

The effect of maternal body mass index (BMI) on IVF cycle outcomes and embryo development in recurrent pregnancy loss (RPL) patients

BMI's role in RPL patients' IVF outcomes

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Abstract

Aim: This study aimed to examine the impact of maternal BMI on embryo quality, euploidy rates, and embryo morphokinetics in recurrent pregnancy loss (RPL) patients.

Material and Methods: We conducted a retrospective analysis of 98 RPL patients and 403 embryos observed using the Time-Lapse Monitoring System (TLM) at Memorial Sisli Hospital, ART, and Genetics Center between April 2021 and November 2022. Patients were categorized into three BMI groups: Group 1 (<25 kg/m²), Group 2 (25-29.9 kg/m²), and Group 3 (≥30 kg/m²). All embryos underwent assessment for quality, ploidy, and morphokinetic characteristics based on BMI. Morphokinetic analysis was performed on euploid embryos (n=168) to eliminate aneuploidy's impact on development dynamics.

Results: Age, Anti-Müllerian hormone (AMH), infertility duration, and previous IVF cycles were similar across groups. Group 2 had a slightly longer ovarian stimulation duration than Group 1 (p=0.040). IVF cycle outcomes, including oocyte retrieval, mature oocytes, and 2PN embryos, were similar. There were no significant differences in euploid embryo numbers among BMI groups. The distribution of good-quality and top-quality embryos was also similar (Group 1: 90.7%, Group 2: 87.7%, Group 3: 87.9%). Maternal BMI significantly affected the time embryos reached the expanded blastocyst stage (tEB) among the groups (p=0.044).

Discussion: This study found that high BMI does not impact embryo ploidy and quality but may prolong the morphokinetic process, specifically the time from morula to blastulation and the attainment of an expanded blastocyst, in cases of recurrent pregnancy loss (RPL).

Keywords

BMI, RPL, Euploidy, Embryo Quality, Morphokinetic Parameters

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Introduction

Recurrent pregnancy loss (RPL) is a complex and emotionally challenging condition affecting IVF patients. Clinicians often have difficulty identifying the factors that cause this condition. Many factors cause RPL, with the most common causes being antiphospholipid syndrome, uterine anomalies and abnormal thyroid function tests [1]. Recently, a number of studies have investigated whether there is a correlation between BMI and pregnancy loss [2-8]. Obesity is a medical condition characterized by excessive body fat accumulation, which may have negative effects on an individual's health. Recently, obesity has become common; at least half of women of reproductive age are overweight or obese. Research on obesity and IVF outcomes is mixed, with some studies showing negative impacts [9-11], and others showing no effect [12, 13]. However, the majority of research suggests obesity may hinder IVF success. Obese women often require higher doses of fertility drugs to stimulate ovulation and have longer stimulations, as their hormones can be imbalanced [9]. Pregnancy rates per IVF cycle appear to be lower in obese women [9-11, 14]. Obese women have 50% lower rates of achieving a clinical pregnancy and live birth. The risk of miscarriage also seems to increase with obesity [10]. Rittenberg et al.'s comprehensive review and meta-analysis of 33 trials and 47,000 cycles examined BMI with IVF results. Obesity, hyperandrogenism, aberrant leptin levels, and insulin resistance impede egg maturation and embryo competence, they said. They also suggested that obese women's follicles and endometrium may have higher IL6 and TNF levels, which could lower egg quality and implantation and increase abortion rates [5]. Some studies have suggested that obesity causes abortion by causing an inflammatory reaction in the uterine environment rather than affecting oocyte or embryo quality because of the high abortion rates in euploid embryo transfers in obese cases [6, 15, 16]. Some of these studies have suggested that BMI leads to pregnancy loss by impairing oocyte and embryo quality [17, 18]. How obesity affects female fertility is complex and still not fully understood. It can negatively affect the reproductive system and may impact the quality of the embryos and oocytes. This study aimed to investigate how maternal BMI affects embryo development, euploidy rates, and the morphokinetic factors involved in the process from fertilization to blastocyst formation in individuals who have had Recurrent Pregnancy Loss (RPL). The morphokinetic parameters play a crucial role in understanding the temporal dynamics of embryonic development and provide valuable insights into the potential impact of maternal BMI on the coordination of early embryogenesis in patients with recurrent pregnancy loss (RPL).

Material and Methods

Ethical Compliance

This study was approved by Yeni Yuzyil University Ethics Committee (approval number: 2022-04-07/24).

