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## Predicting blastocyst formation in low oocyte yield: A retrospective cohort analysis

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### Abstract

**Background:** Poor ovarian response remains a significant challenge in assisted reproductive technology. Patients with low oocyte yield (<5 oocytes) represent a unique subset requiring individualized management strategies.

**Objective:** To evaluate blastocyst formation rates and identify predictive factors for successful embryo development in *in vitro* fertilization (IVF) cycles yielding fewer than five oocytes.

**Methods:** A retrospective cohort analysis was conducted on 47 IVF cycles with <5 oocytes retrieved between July 2016 and December 2024. Parameters analyzed included patient age, semen parameters, oocyte maturity, fertilization rates, and blastocyst conversion rates.

**Results:** The mean patient age was  $36.4 \pm 4.8$  years. The overall blastocyst formation rate was 48.9% (23/47 cycles). Metaphase II (M2) oocyte proportion significantly correlated with blastocyst formation ( $p < 0.05$ ). Cycles achieving at least one blastocyst had higher mean M2 counts ( $2.7 \pm 1.1$  vs  $1.8 \pm 0.9$ ). No significant difference was observed based on sperm parameters.

**Conclusion:** Despite low oocyte numbers, acceptable blastocyst formation rates can be achieved. M2 oocyte count is a key predictor of blastocyst development in poor responders. Individualized stimulation protocols and patient counseling remain essential.

**Keywords:** Blastocyst, embryo development, IVF outcomes, low oocyte yield, poor ovarian response

### Introduction

*In vitro* fertilization (IVF) has revolutionized the management of infertility since the birth of Louise Brown in 1978 [1]. Despite significant advancements in assisted reproductive technology (ART), poor ovarian response (POR) continues to pose substantial challenges for both clinicians and patients [2]. The Bologna criteria, established in 2011, defined POR as the retrieval of three or fewer oocytes following conventional ovarian stimulation [3]. However, clinical practice often encounters patients yielding fewer than five oocytes, representing a broader spectrum of diminished ovarian reserve.

The prevalence of poor ovarian response ranges from 9% to 24% across IVF centers worldwide [4]. Multiple factors contribute to this condition, including advanced maternal age, diminished ovarian reserve, previous ovarian surgery, and genetic predisposition [5]. The management of poor responders remains contentious, with various stimulation protocols proposed to optimize outcomes [6]. These include high-dose gonadotropin protocols, addition of growth hormone, luteal phase stimulation, and the use of adjuvant therapies such as DHEA and CoQ10 [7, 8].

Blastocyst transfer has gained widespread acceptance due to improved implantation rates and better embryo selection compared to cleavage-stage transfer [9]. Extended culture to the blastocyst stage allows for natural embryo selection, as only competent embryos survive to day 5-6 [10]. However, concerns exist regarding the applicability of blastocyst culture in patients with low oocyte yield, where the risk of cycle cancellation due to embryo attrition is higher [11].

The decision to perform day 3 transfer versus extended culture to blastocyst stage in poor responders remains debatable [12]. While some studies suggest comparable outcomes, others advocate for early transfer to minimize the risk of having no embryos for transfer [13]. Understanding the predictive factors for blastocyst formation in this population is crucial for optimizing treatment strategies and patient counseling.

This study aims to evaluate blastocyst formation rates and identify predictive factors influencing successful embryo development to the blastocyst stage in IVF cycles yielding fewer than five oocytes. By analyzing a comprehensive dataset spanning eight years, we seek to provide

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evidence-based guidance for the management of poor ovarian responders in clinical practice.

## Materials and methods

**Study design and setting:** This retrospective cohort study was conducted at a tertiary care fertility center. Data were collected from the center's electronic medical records for all IVF/ICSI cycles performed between July 2016 and December 2024. Informed consent for data utilization was obtained from all participants as part of the standard treatment protocol.

**Patient Selection:** Inclusion criteria comprised IVF/ICSI cycles with fewer than five oocytes retrieved at oocyte pickup. Both autologous and donor oocyte cycles were included. Exclusion criteria included cycles with incomplete data, cycles cancelled before fertilization, and cases where oocytes were retrieved but not subjected to fertilization attempts. A total of 47 cycles meeting these criteria were included in the final analysis.

