   |         | /                             |     |         |         |
| ≥ 7500                           | 1598                  | 54  | 3.38%   |         | /                             |     |         |         |
| Endometrial preparation Protocol |                       |     |         |         |                               |     |         |         |
| Natural cycle                    | /                     |     |         |         | 5204                          | 133 | 2.56%   | 0.145   |
| Artificial cycle                 | /                     |     |         |         | 9558                          | 284 | 2.97%   |         |
| Endometrial thickness (mm)*      |                       |     |         |         |                               |     |         |         |
| Thin (< 7)                       | 385                   | 21  | 5.45%   |         | 357                           | 23  | 6.44%   |         |
| Medium (8–13)                    | 17,901                | 480 | 2.68%   | <0.001  | 7624                          | 238 | 3.12%   | <0.001  |
| Thick (≥ 14)                     | 8420                  | 147 | 1.75%   |         | 607                           | 9   | 1.48%   |         |
| Type of embryos                  |                       |     |         |         |                               |     |         |         |
| Cleavage                         | 22,222                | 582 | 2.62%   | <0.001  | 6503                          | 241 | 3.71%   | <0.001  |
| Blastocyst                       | 5378                  | 90  | 1.67%   |         | 8259                          | 176 | 2.13%   |         |
| No. of embryos transferred       |                       |     |         |         |                               |     |         |         |
| 1                                | 6793                  | 125 | 1.84%   |         | 6343                          | 139 | 2.19%   |         |
| 2                                | 20,328                | 537 | 2.64%   | 0.001   | 7448                          | 229 | 3.07%   | <0.001  |
| 3                                | 479                   | 10  | 2.09%   |         | 971                           | 49  | 5.05%   |         |

Note: \* other protocols include mild stimulation, natural cycle, and short protocol; BMI: body mass index; CP: clinical pregnancy; EP: ectopic pregnancy;

+ missing data existed; endometrial thickness was measured on trigger day in fresh cycle, or on day of ovulation/progesterone administration in frozen thawed cycle

autologous ET cycles [8] However, limited by the relatively low incidence of EP and the small proportion of underweight women in the infertile population, the effect of low BMI per se on EP in women was ignored. To our knowledge, Cai et al. firstly proposed a clear association between low BMI and EP during IVF treatment [15]. In the cohort of 16,378 pregnancies derived from fresh and frozen-thawed ET cycles including 2155 underweight women, low BMI (<18.5 kg/m<sup>2</sup>) was associated with the increased odds of EP (2.92% vs. 2.02%, adjusted OR 1.61; 95% CI, 1.19–2.16; *P*=0.002, compared with the normal BMI group) but not high BMI (>24.9 kg/m<sup>2</sup>).

They conjectured that underweight women might have a nutrition-related unfavorable uterine environment for embryos, which possibly involved in the underlying mechanism of the higher EP risk. However, Bellver J made a dissent that a suboptimal endometrial milieu would be more likely to hamper embryo implantation or ongoing pregnancy in uterus, resulting in lower implantation rates or higher miscarriage rates rather than implanting in fallopian tube, a tissue non-physiologically suitable for conception [11]. And in women undergoing either oocyte donation or autologous euploid embryo transfer, no significant differences in pregnancy

**Table 2** Basic parameters and ectopic pregnancy rates in fresh embryo transfer cycles with different body mass index (kg/m<sup>2</sup>)