Patient Eligibility Criteria

This was a retrospective comparative analysis with 98 RPL patients and 403 embryos that were incubated at the Time-Lapse Monitoring System (TLM) in Memorial Sisli Hospital, ART and Genetics Center between April 2021 and November 2022. Patients aged >38 years, ovarian, endometrial or

uterine abnormalities (Müllerian factor, severe endometriosis/adenomyosis, Asherman syndrome, thin endometrium (<7 mm)) and partners with abnormal sperm parameters according to WHO 2021 criteria were excluded from the study. RPL was diagnosed if the patient had a history of at least three pregnancy losses. Patients were sub-grouped according to their body mass index (BMI) (kg/m²) (Group 1: <25, Group 2: 25-29.9, Group 3: ≥30). In order to exclude the negative effect of aneuploidy on embryo morphokinetics, only euploid embryos (n=168) were used for the comparison between different BMI groups.

Treatment Protocol

All patients were stimulated using the GnRH (Gonadotropin-releasing hormone) antagonist protocol. Controlled ovarian stimulation (COS) was initiated in patients with appropriate dosage according to the patients' individual characteristics. Recombinant follicle-stimulating hormone (rFSH) (Gonal-f; Merck, Switzerland) or a combination of rFSH and recombinant luteinizing hormone (rLH) (Luveris; Merck, Switzerland) or human menopausal gonadotropin (hMG) (hMG, Ferring, Switzerland) were used for COS. GnRH-a (Cetrotide, Merck-Serono) was administered when at least one follicle was ≥ 13 mm. Follicular development was screened by ultrasonography at regular intervals. Once at least three dominant follicles of 17 mm were observed, oocyte retrievals (OPU) were planned at 36 hours following the injection of 250 mcg recombinant human chorionic gonadotropin (rhCG) (Ovitrelle; Merck, Switzerland) or 0.2 mg triptorelin acetate (Gonapeptyl; Ferring, Germany) by transvaginal ultrasound guidance.

Embryo Culture and Morphology Assessment

Single-step embryo culture media (LifeGlobal, Cooper Surgical, Brussels, Belgium) supplemented with 10% Human Serum Albumin (HSA) (Life Global®, Belgium) was used for embryo culture at 25 µl volume of EmbryoSlide® (Vitrolife, Sweden) culture dish wells covered with an overlay of 1.5 ml paraffin oil (Life Global®, Belgium) and incubated overnight at 6% CO₂, 5% O₂, 37 C, with pH 7.26–7.30 in a time-lapse incubator (EmbryoScope™, Sweden) before use. Embryos were cultured until Day 5-6 by refreshing the media on Day 3. Blastocysts were scored before vitrification and after thawing according to Gardner's classification and classified into different groups according to their quality as follows: Top-Quality (TQ): Hatched AA, 6AA, 5AA, 4AA, Good-Quality (GQ): Hatched AB/BA/BB, 5AB/BA/BB, 4AB/BA/BB, 3AA, Moderate-Quality (MQ): 3AB/BA, 2AA, Poor-Quality (PQ): the rest of the embryos.

Embryo Biopsy and Genetical Analysis

Artificial hatching was performed in embryos in the zona pellucida by using a diode laser (RI Saturn 3, England) on the 3rd day of embryo culture before the trophectoderm biopsy procedure. Five to eight cells from the trophectoderm were drawn into a biopsy pipette with a 30 mm inner diameter (Origio, Denmark) by the flicking method. Next Generation Sequencing (NGS) method was used for PGT-A analysis. ReproSeq kit (ThermoFisher, USA) was used according to the manufacturer's instructions using the Ion Torrent™ S5™ System (ThermoFisher, USA). Genetical analyses were performed using the Ion Reporter software suite v5.2 and v5.6 (ThermoFisher, USA).

Statistical Analysis

IBM Statistical Package for the Social Sciences (SPSS) Software Version 25 was used for statistical analyses. Demographics and clinical characteristics of patients and embryo morphokinetic parameters were compared using the non-parametric Kruskal-Wallis test. The chi-square test was used for the analysis of frequencies. The p-value <0.05 was considered statistically significant.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

Demographic and clinical characteristics of the study groups are shown in Table 1. No statistical significance was observed between study groups in terms of age, anti-Müllerian hormone (AMH), basal follicle-stimulating hormone (FSH), duration of infertility, previous IVF cycles, while the duration of ovarian stimulation was higher in Group 2 than in Group 1 (Group 1: 10.90±1.61, Group 2: 11.74±1.28, Group 3: 11.30±1.63, p=0.040, Group 1 vs 2 p=0.011) (Table 1).