**Ovarian Stimulation Protocols:** Controlled ovarian stimulation was performed using various protocols based on individual patient characteristics and physician preference [14]. The majority of patients received gonadotropin-based stimulation using human menopausal gonadotropin (HMG) at doses ranging from 150 to 450 IU daily. Antagonist protocols utilizing cetrorelix or GnRH agonist protocols were employed for pituitary suppression [15]. Trigger for final oocyte maturation was achieved using either recombinant hCG (R-hCG), urinary hCG, GnRH agonist (Decapeptyl/Lupride), or a combination thereof [16]. Some protocols included adjuvants such as clomiphene citrate (Siphene), letrozole, or growth hormone.

## Laboratory Procedures

Oocyte retrieval was performed 34-36 hours post-trigger under transvaginal ultrasound guidance [17]. Retrieved oocytes were classified based on maturity: Metaphase II (M2 - mature oocytes suitable for fertilization), Metaphase I (M1 - immature oocytes that have not completed the first meiotic division), and Germinal Vesicle (GV - immature oocytes at the earliest stage). Intracytoplasmic sperm injection (ICSI) was performed for all cycles. Semen samples were collected on the day of oocyte retrieval, and parameters including volume, concentration, motility, and morphology were assessed according to WHO

2010 guidelines [18]. In cases of severe male factor infertility, testicular sperm aspiration (TESA) or donor sperm was utilized. Fertilization was assessed 16-18 hours post-ICSI by the presence of two pronuclei (2PN). Embryo culture was performed in sequential media under controlled atmospheric conditions (6% CO<sub>2</sub>, 5% O<sub>2</sub>, 37°C) [19]. Embryo quality was assessed on day 1, day 3, and day 5. Blastocyst grading was performed according to the Gardner classification system [20]. Blastocyst formation was defined as the development of at least one embryo to the blastocyst stage by day 5-6 of culture.

## Outcome Measures

The primary outcome measure was blastocyst formation rate, defined as the proportion of cycles achieving at least one blastocyst. Secondary outcomes included fertilization rate (calculated as the number of 2PN embryos divided by the number of M2 oocytes injected), cleavage rate (calculated as the number of day 3 embryos divided by the number of 2PN embryos), and the number of blastocysts available for transfer or cryopreservation. The blastocyst conversion rate was calculated as the number of blastocysts formed divided by the number of 2PN embryos.

## Statistical Analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY). Continuous variables were expressed as mean±standard deviation (SD) and compared using Student's t-test or Mann-Whitney U test as appropriate. Categorical variables were expressed as frequencies and percentages and compared using Chi-square test or Fisher's exact test [21]. Logistic regression analysis was performed to identify independent predictors of blastocyst formation. A p-value of <0.05 was considered statistically significant.

## Results

### Patient Demographics and Cycle Characteristics

A total of 47 IVF/ICSI cycles with fewer than five oocytes retrieved were analyzed (consistent with the inclusion criteria specified in Methods). The baseline demographic and cycle characteristics are presented in Table 1. The mean patient age was 36.4±4.8 years (range: 25-49 years). The majority of cycles (89.4%, n=42) utilized autologous oocytes, while 10.6% (n=5) were donor oocyte cycles.

**Table 1:** Baseline Demographics and Cycle Characteristics (n=47)

| Parameter                                     | Value      |
|---|------------|
| Age (years), Mean ±SD                         | 36.4±4.8   |
| Age Range (years)                             | 25 - 49    |
| Autologous cycles, n (%)                      | 42 (89.4%) |
| Donor oocyte cycles, n (%)                    | 5 (10.6%)  |
| Total Oocytes Retrieved, Mean ±SD             | 2.8±1.2    |
| Metaphase II (M2) Oocytes per cycle, Mean ±SD | 2.3±1.1    |
| Semen Volume (mL), Mean±SD                    | 1.4±0.5    |
| Sperm Concentration (million/mL), Mean ±SD    | 42.1±26.3  |
| Sperm Motility (%), Mean ±SD                  | 28.4±12.6  |

**Abbreviations:** SD, Standard Deviation; n, number of cycles; %, percentage.

- Note:** Metaphase II (M2) oocytes represent mature oocytes that have completed the first meiotic division and are suitable for ICSI fertilization. Values for M2 oocytes represent the mean number of mature oocytes retrieved per cycle.

