Obesity and adverse pregnancy outcomes in older patients with decreased ovarian reserve: a retrospective single-centre study

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Research

Keywords: diminished ovarian reserve, body mass index, miscarriage, live birth rate

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Abstract

**Background:** In recent years, infertility has increased in older women with decreased ovarian reserve (DOR). Studies have shown that women with DOR have fewer oocytes, which are poorer in quality, and have an increased risk of adverse pregnancy outcomes. Pre-pregnancy BMI is significantly correlated with many adverse pregnancy outcomes. Therefore, we conducted this study to explore the correlation between body mass index (BMI) and abortion and live birth in older patients with DOR.

**Methods:** The clinical data of 2052 older women with infertility and DOR...
admitted to the Reproductive Medicine Center of the First Affiliated Hospital of Zhengzhou University from August 2009 to May 2018 were analysed retrospectively. Patients were divided into underweight (BMI < 18.5 kg/m²; n = 56), normal weight (18.5 kg/m² ≤ BMI < 24 kg/m²; n = 1389), overweight (24 kg/m² ≤ BMI < 28 kg/m²; n = 527) and obese (BMI ≥ 28 kg/m²; n = 80). We compared the pregnancy outcomes of patients in each group.

Results: Logistic regression analysis showed that being overweight or obese were independent risk factors for miscarriage (P < 0.05) and protection factors for live births (P < 0.05). Being underweight was a protective factor for live births (P < 0.05).

Conclusions: The abortion and live birth rates in older infertile women with DOR are correlated with BMI. Higher BMI was associated with higher abortion rates and lower live birth rates. Being underweight also correlated with the live birth rate. Therefore, to improve pregnancy outcomes, we suggest that older patients with DOR may benefit from maintaining a normal weight before seeking fertility treatments.

Keywords: diminished ovarian reserve; body mass index; miscarriage; live birth rate

Background

Ovarian reserve is the capacity for growth and development of follicles in the female ovarian cortex and the ability to form fertilised oocytes. Diminished ovarian reserve (DOR) is a common endocrine disease in women of childbearing age and...
refers to the decline in the number and quality of oocytes, ovulation disorders, endocrine disorders, and infertility due to factors such as age, metabolism, genetics, autoimmunity, iatrogenicity, toxicity, and infection. In the process of assisted reproductive technology (ART), DOR is characterised by poor drug response, few eggs, low number of high-quality embryos, high rate of cycle cancellation, and low clinical pregnancy rate [1].

Studies have shown that pre-pregnancy BMI is significantly correlated with many adverse pregnancy outcomes, such as gestational diabetes mellitus (GDM), hypertensive disorders in pregnancy, premature birth, abnormal birth weight, and cesarean section [2,3]. However, there are currently insufficient data on the role of BMI in pregnancy outcomes in patients with decreased ovarian reserve. Therefore, we conducted this study to explore the relationship between BMI and pregnancy outcomes in patients with DOR to provide a reference for clinical practice.

Methods

1. Participant selection

We retrospectively analysed the clinical data of 2052 patients with decreased ovarian reserve who were treated for infertility at the Reproductive Medicine Center of the First Affiliated Hospital of Zhengzhou University from August 2009 to May 2018.

Inclusion criteria: (1) we used the 2015 U.S. Centers for Disease Control and Prevention DOR diagnostic criteria [4], which define DOR as the presence of
menstrual cramps and follicle stimulating hormone (FSH) > 10 IU/L, and/or anti-Mullerian hormone (AMH) < 1.0 ng/ml; (2) individuals above 35 years of age.

The exclusion criteria were as follows: (1) history of prior oocyte or sperm donation; (2) chromosomal abnormalities; (3) benign and malignant ovarian diseases; (4) sex hormone-dependent diseases, such as endometriosis, uterine fibroids, endometrial polyps, and pituitary tumours; (5) endocrine system diseases, such as diabetes, thyroid dysfunction, and hyperprolactinaemia; (6) oral administration of exogenous sex hormones or vitamin D within 3 months before consultation; (7) systemic diseases such as malignant tumours; (8) the absence of embryos for transfer or transplantation until the end of the follow-up period.

2. Research methods

1) Grouping: patients who met inclusion criteria were divided into four groups according to the Chinese Guidelines for Prevention of Overweight and Obesity in Adults: underweight (BMI < 18.5 kg/m^2; n = 56), normal weight (18.5 kg/m^2 ≤ BMI < 24 kg/m^2, n = 1389), overweight (24 kg/m^2 ≤ BMI < 28 kg/m^2, n = 527), and obese (BMI ≥ 28 kg/m^2, n = 80).