IVF cycle outcomes were found similar in terms of retrieved total oocyte count (Group 1: 10.90±6.02, Group 2: 10.28±5.19, Group 3: 12.50±5.31, p>0.05), mature (MII) oocyte count (Group 1: 9.61±5.48, Group 2: 9.10±4.06, Group 3: 9.70±3.36, p>0.05) and 2PN count (Group 1: 7.73±4.54, Group 2: 7.72±3.20, Group 3: 8.50±3.34, p>0.05). No statistical significance was not observed in terms of embryo blastulation rates between the study groups although it was lower in Group 3 (Group 1: 72.61±21.42, Group 2: 73.61±17.69, Group 3: 59.49±24.41, p>0.05) (Table 1).

When the effect of maternal BMI on euploid embryo rate was analyzed, no statistical significance was obtained in terms of euploid embryo count between study groups (Group 1: 45%, Group 2: 56%, Group 3: 44.8%, p>0.05) (Table 2). Similarly, the distribution of good quality (TQ+GQ) embryos was similar among different BMI groups (TQ+GQ %; Group 1: 90.7, Group 2: 87.7%, Group 3: 87.9%) (Table 2).

Regarding the effect of BMI on euploid embryo morphokinetics, time to reach expanded blastocyst (tEB) was found to be

Table 1. Demographic, clinical characteristics and cycle outcomes of the study groups

| Parameters | BMI Groups | | | p-value |
|-------------------------------|----------------|----------------|----------------|------------------------------|
| | Group 1 (n=59) | Group 2 (n=29) | Group 3 (n=10) | |
| Age (year) | 32.68±3.75 | 33.79±3.51 | 33.80±3.82 | 0.320 |
| AMH (ng/ml) | 2.67±2.11 | 2.25±1.61 | 3.45±1.16 | 0.061 |
| Basal FSH (mIU/mL) | 9.65±3.60 | 8.52±3.02 | 6.95±0.90 | 0.372 |
| Infertility duration (years) | 4.09±2.90 | 4.17±2.85 | 6.10±2.84 | 0.130 |
| Previous IVF cycles | 2.75±2.39 | 3.14±1.97 | 2.70±2.05 | 0.412 |
| Duration of Stimulation (Day) | 10.90±1.61 | 11.74±1.28 | 11.30±1.63 | 0.040 (Group 1 vs 2 p=0.011) |
| Total oocytes | 10.90±6.02 | 10.28±5.19 | 12.50±5.31 | 0.452 |
| MI I oocytes | 9.61±5.48 | 9.10±4.06 | 9.70±3.36 | 0.888 |
| 2PN | 7.73±4.54 | 7.72±3.20 | 8.50±3.34 | 0.754 |
| % Blastulation | 72.61±21.42 | 73.61±17.69 | 59.49±24.41 | 0.256 |

Table 2. Effect of female BMI on euploidy and embryo quality

| Female BMI (kg/m²) | Euploid (n=168) | Aneuploid (n=191) | P value |
|--------------------|-----------------|-------------------|---------|
| <25 (n=220) | 99 (45%) | 121 (55%) | 0.176 |
| 25-29.9 (n=100) | 56 (56%) | 44 (44%) | |
| ≥30 (n=29) | 13 (44.8%) | 16 (55.2%) | |

| | Embryo Quality | | p value |
|-----------------|----------------|------------|---------|
| | TQ+GQ (n=192) | MQ (n=42) | |
| <25 (n=248) | 225 (90.7%) | 23 (0.3%) | 0.634 |
| 25-29.9 (n=122) | 107 (87.7%) | 15 (12.3%) | |
| ≥30 (n=33) | 29 (87.9%) | 4 (12.1%) | |