### Oocyte and Embryo Development Outcomes

The distribution of oocyte maturity stages is detailed in Table 2. Among the 132 oocytes retrieved across all 47 cycles, 108 (81.8%) were mature M2 oocytes, 15 (11.4%) were M1 oocytes, and 9 (6.8%) were at the GV stage. The overall fertilization rate was 87.0% (94/108 M2 oocytes). Day 3 embryo development was observed in 85.1% of fertilized oocytes (80/94 2PN embryos).

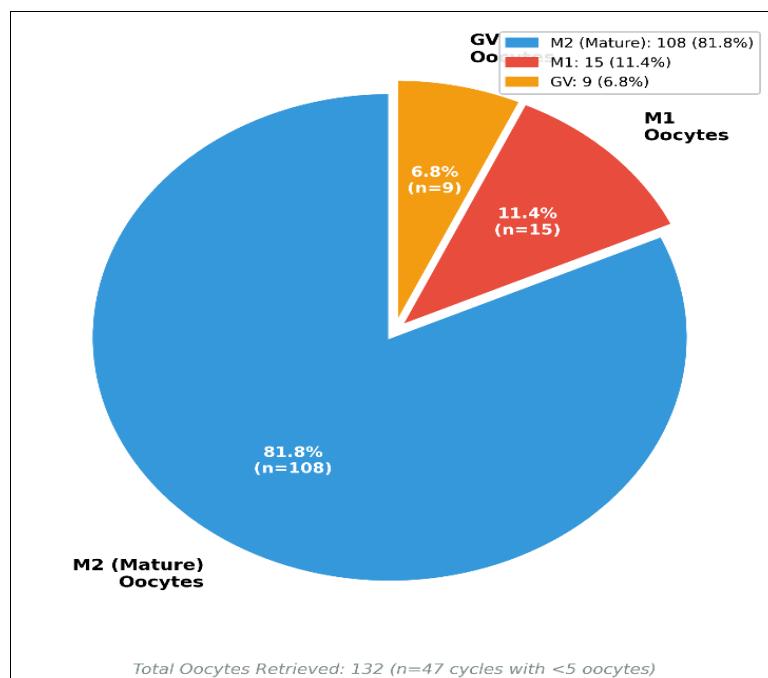
**Table 2:** Distribution of Oocyte Maturity and Embryo Development (n=47 cycles)

| Parameter                     | Number | Percentage |
|-------------------------------|--------|------------|
| Total Oocytes Retrieved       | 132    | -          |
| Metaphase II (M2) Oocytes     | 108    | 81.8%      |
| Metaphase I (M1) Oocytes      | 15     | 11.4%      |
| Germinal Vesicle (GV) Oocytes | 9      | 6.8%       |
| Fertilized Oocytes (2PN)      | 94     | 87.0%*     |
| Day 3 Embryos                 | 80     | 85.1%**    |
| Day 5 Blastocysts             | 46     | 48.9%***   |

**Abbreviations:** M2, Metaphase II (mature oocytes); M1, Metaphase I (immature oocytes); GV, Germinal Vesicle (immature oocytes); 2PN, two pronuclei (indicating successful fertilization).

- \*Fertilization Rate = Number of 2PN embryos / Number of M2 oocytes injected  $\times 100$  (94/108  $\times 100$  = 87.0%)

- \*\*Cleavage Rate = Number of Day 3 embryos / Number of 2PN embryos  $\times 100$  (80/94  $\times 100$  = 85.1%)
- \*\*\*Blastocyst Conversion Rate = Number of Day 5 blastocysts / Number of 2PN embryos  $\times 100$  (46/94  $\times 100$  = 48.9%).

**Fig 1:** Pie chart showing distribution of oocyte maturity stages (M2, M1, GV)

**Blastocyst Formation Outcomes:** Blastocyst formation was achieved in 23 out of 47 cycles (48.9%). The detailed outcomes stratified by the number of blastocysts formed are presented in Table 3. Among cycles achieving blastocyst formation, 8 cycles

(34.8%) produced a single blastocyst, 7 cycles (30.4%) produced two blastocysts, and 8 cycles (34.8%) produced three or more blastocysts. The maximum number of blastocysts achieved in a single cycle was four.