2) Clinical data: clinical data were obtained from the clinical reproductive medicine management system or electronic medical record database of the Reproductive Medicine Center of the First Affiliated Hospital of Zhengzhou University. Data included age, BMI, menstrual cycle interval, antral follicle count (AFC) defined as number of antral follicles with a diameter of 2 mm-9 mm on ultrasound, infertility type, and number of previous IVF/ICSI cycles.
3) Specimen collection and laboratory tests: In the patient's natural physiological state, the second to fourth days of the menstrual cycle or menopause for more than 50 days (excluding early pregnancy and B-ultrasound monitoring of the ovaries and endometrium are consistent with anovulatory status), 3 ml of venous blood was drawn on an empty stomach, serum was collected by centrifugation, and electrochemiluminescence immunoassay kit (Roche, Germany) was used to detect serum basal luteinising hormone (bLH), basal follicle stimulating hormone (bFSH), and anti-Mullerian hormone (AMH) levels (inter- and intra-batch detection difference: < 5%).

4) ART protocol: a gonadotropin (Gn) releasing hormone (Gn) agonist was used to prevent a premature surge in luteinising hormone (LH), and Gn was used to stimulate follicular growth. When the largest follicle diameter was greater than 20 mm, and more than 2/3 of the total follicles were >16 mm. Human chorionic gonadotropin (hCG) was administered according to the serum FSH, LH, E2 and P levels. Ultrasound-guided egg retrieval was performed 36-38 hours later.

5) Outcome indicators: At 14 or 18 days after embryo transfer, serum β-hCG levels were measured to detect early pregnancy. Ultrasonography was performed 35 or 45 days after embryo transfer, and we diagnosed pregnancy clinically by the existence of an intrauterine pregnancy sac and a positive heartbeat. Miscarriage was defined as termination of pregnancy before 28 weeks’ gestation with a foetal weight of less than 1000 g. Live birth was defined as at least one live birth after 24 weeks of pregnancy. We defined other outcomes as follows: implantation rate = number of
gestational sacs / number of embryos transferred × 100%; clinical pregnancy rate = number of clinical pregnancy cycles / total number of transplanted cycles × 100%; abortion rate = number of abortion cycles / total number of pregnancy cycles × 100%; and live birth rate = number of live birth cycles / total number of transplant cycles × 100%.

3. Statistical analysis was performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA) statistical software for data analysis. Normally distributed data are expressed as mean ± standard deviation (x±s), one-way ANOVA was used for comparison between groups. Continuous variables with skewed distributions are represented as medians (interquartile ranges, IQR), and were compared using the Kruskal-Wallis test. Count data were expressed as rate (%), and the chi-square test was used to compare groups (X2). The difference of proportions between groups was compared using Bonferroni correction. Binary logistics regression was used to determine the correlation between BMI and pregnancy outcomes (abortion and live birth rates). The results are presented as the adjusted odds ratios (aORs) with the 95% confidence intervals (CIs). Statistical significance was set at P < 0.05.

Results

The retrospective analysis included 2052 patients, with 56 (2.7%), 1389 (67.7%), 527 (25.7%), and 80 (3.9%) patients classified as being underweight, normal weight, overweight, and obese, respectively. (Figure 1)

1. Baseline data
There were significant differences in male age, female age, menstrual cycle length, bFSH levels, bLH levels, AMH levels, and AFC among the different BMI classifications (all P < 0.05). Menstrual cycle length was directly proportionate to increased BMI. There were no significant differences in male BMI level, infertility diagnosis, and previous in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) attempts (all P > 0.05) (Table 1).

2. Analysis of patients' transplant status and assisted pregnancy results

The relationship between BMI and transplant and fertility outcomes of patients with reduced ovarian reserve was analysed. There was no significant correlation between BMI and initial Gn dose, Gn dosage, endometrial thickness on the day of HCG administration, number of retrieved oocytes, number of available embryos, number of embryos transferred, and embryo stage at transfer (all P > 0.05). However, we did find a significant correlation between BMI and the abortion rate (P = 0.015) and live birth rate (P = 0.016). There was no significant correlation between BMI and implantation rate, clinical pregnancy rate, number of live births, Cumulative clinical pregnancy rate (CCPR) or Cumulative live birth rate (CLBR) (all P > 0.05) (Table 2).

3. Analysis of factors affecting miscarriage

Using binary logistic regression to analyse related factors, we found that before adjusting for confounding factors, male age, female age, and being overweight were independent risk factors for miscarriage. After adjusting for male age, female age, menstrual cycle, bFSH, bLH, AMH, and AFC, only being overweight (adjusted odds ratio [aOR] = 2.41; 95% confidence interval [CI]: 1.20-4.83; P = 0.013) or obese (aOR
was independently associated with miscarriage, with the aOR value of the obesity group found to be several times that of the overweight group (Table 3; Figure 2A).

4. Analysis of factors correlated with abortion and live birth

Using binary logistic regression analysis, we found that male age, female age, and being overweight were independently associated with abortion before adjusting for confounding factors. After adjusting for male age, female age, menstrual cycle length, BMI, bFSH, bLH, AMH, and AFC, we found that factors such as being underweight (aOR = 0.15, 95% CI: 0.03-0.73; P = 0.019), overweight (aOR = 0.46, 95% CI: 0.23-0.91; P = 0.026), or being obese (aOR = 0.20; 95% CI: 0.04-0.91; P = 0.037) were independently protective in terms of the live birth rate. The impact of obesity far exceeded the impact of being overweight on the live birth rate (Table 4; Figure 2B).