|  | < 18.5            | 18.5–23.9         | ≥ 24              | P value |
|--|-------------------|-------------------|-------------------|---------|
| No.                                      | 1703              | 16,840            | 9057              |         |
| Age (year)                               | 28.93 ± 3.80      | 30.26 ± 4.30      | 30.74 ± 4.55      | < 0.001 |
| BMI (kg/m <sup>2</sup> )                 | 17.72 ± 0.66      | 21.35 ± 1.47      | 26.56 ± 2.12      | < 0.001 |
| Infertility type                         |                   |                   |                   | < 0.001 |
| Primary                                  | 1097 (64.42%)     | 9437 (56.04%)     | 4731 (52.24%)     |         |
| Secondary                                | 606 (35.58%)      | 7403 (43.96%)     | 4326 (47.76%)     |         |
| Tubal factor existed                     |                   |                   |                   | < 0.001 |
| Yes                                      | 772 (45.33%)      | 6740 (40.02%)     | 3463 (38.24%)     |         |
| No                                       | 931 (54.67%)      | 10,100 (59.98%)   | 5594 (61.76%)     |         |
| Peak Estrogen (pg/ml), Mean (25th, 75th) | 3787 (2513, 5356) | 3324 (2191, 4891) | 2676 (1752, 4034) | < 0.001 |
| Endometrial thickness (mm)               | 12.31 ± 2.45      | 12.42 ± 2.59      | 12.40 ± 2.61      | < 0.001 |
| Type of embryos                          |                   |                   |                   | < 0.001 |
| Cleavage                                 | 1424 (83.62%)     | 13,807 (81.99%)   | 6991 (77.19%)     |         |
| Blastocyst                               | 279 (16.38%)      | 3033 (18.01%)     | 2066 (22.81%)     |         |
| No. of embryos transferred               | 1.79 ± 0.43       | 1.79 ± 0.45       | 1.73 ± 0.48       |         |
| 1  | 367 (21.55%)      | 3818 (22.67%)     | 2608 (28.80%)     |         |
| 2  | 1322 (77.63%)     | 12,694 (75.38%)   | 6312 (69.69%)     | < 0.001 |
| 3  | 14 (0.82%)        | 328 (1.95%)       | 137 (1.51%)       |         |
| Ectopic pregnancy, n (%)                 | 56 (3.29%)        | 386 (2.29%)       | 230 (2.54%)       | 0.029   |

Note Data were shown as mean ± standard deviation unless otherwise indicated; BMI, body mass index

outcomes have been previously reported between underweight and normal weight groups [22–25], which did not support the premise that being underweight might have an adverse effect on the uterine environment. In our study, neither an independent effect of female BMI on EP in fresh cycles after adjusting for potential confounders nor a significant difference in EP rates among the three BMI groups in FET cycles was demonstrated, which differs from the findings of Cai et al. This difference may be attributed to discordant BMI classification criteria and variations in the proportion of the subjects among three groups: 6.2% (1703/27,600) of underweight women, 61.0% (16,840/27,600) of women with normal weight, and 32.8% (9057/27,600) women with overweight/obesity were included in the present study according to the standards of WGO and ILSI, whereas the proportions were 12.1% (1324/10,930), 82.8% (9047/10,930) and 5.1%

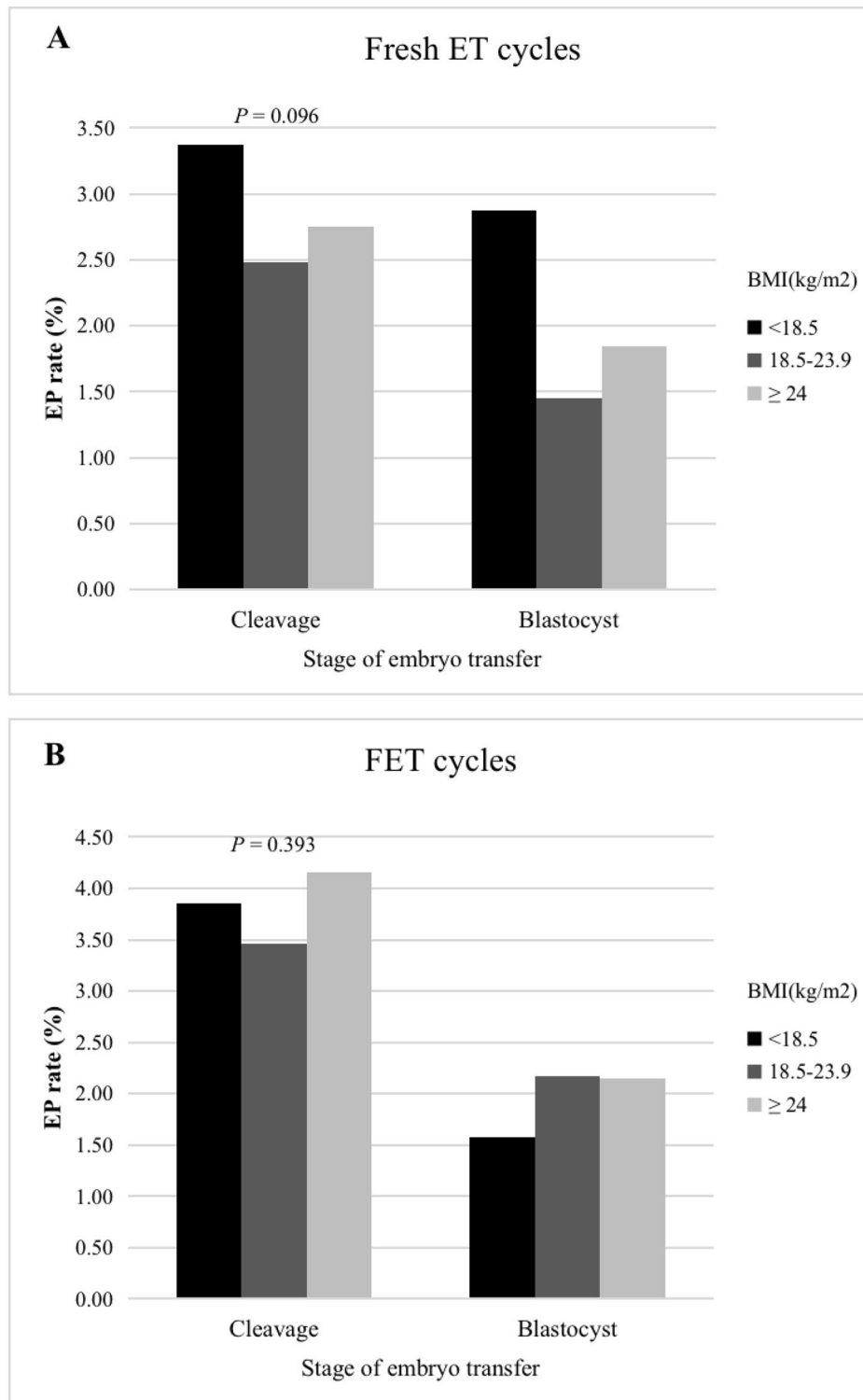
**Table 3** Basic parameters and ectopic pregnancy rate in frozen-thawed embryo transfer cycles with different body mass index (kg/m<sup>2</sup>)