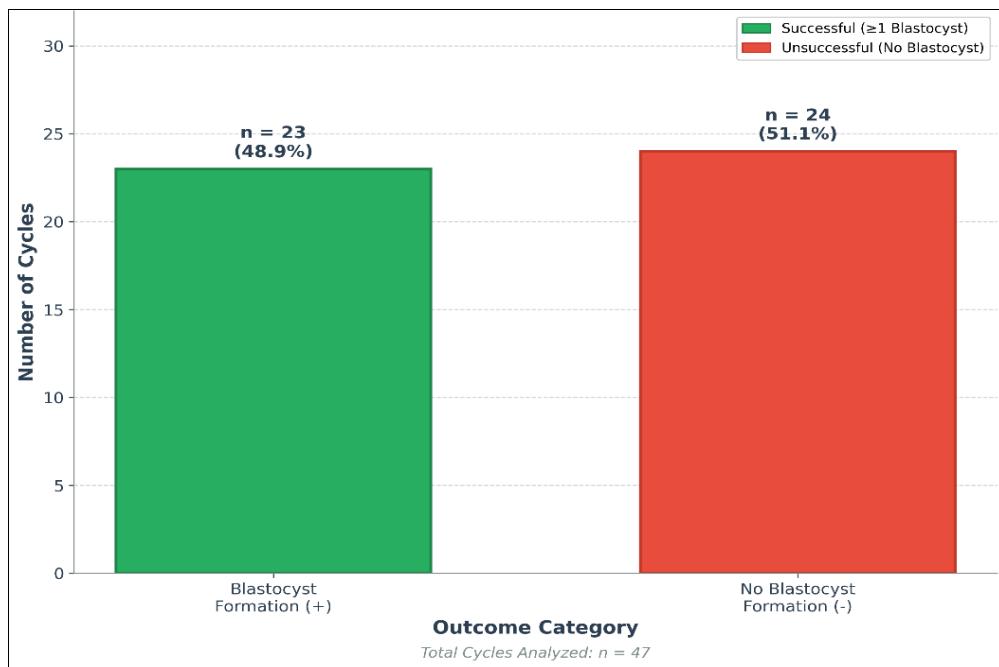
**Table 3:** Blastocyst Formation Outcomes by Cycle (n=47 cycles)

| Outcome Category                            | Number (n) | Percentage (%) |
|---|------------|----------------|
| No Blastocyst Formation                     | 24         | 51.1%          |
| Blastocyst Formation ( $\geq 1$ blastocyst) | 23         | 48.9%          |
| - Single Blastocyst (1 blastocyst)          | 8          | 34.8%*         |
| - Two Blastocysts                           | 7          | 30.4%*         |
| - Three or More Blastocysts                 | 8          | 34.8%*         |
| Fresh Embryo Transfer                       | 12         | 25.5%          |
| Freeze-All Strategy (Cryopreservation)      | 11         | 23.4%          |

**Abbreviations:** n, number of cycles; %, percentage.

\*Percentages calculated among cycles with successful blastocyst formation (n=23). All other percentages calculated from total cycles (n=47).

**Predictive Factors for Blastocyst Formation:** Comparative analysis between cycles with and without blastocyst formation is presented in Table 4. Cycles achieving blastocyst formation had significantly higher mean M2 oocyte counts ( $2.7 \pm 1.1$  vs  $1.8 \pm 0.9$ ,  $p=0.003$ ). Patient age showed a trend toward significance ( $35.2 \pm 4.5$  vs  $37.6 \pm 5.0$ ,  $p=0.08$ ). Semen parameters including volume, concentration, and motility did not demonstrate significant differences between groups.