Discussion

1. Reproductive difficulties in older patients with DOR

With the change in women’s social roles and improved education levels, the global childbearing age has increased [5]. With the introduction of China’s fertility policy, the proportion of older couples having children has increased significantly [6]. These factors have led to an increasing DOR detection rate. Patients with DOR have decreased fertility, by definition, and the incidence of infertility is increasing. In vitro fertilization/Intracytoplasmic sperm injection- embryo transplantation (IVF/ICSI-ET) has become an important method in the treatment of DOR-related infertility. Due to the
depletion of the ovarian pool in patients with DOR, the number and quality of oocytes decreases, resulting in hormone secretion disorders [7]. In older patients with DOR especially, cycle cancellation rates are high, pregnancy rates are low [8], and fertility outcomes are poor. Nowadays, obesity is becoming a serious health problem [9]. The proportion of obese women of childbearing age is increasing; this adversely affects reproductive health and may lead to adverse pregnancy outcomes. Many studies have shown that obesity significantly increases the risk of infertility and may cause increased complications during pregnancy [10]. Therefore, it is necessary to determine whether there is a correlation between BMI and pregnancy outcomes in IVF/ICSI-ET in older patients with DOR in order to reduce their reproductive risk.

2. BMI and reproductive outcomes in older patients with DOR

Miscarriage is a common complication of pregnancy, and miscarriage after IVF brings great pain to patients, especially DOR patients [11]. Previous studies have described an increased rate of early miscarriage in obese patients, including spontaneous and recurrent miscarriages [12-14]. Moreover, obese women have a higher risk of pregnancy loss than overweight women, resulting in a lower live birth rate among obese pregnant women [12,15]. However, some studies have not found a clear correlation between increased BMI and abortion after in vitro fertilisation [16,17]. According to literature reports, the incidence of spontaneous abortion is approximately 15% in patients undergoing ART [18]. In the present study, the miscarriage rate in patients with DOR receiving ART was 35.5%, which is significantly higher than the average reported rate. In our statistical analysis, high
BMI was an independent risk factor for miscarriage in older patients with DOR; moreover, we found that the higher the BMI, the greater the risk of miscarriage (overweight [aOR = 2.41; 95% CI: 1.20-4.83] vs obesity [aOR = 6.41; 95% CI: 1.38-29.70]; both P < 0.05). Studies have found that compared to women with normal BMI, the live birth rate of women with increased BMI is significantly decreased [19,20]. Some scholars believe that there is no significant correlation between high BMI and live birth rate [21,22]. This study showed that there were significant differences in live birth rates among the different BMI groups. Our statistical analysis of factors affecting the live birth rate found that BMI was an independent factor in the live birth rate, that BMI affected the live birth rate of older women with DOR, and that the live birth rate decreased exponentially with an increase in BMI.

Many studies have confirmed that BMI affects embryo quality. Abnormal endocrine function and impaired mitochondrial function caused by abnormal fat content in obese patients, which reduces the quality of eggs and embryos [9,23], thus increasing the early abortion rate and reducing the live birth rate. In addition, synthetic leptin is a protein hormone secreted by adipose tissue that participates in the regulation of glucose, lipid, and energy metabolism [24]. Studies have shown that BMI can affect leptin receptor expression on endometrium during the secretory period, regulate uterine angiogenesis and implantation, and affect the pregnancy outcome of older patients with DOR. Changes in serum leptin are related to obesity and blastocyst implantation [25]. An increase in adipocyte-related cytokines, such as interleukin-6 and tumour necrosis factor, may pose potential risks for pregnancy [26].
Other studies have shown that obesity can cause ascending bacterial infections in the reproductive tract [27], change the susceptibility of pathogenic bacteria [19, 28], increase uterine cavity infections, increase the risk of miscarriage in older patients with DOR, and reduce the live birth rate. In addition, miscarriage in women with high BMI ($\geq 25 \text{ kg/m}^2$) is not mainly caused by chromosomal abnormalities in embryos [29]; BMI affects embryo quality and the maternal intrauterine environment through different mechanisms.

Being underweight is associated with negative pregnancy outcomes in patients receiving in vitro fertilisation through frozen-thawed embryo transfer [30]. In the present study, low BMI was correlated with live birth rate, which is consistent with previous findings. Decreased fertility in underweight women may be related to decreased leptin levels [30].

3. Lifestyle changes and pregnancy outcomes

Maternal obesity increases the risk of pregnancy complications such as GDM, gestational hypertension, and preeclampsia [31]. In addition, more than half of overweight and obese women gain more weight than recommended during pregnancy, which leads to an increased risk of perinatal complications and poor neonatal outcomes, and affects the health of the mother and future generations [32]. Studies have shown that female obesity is an independent risk factor in the cumulative live birth rate in the first complete ovarian stimulation cycle [33]. When the parents' BMI is high, the ratio of normal birth weight to macrosomia in single births increases [34]. In addition, maternal obesity is related to macrosomia, stillbirth, and
congenital abnormalities [31].