|                                  | < 18.5       | 18.5–23.9     | ≥ 24          | P value |
|----------------------------------|--------------|---------------|---------------|---------|
| No.                              | 883          | 8840          | 5039          |         |
| Age (year)                       | 29.03 ± 4.11 | 30.50 ± 4.46  | 31.03 ± 4.84  | < 0.001 |
| BMI (kg/m <sup>2</sup> )         | 17.68 ± 0.67 | 21.40 ± 1.47  | 26.69 ± 2.22  | < 0.001 |
| Infertility type                 |              |               |               | < 0.001 |
| Primary                          | 496 (56.17%) | 4122 (46.63%) | 2161 (42.89%) |         |
| Secondary                        | 387 (43.83%) | 4718 (53.37%) | 2878 (57.11%) |         |
| Tubal factor existed             |              |               |               | < 0.001 |
| Yes                              | 383 (43.37%) | 3587 (40.58%) | 1932 (38.34%) |         |
| No                               | 500 (56.63%) | 5253 (59.42%) | 3107 (61.66%) |         |
| Endometrial preparation Protocol |              |               |               | < 0.001 |
| Natural cycle                    | 350 (39.64%) | 3415 (38.63%) | 1439 (28.56%) |         |
| Artificial cycle                 | 533 (60.36%) | 5425 (61.37%) | 3600 (71.44%) |         |
| Endometrial thickness (mm)       | 10.50 ± 1.95 | 10.39 ± 1.99  | 10.30 ± 2.03  | 0.034   |
| Type of embryos                  |              |               |               | < 0.001 |
| Cleavage                         | 441 (49.94%) | 4043 (45.74%) | 2019 (40.07%) |         |
| Blastocyst                       | 442 (50.06%) | 4797 (54.26%) | 3020 (59.93%) |         |
| No. of embryos transferred       | 1.72 ± 0.58  | 1.65 ± 0.60   | 1.59 ± 0.60   | < 0.001 |
| 1                                | 310 (35.11%) | 3666 (41.47%) | 2367 (46.97%) |         |
| 2                                | 512 (57.98%) | 4558 (51.56%) | 2378 (47.19%) | < 0.001 |
| 3                                | 61 (6.91%)   | 616 (6.97%)   | 294 (5.84%)   |         |
| Ectopic pregnancy, n (%)         | 24 (2.72%)   | 244 (2.76%)   | 149 (2.96%)   | 0.782   |

Note Data were shown as mean ± standard deviation unless otherwise indicated; BMI, body mass index

(559/10,930) respectively in the study by Cai et al. based on the WHO criteria.