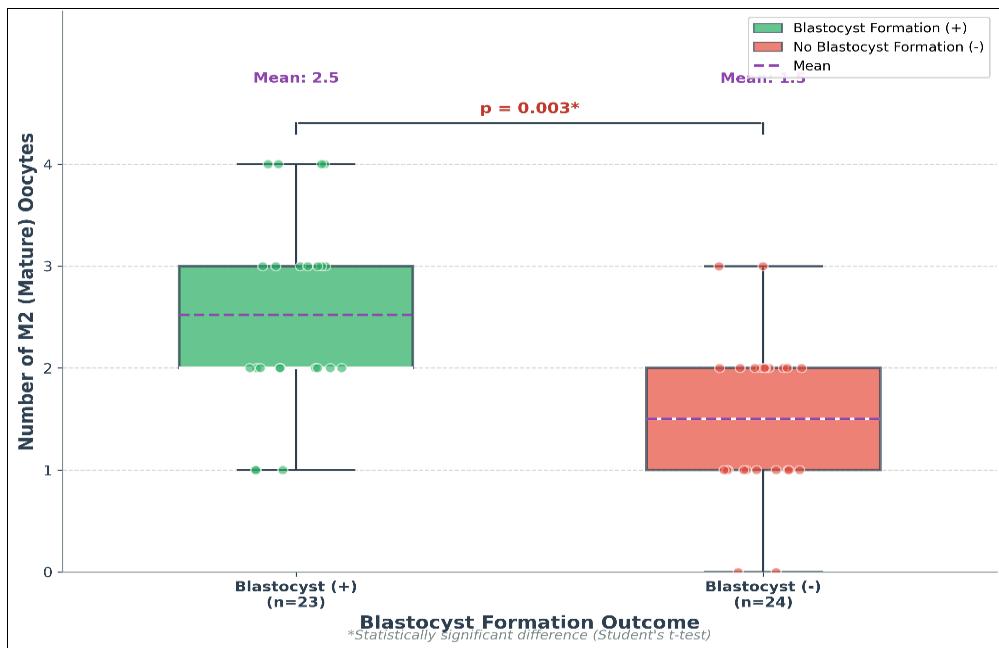
**Fig 2:** Bar chart comparing cycles with vs. without blastocyst formation**Table 4:** Comparison of Parameters between Blastocyst Formation Groups

| Parameter                           | Blastocyst Formation (+) | No Blastocyst Formation (-) | p-value |
|-------------------------------------|--------------------------|-----------------------------|---------|
| Number of cycles (n)                | 23                       | 24                          | -       |
| Age (years), Mean±SD                | 35.2±4.5                 | 37.6±5.0                    | 0.08    |
| Total Oocytes, Mean±SD              | 3.1±1.0                  | 2.5±1.2                     | 0.06    |
| M2 Oocytes, Mean±SD                 | 2.7±1.1                  | 1.8±0.9                     | 0.003†  |
| Semen Volume (mL), Mean±SD          | 1.5±0.5                  | 1.3±0.5                     | 0.21    |
| Sperm Concentration (M/mL), Mean±SD | 44.2±27.1                | 40.0±25.8                   | 0.58    |
| Sperm Motility (%), Mean±SD         | 29.8±11.9                | 27.0±13.4                   | 0.45    |
| Fertilization Rate (%), Mean±SD     | 91.2±12.4                | 82.6±18.1                   | 0.06    |

**Abbreviations:** SD, Standard Deviation; n, number of cycles; M2, Metaphase II (mature) oocytes; M/mL, million per milliliter; %, percentage.

- Blastocyst Formation (+): Cycles that achieved at least one blastocyst by Day 5-6 of embryo culture.

- No Blastocyst Formation (-): Cycles that did not achieve any blastocyst development.
- †Statistically significant ( $p<0.05$ ) by Student's t-test. All continuous variables compared using Student's t-test.