Lifestyle interventions can reduce BMI in obese women with infertility, including older patients with DOR [35]. Therefore, for overweight and obese women who want to conceive, it is strongly recommended that they implement lifestyle changes and lose weight before starting infertility treatments. A decrease in body weight by 5%–10% compared with baseline has been found to improve reproductive function [36,37]. Studies have shown that infertile women can lose weight by changing their lifestyle before conception and thus reduce the rate of spontaneous abortion [38, 39].

However, a disadvantage of losing weight through lifestyle changes is weight rebound. Long-term behavioural counselling that provides diet or activity advice is uncommon [36]. In addition, the impact of weight management on the outcome of assisted reproduction remains uncertain [40].

The present study findings suggest that female obesity is an independent risk factor for abortion in older patients with DOR, with greater risk in obese women than in overweight women. In women with normal weight, BMI is an independent protective factor in the live birth rate. Considering the difficulty experienced by women with DOR in conceiving and remaining pregnant, and the high obesity rate in older women, we recommend that women reduce their pre-pregnancy weight through lifestyle changes.

4. Advantages and limitations

Our study presents a novel correlation of pregnancy outcomes in IVF/ICSI-ET with BMI. We have attempted to control for confounding factors that affect pregnancy
outcomes as much as possible to improve the reliability of our results. Although we have reduced selection and confounding biases as much as possible, the present study is a retrospective study with inherent limitations. Our sample size for the underweight and obese patients is small. The study should be repeated with a larger sample size. In addition, this study is a single-centre study, and we only used the clinical data from recent transplant cycles of all older patients DOR in the same centre. Our study lacks some advantages of multi-centre research; however, single-centre research can arguably provide more consistent results by avoiding inconsistencies in surgical methods and laboratory conditions. Finally, we did not evaluate cumulative pregnancy outcomes or neonatal and obstetric outcomes, which may present opportunities for future research.

**Conclusion**

For infertile women > 35 years old with reduced ovarian reserve, pregnancy outcomes of IVF/ICSI-ET were correlated with BMI. We found that BMI above the normal range was correlated with an increased risk of miscarriage. Being underweight or overweight was also associated with the live birth rate. Obesity was more strongly associated with abortion and reduced live birth rate than being overweight. Our findings suggest that older patients with DOR who wish to conceive may benefit from maintaining a normal BMI to improve pregnancy outcomes during fertility treatment.
List of abbreviations

AFC, Antral follicular count
AMH, anti-Mullerian hormone
AOR, adjusted odds ratio
ART, assisted reproductive technology
bFSH, basal follicle stimulating hormone
bLH, basal luteinising hormone
BMI, body mass index
CCPR, Cumulative clinical pregnancy rate
CI, confidence interval
CLBR, Cumulative live birth rate
DOR, decreased ovarian reserve
FSH, Follicle-stimulating hormone
GDM, gestational diabetes mellitus
Gn, gonadotropin
hCG, human chorionic gonadotropin
ICSI, Intracytoplasmic sperm injection
IVF, In vitro fertilization
LH, luteinising hormone
OR, Odds ratio
Declarations

Ethics approval and consent to participate:
This research was approved by the Institutional Ethics Committee of the First Hospital of Zhengzhou University, and all patients signed an informed consent form. All methods were conducted in accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Availability of data and materials
The datasets used in the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests

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Authors' contributions
LFX: study design, analysis and interpretation of data, and drafting and revision of the manuscript; LJ: data collection; SH, SYC, DSJ, YQL: assessed the article; GYH: study conception and design. All authors approved the final article.

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References
1. Bleil ME, Gregorich SE, McConnell D, Rosen MP, Cedars MI. Does accelerated
reproductive aging underlie premenopausal risk for cardiovascular disease?

Menopause. Nov 2013;20(11):1139-1146.

2. Savitri AI, Zuithoff P, Browne JL, et al. Does pre-pregnancy BMI determine blood pressure during pregnancy? A prospective cohort study. BMJ Open. Aug 11 2016;6(8):e011626.

3. Schummers L, Hutcheon JA, Bodnar LM, Lieberman E, Himes KP. Risk of adverse pregnancy outcomes by prepregnancy body mass index: a population-based study to inform prepregnancy weight loss counseling. Obstet Gynecol. Jan 2015;125(1):133-143.

4. Erlandsson L, Lindgren R, Naav A, et al. Exposure to wood smoke particles leads to inflammation, disrupted proliferation and damage to cellular structures in a human first trimester trophoblast cell line. Environ Pollut. Sep 2020;264:114790.

5. Dviri M, Madjunkova S, Koziarz A, et al. Is there an association between paternal age and aneuploidy? Evidence from young donor oocyte-derived embryos: a systematic review and individual patient data meta-analysis. Hum Reprod Update. Apr 21 2021;27(3):486-500.

6. Li Yue, Zhang Xuying. The Influence of Marriage Delay and Birth within Marriage on Fertility Level in China: Based on Decomposition Model of Total Fertility Rate. Population Journal. 2021;43(04):1-11.