In this study, the EP rates in fresh ET and FET cycles were 2.43% and 2.82% respectively. Our data also identified some risk factors for EP related to IVF treatment, such as tubal factor, elevated peak estrogen levels after COH, endometrial thickness, and stage of embryo for transfer in fresh ET cycles, which concurred with those previously reported [7, 10, 19]. For fresh ET cycles, the results of multivariate regression analysis suggested that the uneven distribution of these factors among the three groups potentially contributed to the higher EP rates in underweight women rather than the independent effect of low female BMI.



**Fig. 2** Ectopic pregnancy rates according to female body mass index classification and the stage of embryo transfer. **(A)** EP rates in different BMI groups in fresh ET cycles. **(B)** EP rates in different BMI groups in FET cycles. *Note:* BMI: body mass index; EP: ectopic pregnancy; FET: frozen-thawed embryo transfer; EP rate given as  $n = \text{ectopic pregnancy} / N = \text{clinical pregnancy}$ . Statistical differences analyzed by chi-squared test

**Table 4** Multivariate logistic regression analysis of risk factors for ectopic pregnancy

|                             | Fresh embryo transfer |         | Frozen-thawed embryo transfer |       |
|-----------------------------|-----------------------|---------|-------------------------------|-------|
|                             | Adjusted OR (95% CI)  | P       | Adjusted OR (95% CI)          | P     |
| BMI (kg/m <sup>2</sup> )    |                       |         |                               |       |
| < 18.5                      | Reference             |         | Reference                     |       |
| 18.5–23.9                   | 0.98 (0.70–1.37)      | 0.894   | 1.15 (0.89–2.05)              | 0.513 |
| ≥ 24                        | 0.89 (0.75–1.06)      | 0.205   | 1.24 (0.94–2.65)              | 0.367 |
| Infertility type            |                       |         | /                             |       |
| Primary                     | Reference             |         | /                             |       |
| Secondary                   | 1.19 (1.02–1.40)      | 0.032   | /                             |       |
| Tubal factor existed        |                       |         |                               |       |
| Yes                         | 1.57 (1.24–2.58)      | 0.017   | 1.32 (0.87–1.93)              | 0.081 |
| No                          | Reference             |         | Reference                     |       |
| Peak Estrogen level (pg/ml) |                       |         | /                             |       |
| < 2500                      | Reference             |         | /                             |       |
| [2500–5000)                 | 1.04 (0.86–1.25)      | 0.686   | /                             |       |
| [5000–7500)                 | 1.38 (1.08–1.75)      | 0.009   | /                             |       |
| ≥ 7500                      | 1.75 (1.27–2.39)      | 0.001   | /                             |       |
| Endometrial thickness (mm)  |                       |         |                               |       |
| Thin (< 7)                  | 3.04 (1.88–4.93)      | < 0.001 | 5.10 (2.33–10.17)             | 0.000 |
| Medium (8–13)               | 1.50 (1.24–1.81)      | < 0.001 | 2.14 (1.09–4.18)              | 0.027 |
| Thick (≥ 14)                | Reference             |         | Reference                     |       |
| Type of embryos             |                       |         |                               |       |
| Cleavage                    | 1.62 (1.09–2.42)      | 0.018   | 1.63 (1.13–2.33)              | 0.008 |
| Blastocyst                  | Reference             |         | Reference                     |       |
| No. of embryos transferred  |                       |         |                               |       |
| 1                           | Reference             |         | Reference                     |       |
| 2                           | 1.22 (0.58–2.57)      | 0.602   | 1.24 (0.83–2.96)              | 0.291 |
| 3                           | 1.31 (0.67–2.55)      | 0.432   | 1.47 (0.96–3.41)              | 0.072 |

Note BMI, body mass index; OR, odds ratio; CI, confidence interval

In fresh cycles, adjusted factors were BMI, infertility type, tubal factor, peak estrogen level, endometrial thickness, type of embryos, and number of embryos transferred. In frozen thawed cycles, adjusted factors were BMI, tubal factor, endometrial thickness, type of embryos, and number of embryos transferred

Supraphysiologic hormonal milieu after COH in fresh ET cycles is known to increase the risk of EP during IVF treatment, and high estradiol levels may interfere the regulation of tubal physiologic process, which possibly plays a role in the pathophysiological mechanism of tubal EP development after embryos transfer [26]. Wang et al. reported that in fresh ET cycles, high estradiol levels [estradiol on hCG day > 4085 pg/mL] were associated with the increased EP risk (3.4% vs. 2.0%, adjusted