**Fig 3:** Box plot comparing M2 oocyte counts between groups with and without blastocyst formation

### Age-Stratified Analysis

When stratified by age groups, blastocyst formation rates varied considerably (Table 5). Patients aged  $\leq 35$  years demonstrated the highest blastocyst formation rate (58.8%, 10/17), followed

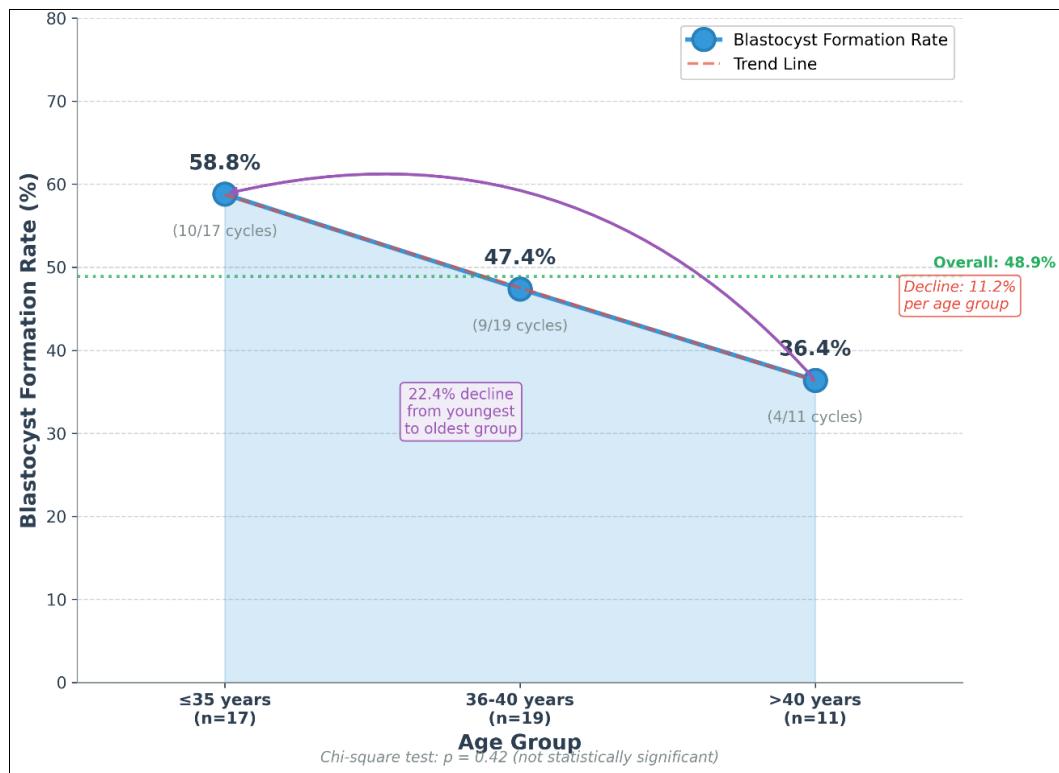
by those aged 36-40 years (47.4%, 9/19). Patients older than 40 years showed the lowest rate (36.4%, 4/11), although the difference did not reach statistical significance ( $p=0.42$ ).

**Table 5:** Blastocyst Formation Rate Stratified by Maternal Age Group (n=47 cycles)

| Age Group       | Total Cycles (n) | Blastocyst Formation (+) (n) | Rate (%) |
|-----------------|------------------|------------------------------|----------|
| $\leq 35$ years | 17               | 10                           | 58.8%    |
| 36-40 years     | 19               | 9                            | 47.4%    |
| $>40$ years     | 11               | 4                            | 36.4%    |
| Overall         | 47               | 23                           | 48.9%    |

**Abbreviations:** n, number of cycles; %, percentage.

- **Blastocyst Formation (+):** Cycles achieving at least one blastocyst by Day 5-6.
- **Rate (%):** Blastocyst Formation Rate = (Number of cycles with blastocyst / Total cycles in age group)  $\times 100$ .
- **Statistical Analysis:** Chi-square test,  $p = 0.42$  (not statistically significant at  $\alpha = 0.05$ ).



**Fig 4:** Line graph showing declining blastocyst formation rate with increasing age groups

### Discussion

This retrospective cohort analysis provides valuable insights into blastocyst formation outcomes in patients with low oocyte yield. Our findings demonstrate that despite retrieving fewer than five oocytes, nearly half of the cycles (48.9%) achieved blastocyst formation, challenging the conventional notion that blastocyst culture should be avoided in poor responders.

The blastocyst formation rate observed in our study is consistent with findings from other centers treating poor responders. Ubaldi *et al.* reported blastocyst development rates of 40-50% in patients with diminished ovarian reserve [22]. Similarly, a multicenter study by Polyzos *et al.* demonstrated comparable outcomes in poor responders undergoing extended culture [23]. These findings collectively suggest that oocyte quality, rather than quantity alone, plays a crucial role in determining embryo developmental potential.

