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**ASSESSING THE REPRODUCTIVE POTENTIAL OF LATE MATURE OOCYTES IN EGG FREEZING CYCLES**

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**OBJECTIVE:**

Following vaginal oocyte retrieval (VOR), oocytes are assessed for germinal vesicle (GV) meiosis I (MI), or meiosis II (MII) development prior to cryopreservation. Given evidence of improved maturation rates of immature oocytes prior to cryopreservation rather than post thaw, GV and MI oocytes may be cultured in the laboratory to reassess maturation before cryopreservation. It is not established whether oocytes with delayed maturation have reduced reproductive potential. The objective of this study is to assess clinical outcomes in oocytes that reached the MII stage of development either on first assessment after VOR or later the same day.

**MATERIALS AND METHODS:**

This retrospective cohort study analyzed all autologous oocyte cryopreservation cycles with at least one late mature oocyte frozen that underwent subsequent thaw for in-vitro fertilization from January 2016 – February 2024. All cryopreserved oocytes from each cycle, including MIIs and late mature MIIs, were included. Group 1 included oocytes that were initially classified as immature (MI or GV) that matured at least 4 hours later the same day (“late mature MII”) and Group 2 included all MII oocytes mature on first assessment 1 hour following VOR (“MII”). Comparative statistics and univariate analysis were performed using Wilcoxon Ranks and chi-square. Logistic regression fitted with GEE controlling for oocyte age, AMH, BMI, year of cryopreservation, and year of thaw was performed on the outcomes of thaw survival, fertilization, blastulation, and ploidy.

**RESULTS:**

A total of 179 patients underwent 211 oocyte cryopreservation cycles and 191 oocyte thaw cycles. Group 1 included 337 late mature MIIs and Group 2 included 1907 MIIs. Thaw survival rate was similar between Group 1 and Group 2 (77.2% vs 79.8%,  $p=0.51$ ). Fertilization rate per surviving oocyte was lower in Group 1 (74.2%) compared to Group 2 (86.7%), with a



significantly lower odds of fertilization in late mature oocytes compared with controls on multivariate logistic regression (OR 0.43 CI 0.31-0.61,  $p < 0.0001$ ). In the adjusted analysis, the odds of a fertilized oocyte developing to a blastocyst for fresh transfer or cryopreservation were comparable between groups (OR 0.74 CI 0.50-1.10,  $p = 0.134$ ). There was no difference in blastocyst euploidy rate when tested using NGS ( $p = 0.177$ ). This study had an 80% power to detect a 10% difference in blastulation with an alpha of 0.05.

#### **CONCLUSIONS:**

Extended culture of immature oocytes maximizes the number of cryopreserved oocytes per cycle and improves overall prognosis. Oocytes with delayed maturation had similar odds of thaw survival but lower fertilization rates than early mature oocytes, possibly due to dysynchrony of nuclear and cytoplasmic maturation. Late mature MII oocytes that fertilize appear to have similar odds of developing into a euploid blastocyst. Overall, patients can be reassured that oocytes with delayed maturation can serve to benefit future family building.

#### **IMPACT STATEMENT:**

While cryopreserved late mature oocytes show lower fertilization rates, those that do fertilize exhibit high reproductive potential in terms of oocyte thaw survival, blastulation, and ploidy results.

#### **REFERENCES:**

Lee, J. Barritt, J. Moshini, R.M. Slifkin, R. Copperman, A.B. Optimizing human oocyte cryopreservation for fertility preservation patients: should we mature then freeze or freeze then mature?