Table 3. Effect of BMI on euploid embryo morphokinetics in RPL patients

| Morphokinetic Parameters | Female BMI (kg/m²) | | | p-value |
|--------------------------|--------------------|----------------|-------------|--|
| | <25 (n=99) | 25-29.9 (n=56) | ≥30 (n=13) | |
| tPNa | 8.00±1.52 | 7.86±1.66 | 8.11±1.69 | 0.959 |
| PNf | 23.48±3.08 | 23.09±3.55 | 24.15±2.11 | 0.465 |
| t2 | 25.83±3.30 | 25.55±3.65 | 26.25±2.31 | 0.633 |
| t3 | 36.69±4.31 | 35.72±5.16 | 38.00±2.79 | 0.150 |
| t4 | 38.37±4.09 | 37.48±4.65 | 38.76±2.72 | 0.285 |
| t5 | 49.14±5.87 | 48.86±7.72 | 51.59±5.72 | 0.322 |
| t6 | 52.42±5.73 | 52.01±7.39 | 54.16±4.56 | 0.354 |
| t7 | 54.57±6.40 | 53.85±7.73 | 56.67±6.42 | 0.426 |
| t8 | 56.90±7.97 | 56.72±9.07 | 57.36±6.32 | 0.887 |
| t9 | 68.13±8.11 | 65.22±11.23 | 65.61±9.64 | 0.349 |
| tSC | 71.10±9.42 | 76.59±10.47 | 76.76±7.00 | 0.988 |
| tM | 85.99±8.76 | 86.79±8.68 | 84.69±8.12 | 0.771 |
| tSB | 97.21±8.06 | 94.09±10.18 | 98.11±7.01 | 0.252 |
| tB | 103.51±8.06 | 102.69±8.78 | 105.44±5.60 | 0.567 |
| tEB | 109.32±7.36 | 109.27±8.18 | 144.19±4.58 | 0.044 (Group 1 vs 3 p=0.017, Group 2 vs 3 p=0.015) |
| t2-t8 | 30.95±6.42 | 31.09±7.01 | 31.11±4.73 | 0.816 |
| t8-tM | 29.95±10.49 | 32.21±11.41 | 27.32±5.58 | 0.208 |
| tSC-tM | 9.04±4.33 | 10.20±5.83 | 7.92±2.41 | 0.638 |
| t8-tSC | 21.00±10.74 | 22.00±11.31 | 19.40±5.35 | 0.858 |
| tM-tSB | 12.19±10.86 | 9.59±3.85 | 14.35±4.06 | 0.013 (Group 1 vs 3 p=0.034, Group 2 vs 3 p=0.004) |
| tSB-tEB | 13.77±3.11 | 16.74±7.74 | 16.89±7.10 | 0.157 |

statistically significant between the study groups (p=0.044). When we performed Bonferroni Post Hoc test in order to find out the statistical significance between the two groups, it was observed that the tEB was longer in Group 3 than both in Group 1 and 2 (tEB; Group 1: 109.32±7.36, Group 2: 109.27±8.18, Group 3: 144.19±4.58. Group 1 vs 3 p= 0.017, Group 2 vs 3 p=0.015). Similarly, time from morula stage to the start of blastulation (tM-tSB) was statistically significant in different BMI groups (p=0.013), and it was longer in Group 3 compared to Group 1 and Group 2 (tM-tSB; Group 1: 12.19±10.86, Group 2: 9.59±3.85, Group 3: 14.35±4.06. Group 1 vs 3 p= 0.034, Group 2 vs 3 p=0.004) (Table 3).

Values were presented as number (n) and percentage (%). BMI, body mass index; AMH, anti-mullerian hormone; FSH, follicle-stimulating hormone; MII, metaphase II; PN, pronucleus. The

Kruskal-Wallis test was used for inter-group comparisons. The Bonferroni test was used for statistically significant values. Bold values are statistically significant ($p < 0.05$).

BMI, body mass index; TQ, top-quality embryos; GQ, good quality embryos, MQ; moderate quality embryos. The Chi-square test was used for intergroup comparisons.

Values were presented as mean \pm standard deviation. Times indicate hours post ICSI. PNa, PN onset time; Pnf, PN fading time; t2, time to reach two cells; t3, time to reach three cells; t4, time to reach four cells; t5, time to reach five cells; t6, time to reach six cells; t7, time to reach seven cells; t8, time to reach eight cells; t9, time to reach nine cells; tSC, time to start compaction; tM, time for reaching morula; tSB, time of starting blastulation; tB, time to reach the blastocyst; tEB, time to reach expanded blastocyst. Intergroup comparisons were made using the Kruskal-Wallis test. Significant values were further analyzed using the Bonferroni Post Hoc test. Bold values were statistically significant ($p < 0.05$).