Our analysis identified M2 oocyte count as a significant predictor of blastocyst formation. Cycles achieving blastocyst development had significantly higher mean M2 oocyte counts

compared to those without blastocyst formation (2.7 vs 1.8,  $p=0.003$ ). This finding aligns with the fundamental principle that oocyte maturity is essential for successful fertilization and subsequent embryo development [24]. The high proportion of M2 oocytes (81.8%) in our cohort reflects appropriate trigger timing and stimulation protocols.

Interestingly, semen parameters did not significantly influence blastocyst formation rates in our study. This observation can be attributed to the universal application of ICSI, which bypasses natural sperm selection barriers and minimizes the impact of suboptimal semen quality on fertilization outcomes [25]. However, it should be noted that ICSI cannot overcome issues related to sperm DNA fragmentation, which was not routinely assessed in our cohort.

The age-stratified analysis revealed a declining trend in blastocyst formation rates with advancing maternal age, although the difference did not reach statistical significance. Patients aged  $\leq 35$  years achieved the highest rate (58.8%), while those  $>40$  years showed a lower rate (36.4%). This trend is

consistent with the well-established negative impact of maternal age on oocyte quality and aneuploidy rates [26]. The lack of statistical significance may be attributed to the relatively small sample size in each age subgroup.

The management of poor ovarian responders continues to evolve with various strategies proposed to optimize outcomes. Our data support the use of individualized stimulation protocols, with the majority of patients receiving high-dose gonadotropin stimulation combined with GnRH antagonist for pituitary suppression. The addition of adjuvants such as clomiphene citrate or letrozole in some protocols reflects attempts to enhance follicular recruitment through alternative mechanisms [27].

An important clinical consideration emerging from our study is the decision regarding fresh transfer versus freeze-all strategy. Among cycles achieving blastocyst formation, approximately half (52.2%) proceeded with fresh embryo transfer, while the remainder opted for cryopreservation. The freeze-all approach offers advantages including avoiding OHSS risk, allowing for endometrial preparation, and potentially improving implantation rates in subsequent frozen embryo transfer cycles [28].

The finding that approximately one-third of successful cycles produced three or more blastocysts is noteworthy. This challenges the perception that poor responders invariably have compromised oocyte quality. It suggests that within the population of poor responders, there exists a subset with preserved oocyte competence who can achieve favorable outcomes with appropriate management [29].

Our study has several limitations that warrant consideration. The retrospective design introduces potential selection bias and limits causal inference. The heterogeneous stimulation protocols employed make it challenging to attribute outcomes to specific treatment approaches. Additionally, clinical pregnancy and live birth rates were not analyzed, which would provide more comprehensive assessment of treatment success. The relatively small sample size limits the statistical power for subgroup analyses.

Future prospective studies with larger cohorts are needed to validate these findings and identify additional predictive factors for blastocyst formation in poor responders. The role of adjuvant therapies, specific stimulation protocols, and the potential benefit of preimplantation genetic testing in this population deserves further investigation [30].

## Conclusion

This retrospective cohort analysis demonstrates that acceptable blastocyst formation rates can be achieved in *in vitro* fertilization cycles with low oocyte yield. The blastocyst formation rate of 48.9% in cycles with fewer than five oocytes supports the consideration of extended culture in selected poor responders. M2 oocyte count emerged as a significant predictor of blastocyst development, emphasizing the importance of optimizing stimulation protocols to maximize mature oocyte retrieval.

While maternal age showed a declining trend in blastocyst formation rates, the absence of statistical significance suggests that individual patient factors may outweigh chronological age in determining outcomes. Semen parameters, when ICSI is employed, do not appear to significantly impact blastocyst development in this population.

These findings have important implications for patient counseling and clinical decision-making. Rather than universally avoiding blastocyst culture in poor responders, an individualized approach considering oocyte maturity, patient age, and overall

embryo quality should guide treatment strategies. Further prospective studies are warranted to establish evidence-based guidelines for the management of patients with low oocyte yield in assisted reproduction.

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