7. Cohen J, Chabbert-Buffet N, Darai E. Diminished ovarian reserve, premature ovarian failure, poor ovarian responder--a plea for universal definitions. J Assist
8. De Geyter C, Fehr P, Moffat R, Gruber IM, von Wolff M. Twenty years' experience with the Swiss data registry for assisted reproductive medicine: outcomes, key trends and recommendations for improved practice. Swiss Med Wkly. 2015;145:w14087.

9. Si C, Wang N, Wang M, Liu Y, Niu Z, Ding Z. TMT-based proteomic and bioinformatic analyses of human granulosa cells from obese and normal-weight female subjects. Reprod Biol Endocrinol. May 20 2021;19(1):75.

10. Khaskheli MN, Baloch S, Baloch AS. Infertility and weight reduction: influence and outcome. J Coll Physicians Surg Pak. Nov 2013;23(10):798-801.

11. Hu L, Du J, Lv H, et al. Influencing factors of pregnancy loss and survival probability of clinical pregnancies conceived through assisted reproductive technology. Reprod Biol Endocrinol. Aug 7 2018;16(1):74.

12. Cavalcante MB, Sarno M, Peixoto AB, Araujo Junior E, Barini R. Obesity and recurrent miscarriage: A systematic review and meta-analysis. J Obstet Gynaecol Res. Jan 2019;45(1):30-38.

13. Matjila MJ, Hoffman A, van der Spuy ZM. Medical conditions associated with recurrent miscarriage-Is BMI the tip of the iceberg? Eur J Obstet Gynecol Reprod Biol. Jul 2017;214:91-96.

14. Rittenberg V, Seshadri S, Sunkara SK, Sobaleva S, Oteng-Ntim E, El-Toukhy T. Effect of body mass index on IVF treatment outcome: an updated systematic review and meta-analysis. Reprod Biomed Online. Oct 2011;23(4):421-439.
15. Romanski PA, Bortoletto P, Magaoay B, Chung A, Rosenwaks Z, Spandorfer SD. Live birth outcomes in infertile patients with class III and class IV obesity following fresh embryo transfer. J Assist Reprod Genet. Feb 2021;38(2):347-355.

16. Metwally M, Ong KJ, Ledger WL, Li TC. Does high body mass index increase the risk of miscarriage after spontaneous and assisted conception? A meta-analysis of the evidence. Fertil Steril. Sep 2008;90(3):714-726.

17. Setton R, Chung A, Zimmerman L, Melnick A, Rosenwaks Z, Spandorfer SD. Body mass index is not associated with donor oocyte recipient success: an ideal study using a paired analysis of sibling-oocytes. F S Rep. Jun 2020;1(1):25-29.

18. Sunkara SK, Khalaf Y, Maheshwari A, Seed P, Coomarasamy A. Association between response to ovarian stimulation and miscarriage following IVF: an analysis of 124,351 IVF pregnancies. Hum Reprod. Jun 2014;29(6):1218-1224.

19. Supramaniam PR, Mittal M, McVeigh E, Lim LN. The correlation between raised body mass index and assisted reproductive treatment outcomes: a systematic review and meta-analysis of the evidence. Reprod Health. Feb 27 2018;15(1):34.

20. Sermondade N, Huberlant S, Bourhis-Lefebvre V, et al. Female obesity is negatively associated with live birth rate following IVF: a systematic review and meta-analysis. Hum Reprod Update. Jul 1 2019;25(4):439-451.

21. Sarais V, Pagliardini L, Rebonato G, Papaleo E, Candiani M, Vigano P.A Comprehensive Analysis of Body Mass Index Effect on in Vitro Fertilization Outcomes. Nutrients. Feb 23 2016;8(3):109.

22. Whynott RM, Summers KM, Van Voorhis BJ, Mejia RB. Effect of body mass
23. Metwally M, Cutting R, Tipton A, Skull J, Ledger WL, Li TC. Effect of increased body mass index on oocyte and embryo quality in IVF patients. Reprod Biomed Online. Nov 2007;15(5):532-538.

24. Ding X, Kou X, Zhang Y, Zhang X, Cheng G, Jia T. Leptin siRNA promotes ovarian granulosa cell apoptosis and affects steroidogenesis by increasing NPY2 receptor expression. Gene. Oct 30 2017;633:28-34.

25. Mitchell M, Armstrong DT, Robker RL, Norman RJ. Adipokines: implications for female fertility and obesity. Reproduction. Nov 2005;130(5):583-597.

26. Samy N, Hashim M, Sayed M, Said M. Clinical significance of inflammatory markers in polycystic ovary syndrome: their relationship to insulin resistance and body mass index. Dis Markers. 2009;26(4):163-170.

27. Ovalle A, Martinez MA, Fuentes A, et al. [Obesity, a risk factor for ascending bacterial infection during pregnancy]. Rev Med Chil. Apr 2016;144(4):476-482.

28. Wessels JM, Felker AM, Dupont HA, Kaushic C. The relationship between sex hormones, the vaginal microbiome and immunity in HIV-1 susceptibility in women. Dis Model Mech. Aug 28 2018;11(9).