Discussion

Our analysis of embryo quality rates showed no statistically significant difference between BMI groups. This finding suggests that maternal BMI may not be an important factor affecting the quality of embryos in RPL patients undergoing IVF. Furthermore, the distribution of good-quality embryos (TQ+GQ) was similar among different BMI groups, suggesting that embryo quality remains consistent regardless of maternal BMI. Research on this topic provides compelling evidence that obesity negatively impacts embryo quality and IVF outcomes. Multiple studies have found that obesity, especially in higher BMI categories like class II and III, led to poorer embryo development and quality [10, 19, 20]. Obese women had fewer mature and normally fertilized oocytes as well as lower estradiol levels [10], indicating a poorer ovarian response. Embryos from obese women were also slower to develop and had lower morphological grades [21]. The mechanisms of the negative effects of obesity appear to be inflammation and oxidative stress. Excess fat tissue promotes systemic and ovarian inflammation, damaging ovarian cells and oocytes. Inflammation and oxidative stress disrupt oocyte maturation, fertilization, and embryo development. They may also alter the ovarian environment, disrupting steroidogenesis and folliculogenesis [22]. In Luke et al.'s review of 45 163 ART embryo transfers in which maternal height and weight were recorded, increasing obesity was associated with a significant increase in failure to achieve clinical pregnancy with the use of autologous oocytes ($p < 0.0001$), but not with the use of donor oocytes [18]. While some studies agree with us that obesity does not directly affect embryo quality or development based on morphological assessments [9, 10, 15, 23], they have suggested other mechanisms for how obesity may indirectly affect IVF outcomes. Possible explanations include hormonal disturbances (higher LH and lower estradiol) in obese women [23], poorer uterine receptivity [10], and metabolic or inflammatory changes [9].

In our study, we did not find any difference in euploidy rates between BMI groups in cases with a history of RPL. This suggests that the negative impact of excess weight on reproductive outcomes is independent of the embryo's ploidy status. In

cases with euploid embryo transfer, higher miscarriage rates in the high BMI group compared to the normal BMI group were not associated with embryo ploidy. Studies showing that obesity increases abortion rates in euploid embryo transfer cycles show no association with oocyte or embryo quality. The increased abortion rates seen with increasing BMI may be due to obesity-associated changes in inflammatory factors (such as interleukin 6 and tumor necrosis factor) as well as the influence of endometrial receptivity [16, 17, 25, 26].

Our findings showed a correlation between BMI and embryo morphokinetic in cases with a history of RPL. Notably, embryos from patients with a BMI below 25 kg/m² took longer to reach the point of pronucleus appearance (tPNa) than embryos from patients with a BMI between 25 kg/m² and 29 kg/m². Although this difference is not statistically significant, its clinical significance may require further investigation. Similar to our study results, Piquette et al.'s comparison of embryo division timings revealed that embryos from individuals with obesity reached the two-cell embryo stage (T2) more quickly than those from individuals with a normal weight. However, when examining BMI as a continuous variable, no statistically significant correlation was found between BMI and the timing of embryo division [24].

In our study, the time intervals between certain developmental stages, such as the eight-cell (t8) and morula stage (tM), appeared to decrease as maternal BMI decreased. Similarly, Kassi et al. stated that embryos from women with underweight status were found to reach the 8-cell stage more rapidly than those from women with normal weight or obesity. This study underscores the role of weight as a potential factor influencing embryo development, as evidenced by observations made through TLM [25].

Another finding was that embryos in the low BMI group took longer to reach the blastulation stage (tSB) than those in the high BMI group. These findings suggest that maternal BMI may have a nuanced effect on the rate of embryo development. The precise mechanisms behind these variations remain unclear and require further investigation.

To summarize, our study adds to the growing body of research suggesting that maternal BMI could be a contributing factor to morphokinetics of embryos in RPL patients. The discrepancies observed in embryo development rates at different stages could indicate underlying physiological differences associated with obesity. However, it is important to note that more extensive prospective studies are necessary to affirm these results and provide a more comprehensive understanding of the correlation between maternal BMI, embryo development, and recurrent pregnancy loss.

In conclusion, understanding the complex interactions between maternal factors, embryonic development, and recurrent pregnancy loss is crucial for improving diagnostic and treatment strategies for RPL patients, providing them with hope and better prospects for successful pregnancies in the future.

Conclusion

Our findings showed that maternal BMI could affect embryo morphokinetic. In this small-sized and retrospective study, we mainly found that the timing to start the blastulation from the morula stage, as well as blastocyst expansion, takes longer

when the maternal BMI is above 30 (kg/m²). The prevalence of total, MII, 2PN oocytes, blastulation rate, euploid embryos and good quality embryos were similar among different BMI groups in our study. Our findings should be confirmed with further large-scale studies, including analysis of clinical outcomes.

Limitation of Study

The major limitation of this small-sized, retrospective study is that it included a small number of cases, and the clinical outcomes of embryos in different BMI groups were not assessed.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content, including study design, data collection, analysis and interpretation, writing, preparation and scientific review of the contents, and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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