OR, 1.99; 95% CI, 1.19–3.35;  $P=0.009$ ) in women without polycystic ovary syndrome [27]. In our study, lean women accompanied with higher estradiol levels on average yielded higher EP rates in fresh ET cycles, and high peak estradiol levels were related to an increased risk of EP after adjusting potential confounding factors, which was consistent with our previous findings in both tubal infertile women and non-tubal infertile women undergoing fresh ET cycles [7]. For FET cycles without exogenous high-dose gonadotropin supplementation, hormone levels could be considered close to natural condition, and EP rates were similar among three BMI groups. Taken together, our findings supported the conclusions of Wang et al., and we noticed that lean women appeared to have a sensitivity to ovarian stimulation and develop higher estradiol levels after COH [28]. Thus, when exploring the independent impact of female BMI on EP risk, attention should be paid to eliminating the interference of estradiol levels.

Our findings also confirmed that thin endometrium thickness (EMT) contributed to an increased risk of EP in both fresh cycles and FET cycles, which is in line with multiple existing reports [1, 5]. One potential explanation for the relationship between thin endometrium and higher EP rates is the difference in oxygen tension between the thin endometrium and the fallopian tube. In the thin endometrium with a thin or absent functional layer, the implanting embryos would be closer to the spiral arteries in the basal endometrium layer where the embryos are exposed to higher oxygen concentrations, which may be detrimental to their growth. By contrast, the oxygen tension is relatively lower in the fallopian tube [29]. Consistent with many past studies, this study also presented similar higher EP rates for cleavage-stage embryos compared to blastocyst transfer [7, 9, 30]. Several speculations have been proposed: transferring blastocysts into the uterine cavity seems closer to the physiological state than that of cleavage-stage embryos, requiring a shorter interval for further development before implantation; the size of a blastocyst is larger than that of a cleavage-stage embryo, all of which may reduce the chance of embryos migrating to the fallopian tube [9, 31].

The strength of the current study is the large sample size with the adequate number of underweight women in a single center. Given that the characteristics and distribution of BMI vary by race and region, BMI categories were based on the recommendations on cut-off points of BMI in Chinese adults by WGOC. Therefore, the results were more suitable to provide counseling and guidance for Chinese women undergoing IVF treatment. And the independent effect of female BMI on EP development was discussed in fresh ET and FET cycles respectively. It must be acknowledged that this study has some following



limitations. First, as a retrospective study, some potential confounders, such as the volume of transfer fluid, transfer depth and smoking habits, were not available in our database. Second, when investigating the association between development stages of embryos transferred and the occurrence of EP, our study mainly focused on the comparison between cleavage-stage embryos and blastocysts without differentiating the specific stage of a certain blastocyst. In addition, this study spans a long duration over ten years when IVF technology has progressed rapidly, and thus there might be potential biases related to the evolution of IVF behind the results. More well-designed prospective studies with large cohort are needed to evaluate the effect of female underweight or excess weight on EP development after IVF treatment, and possible underlying mechanisms.

## Conclusion

This study suggested that female BMI might be not associated with the risk of ectopic pregnancy in either fresh ET or FET cycles. It may be too early to say with certainty that female underweight, or overweight/obesity is to blame for the higher risk of EP after IVF treatment.

## Abbreviations

|      |                                     |
|------|-------------------------------------|
| BMI  | Body mass index                     |
| EP   | Ectopic pregnancy                   |
| ET   | Embryo transfer                     |
| ART  | Assisted reproduction techniques    |
| IVF  | In vitro fertilization              |
| ICSI | Intracytoplasmic sperm injection    |
| GnRH | Gonadotropin-releasing hormone      |
| OHSS | Ovarian hyperstimulation syndrome   |
| FET  | Frozen-thawed embryo transfer       |
| COH  | Controlled ovarian hyperstimulation |
| hCG  | Human chorionic gonadotropin        |

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## Authors' contributions

YS and ZB were responsible for the conception of study. XG contributed to design this study, statistical analyses, and write this manuscript. KW handled patient recruitment and data collection. All authors were involved in revising the article and have approved this final version.

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## Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

This study was approved by the Ethics Review Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent for participation was not required due to the retrospective nature of this study, in accordance with the national legislation and institutional requirements.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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