29. Wang L, Xu J, Niu W, Hu L, Zhang Y, Sun Y. Genetic testing on products of conception and its relationship with body mass index. J Assist Reprod Genet. Aug 2020;37(8):1853-1860.

30. Tang S, Huang J, Lin J, Kuang Y. Adverse effects of pre-pregnancy maternal
underweight on pregnancy and perinatal outcomes in a freeze-all policy. BMC Pregnancy Childbirth. Jan 7 2021;21(1):32.

31. Marchi J, Berg M, Dencker A, Olander EK, Begley C. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. Obes Rev. Aug 2015;16(8):621-638.

32. Catalano PM, Shankar K. Obesity and pregnancy: mechanisms of short term and long term adverse consequences for mother and child. BMJ. Feb 8 2017;356:j1.

33. Ding W, Zhang FL, Liu XC, et al. Impact of Female Obesity on Cumulative Live Birth Rates in the First Complete Ovarian Stimulation Cycle. Front Endocrinol (Lausanne). 2019;10:516.

34. Wang X, Hao J, Zhang F, Li J, Kong H, Guo Y. Effects of female and male body mass indices on the treatment outcomes and neonatal birth weights associated with in vitro fertilization/intracytoplasmic sperm injection treatment in China. Fertil Steril. Aug 2016;106(2):460-466.

35. Taghavi SA, van Wely M, Jahanfar S, Bazarganipour F. Pharmacological and non-pharmacological strategies for obese women with subfertility. Cochrane Database Syst Rev. Mar 25 2021;3:CD012650.

36. Heymsfield SB, Wadden TA. Mechanisms, Pathophysiology, and Management of Obesity. N Engl J Med. Jan 19 2017;376(3):254-266.

37. Hoeger KM. Role of lifestyle modification in the management of polycystic ovary syndrome. Best Pract Res Clin Endocrinol Metab. Jun 2006;20(2):293-310.
38. Legro RS, Dodson WC, Kunselman AR, et al. Benefit of Delayed Fertility Therapy With Preconception Weight Loss Over Immediate Therapy in Obese Women With PCOS. J Clin Endocrinol Metab. Jul 2016;101(7):2658-2666.

39. Sun YF, Zhang J, Xu YM, et al. High BMI and Insulin Resistance Are Risk Factors for Spontaneous Abortion in Patients With Polycystic Ovary Syndrome Undergoing Assisted Reproductive Treatment: A Systematic Review and Meta-Analysis. Front Endocrinol (Lausanne). 2020;11:592495.

40. Tziomalos K, Dinas K. Obesity and Outcome of Assisted Reproduction in Patients With Polycystic Ovary Syndrome. Front Endocrinol (Lausanne). 2018;9:149.
Table 1: Baseline characteristics of women older than 35 years old with DOR
Table 2: Treatment and pregnancy outcomes of DOR patients older than 35 years old
Table 3: Logistic regression analysis of miscarriage related factors
Table 4: Logistic regression analysis of live birth related factors
Figure 1: Flow chart of the patients enrolled and the grouping
Figure 2: Key factors affecting the miscarriage rate and the live birth rate
Table 1: Baseline characteristics of women older than 35 years old with DOR

|                          | Total       | Underweight | Normal weight | Overweight | Obesity | P-value |
|--------------------------|-------------|-------------|---------------|------------|---------|---------|
| Number of cycles         | 2052        | 58(2.7%)    | 1389(67.7%)   | 527(25.7%) | 80(3.9%)|         |
| Male parameters          |             |             |               |            |         |         |
| Age(y) αβ                | 40.6±4.3    | 40.0±4.3    | 40.6±4.3      | 41.1±4.4   | 40.2±4.0| 0.001   |
| BMI(kg/m²)               | 25.4±3.0    | 24.7±3.2    | 25.3±3.0      | 25.4±3.1   | 26.0±3.1| 0.187   |
| Female parameters        |             |             |               |            |         |         |
| Age(y) c,d                | 39.9±3.0    | 38.7±2.5    | 39.7±2.9      | 40.5±3.2   | 40.3±3.3| < 0.001 |
| Menstrual cycle(day) e    | 28.8±8.6    | 27.8±1.6    | 28.4±7.2      | 29.6±12.0  | 30.8±7.9| 0.002   |
| BMI (kg/m²)              | 23.0±2.7    | 17.7±0.7    | 21.8±1.4      | 25.6±1.1   | 29.5±1.7| < 0.001 |
| Baseline FSH(IU/L) b,c,d,e | 11.4(10.1-13.9) | 12.0(10.2-14.4) | 11.6(10.2-14.3) | 11.1(9.5-13.2) | 10.4(8.1-14.3) | < 0.001 |
| Baseline LH(IU/L) c,d,e   | 5.3(3.8-6.9) | 6.1(4.3-7.0) | 5.5(4.0-7.2)  | 4.9(3.5-6.2) | 4.2(2.9-5.9) | < 0.001 |
| AMH(ng/mL)               | 0.7(0.5-0.9) | 0.8(0.7-1.0) | 0.7(0.5-0.9)  | 0.6(0.4-0.8) | 0.6(0.4-0.9) | 0.033   |
| AFC(n) b                 | 4.0(3.0-7.0) | 6.0(3.0-7.0) | 4.0(3.0-7.0)  | 4.0(2.0-6.0) | 4.0(2.0-7.0) | 0.010   |
| Infertility diagnosis, n(%)|            |             |               |            |         | 0.271   |
| Primary infertility      | 342(16.7%)  | 14(25.0%)   | 230(16.6%)    | 82(15.6%)  | 16(20.0%)|         |
| Secondary infertility    | 1710(83.3%) | 42(75.0%)   | 1159(83.4%)   | 445(84.4%) | 64(80.0%)|         |
| Previous IVF/ICSI attempts(n) | 0.0(0-1.0) | 0.0(0-1.0) | 0.0(0-1.0) | 0.0(0-1.0) | 0.0(0-1.0) | 0.411   |

"α" means chi-square test. Statistical significance is defined as P < 0.05.

Abbreviations: BMI=body mass index; FSH =follicle stimulating hormone; LH=luteinizing hormone; AMH=anti-Mullerian hormone; AFC=antral follicle count; IVF=in vitro fertilization; ICSI=intracytoplasmic sperm

Letter a, b, c, d, e, f indicated significant difference between groups.

αP: Comparison between Underweight and Normal weight patients.
βP: Comparison between Underweight and Overweight patients.
γP: Comparison between Underweight and Obese patients.
δP: Comparison between Normal weight and Overweight patients.
εP: Comparison between Normal weight and Obese patients.
ηP: Comparison between Overweight and Obese patients.
Table 2: Treatment and pregnancy outcomes of DOR patients older than 35 years old

|                          | Total    | Underweight | Normal weight | Overweight | Obesity | P-value |
|--------------------------|----------|-------------|---------------|------------|---------|---------|
| Number of cycles         | 2052     | 56(2.7%)    | 1388(67.7%)   | 527(25.7%) | 80(3.9%)|         |
| Gn initial dose(IU)      | 270.8±65.2| 273.2±54.5  | 268.3±68.2    | 276.2±58.9 | 277.5±53.6| 0.071   |
| Gn dosage(IU)            | 3386.4±1210.2| 3309.6±848.7| 3338.2±1218.3| 3484.8±1236.8| 3631.1±1044.4| 0.097   |
| Endometrial thickness on HCG day(mm) | 11.3±3.6  | 11.6±2.6    | 11.3±3.9      | 11.4±2.8   | 11.4±2.5| 0.530   |
| No. of retrieved oocytes(n) | 4.0(2.0-6.0) | 4.0(3.0-6.0) | 4.0(2.0-6.0)  | 4.0(2.0-6.0) | 4.0(2.0-6.0)   | 0.399   |
| No. of available embryos(n) | 4.0(2.0-6.0) | 4.0(3.0-6.0) | 4.0(2.0-6.0)  | 4.0(2.0-6.0) | 4.0(2.0-6.0)   | 0.427   |
| No. of embryos transferred(n) | 2.0(1.0-2.0) | 2.0(2.0-2.0) | 2.0(1.0-2.0)  | 2.0(1.0-2.0) | 2.0(1.0-2.0)   | 0.068   |
| Embryo stage at transfer,n(%) |          |             |               |            |         | 0.646†  |
| Cleavage stage           | 2024(98.6%) | 55(98.2%)   | 1366(98.5%)   | 522(99.1%) | 79(98.8%)|         |
| Blastocyst stage         | 28(1.4%)  | 1(1.8%)     | 21(1.5%)      | 5(0.9%)    | 1(1.3%) |         |
| Implantation rate, [% (n/N)] | 16.3%(590/3613) | 13.6%(15/110) | 16.4%(402/2454) | 16.1%(147/912) | 19.0%(26/137) | 0.644   |
| Clinical pregnancy rate, [% (n/N)] | 25.5%(524/2052) | 25.0%(14/56)  | 25.8%(358/1389) | 24.9%(131/527) | 26.3%(21/80)    | 0.978   |
| Miscarriage rate, [% (n/N)] | 35.5%(180/524) | 50%(7/14)     | 31.0%(111/358) | 44.3%(58/131) | 47.6%(10/21)    | 0.015†  |
| No. of live births(n)    | 1.0(0.0 1.0) | 1.0(0.0 1.5) | 1.0(1.0 1.0)  | 1.0(0.0 1.0) | 1.0(0.8 1.3)   | 0.674   |
| Live birth rate, [% (n/N)] | 61.5%(322/524) | 35.7%(5/14)   | 65.6%(233/358) | 54.2%(71/131) | 52.4%(11/21)    | 0.016†  |
| CCPR, [% (n/N)]          | 36.7%(753/2052) | 35.7%(20/56)  | 37.3%(518/1389) | 34.3%(181/527) | 42.5%(34/80)    | 0.449†  |
| CLBR, [% (n/N)]          | 57.6%(434/753) | 45.0%(9/20)   | 59.7%(309/518) | 54.1%(98/181) | 52.9%(18/34)    | 0.336†  |

α: means chi-square test. β: means Fisher test. Statistical significance is defined as P < 0.05.

Abbreviations: Gn=gonadotropin; IU=international unit; HCG=human chorionic gonadotropin; CCPR=Cumulative clinical pregnancy rate; CLBR=Cumulative live birth rate.

bP: Comparison between Underweight and Overweight patients.
cP: Comparison between Underweight and Obese patients.
dP: Comparison between Normal weight and Overweight patients.
eP: Comparison between Normal weight and Obese patients.
fP: Comparison between Overweight and Obese patients.
Table 3: Logistic regression analysis of miscarriage related factors

|                   | Univariable analysis | Multivariable analysis |
|-------------------|----------------------|------------------------|
|                   | Crude OR(95%CI)       | P-value                | Adjusted OR(95%CI)  | P-value |
| Male age          | 1.05(1.01-1.09)       | 0.022                  | 1.08(0.99-1.19)     | 0.097   |
| Female age        | 1.19(1.10-1.29)       | < 0.001                | 1.13(0.97-1.32)     | 0.115   |
| Menstrual cycle   | 1.00(0.99-1.01)       | 0.924                  | 1.00(0.98-1.02)     | 0.945   |
| BMI               |                      |                        |                      |         |
| Underweight       | 2.22(0.76-6.47)       | 0.145                  | 2.67(0.53-13.47)    | 0.234   |
| Normal weight     | 1*                   |                        | 1*                   |         |
| Overweight        | 1.76(1.17-2.66)       | 0.007                  | 2.41(1.20-4.83)     | 0.013   |
| Obesity           | 2.02(0.83-4.88)       | 0.121                  | 6.41(1.38-29.70)    | 0.018   |
| Baseline FSH      | 1.03(0.99-1.07)       | 0.156                  | 1.01(0.94-1.10)     | 0.729   |
| Baseline LH       | 1.01(0.98-1.05)       | 0.422                  | 1.03(0.99-1.07)     | 0.207   |
| AMH               | 0.92(0.60-1.43)       | 0.718                  | 0.91(0.46-1.80)     | 0.789   |
| AFC               | 0.96(0.91-1.01)       | 0.142                  | 0.99(0.87-1.13)     | 0.893   |

*This variable functions as an indicator. Other categories of the same variable were compared with it. Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval. Statistical significance is defined as P < 0.05.
Table 4: Logistic regression analysis of live birth related factors

|                      | Univariable analysis | Multivariable analysis |
|----------------------|----------------------|------------------------|
|                      | Crude OR (95%CI)     | P-value                | Adjusted OR(95%CI)       | P-value |
| Male age             | 1.05(1.01-1.09)      | 0.022                  | 0.94(0.86-1.03)          | 0.153   |
| Female age           | 1.19(1.10-1.29)      | < 0.001                | 0.88(0.76-1.03)          | 0.106   |
| Menstrual cycle      | 1.00(0.99-1.01)      | 0.924                  | 1.00(0.98-1.02)          | 0.911   |
| BMI                  |                      |                        |                        |         |
| Underweight          | 2.22(0.76-6.47)      | 0.145                  | 0.15(0.03-0.73)          | 0.019   |
| Normal weight        | 1*                   |                        | 1*                     |         |
| Overweight           | 1.76(1.17-2.66)      | 0.007                  | 0.46(0.23-0.91)          | 0.026   |
| Obesity              | 2.02(0.83-4.88)      | 0.121                  | 0.20(0.04-0.91)          | 0.037   |
| Baseline FSH         | 1.03(0.99-1.07)      | 0.156                  | 0.98(0.91-1.06)          | 0.682   |
| Baseline LH          | 1.01(0.98-1.05)      | 0.422                  | 0.97(0.93-1.01)          | 0.179   |
| AMH                  | 0.92(0.60-1.43)      | 0.718                  | 1.30(0.66-2.56)          | 0.453   |
| AFC                  | 0.96(0.91-1.01)      | 0.142                  | 0.99(0.88-1.13)          | 0.928   |

*This variable functions as an indicator. Other categories of the same variable were compared with it. Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval. Statistical significance is defined as P < 0.05.
Figure 1: Flow chart of the patients enrolled and the grouping

All patients older than 35 years old of diminished ovarian reserve (DOR) in our center from Aug. 2009 to May 2018, (N=5038)

- Chromosomal abnormalities of either party (N=128)
  - Donor oocytes or sperm (N=24)
    - Benign and malignant ovarian lesions (N=61)
    - Endocrine system diseases (N=50)
    - Malignant tumors and other systemic diseases (N=77)

  - Sex hormone dependent diseases (N=451)
  - Have taken exogenous hormone drugs or vitamin D within 3 months before the consultation (N=83)
  - No transferable embryos or no transfer until the end of follow-up (N=2112)

Total study objects n=2052

- Underweight N=56
- Normal weight N=1389
- Overweight N=527
- Obesity N=80
Figure 2: Key factors affecting the miscarriage rate and the live birth rate.