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Inverted microscopy-based assessment reveals major maturation gap between denuded and non-denuded GV oocytes: A novel r-IVM approach

İnvert mikroskopi ile yapılan değerlendirme, denüdasyon uygulanan ve uygulanmayan GV oositler arasında belirgin bir maturasyon farkı ortaya koyuyor: Yenilikçi bir r-IVM yaklaşımı

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Abstract

Objective: To compare the maturation rate and developmental potential of immature oocytes subjected to and spared from cumulus-oocyte complex (COC) denudation.

Materials and Methods: This single-center prospective observational study was conducted between 15 November-15-December 2025. Germinal vesicle (GV) oocytes were allocated to two groups: Group 1 included oocytesobtained from follicles >10 mm and identified as GV following denudation, whereas group 2 included immature oocytes retrieved from non-dominant follicles with diameter <10 mm, and assessed under an inverted microscope immediately after oocyte retrieval and placed into culture medium without being denuded. All immature oocytes were cultured separately in Continuous Single Culture-NX Complete medium, supplemented with gentamicin and human serum albumin, for 24 hours. COCs in group 2 were subsequently denuded and evaluated for nuclear maturation. Oocytes reaching metaphase II (MII) underwent intracytoplasmic sperm injection. The primary outcome was the MII maturation rate; secondary outcomes included 2PN formation rate and cleavage-stage embryo rate.

Results: A total of 885 oocytes were retrieved from 52 patients. Group 1 included 84 denuded GV oocytes, and group 2 comprised 141 non-denuded COCs. After 24 hours of culture, maturation rates in groups 1 and 2 were 3/84 (2.37%) and 52/141 (36.9%), respectively. In group 1, only one oocyte was fertilized, and the resulting embryo arrested on day 3. In group 2, the fertilization and day-3 embryo rates were 23/48 (47.9%) and 14/23 (73.4%), respectively.

Conclusion: Non-denuded immature oocytes demonstrated significantly higher maturation, fertilization, and embryo development rates compared with denuded oocytes.

Keywords: Inverted microscope, germinal vesicle, immature oocyte, in vitro maturation

Öz

Amaç: Kümülüs-oosit kompleksi (KOK) denüdasyonu uygulanan ve uygulanmayan immatür oositlerin 24 saatlik kültür sonrası maturasyon oranı ve embriyo gelişim potansiyelinin karşılaştırılması amaçlandı.

Gereç ve Yöntemler: Bu tek merkezli prospektif gözlemsel çalışma 15 Kasım-15 Aralık 2025 tarihleri arasında gerçekleştirildi. İşlem sırasında elde edilen germinal vezikül (GV) evresindeki oositler iki gruba ayrıldı. Grup 1, denüdasyon sonrası GV olarak tanımlanan ve 10 mm üzeri foliküllerden

PRECIS: The maturation rate of non-denuded immature oocytes was found to be significantly higher than that of denuded oocytes.

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elde edilen oositlerden; grup 2 ise KOK denüdasyonu yapılmaksızın inverted mikroskop altında değerlendirilen ve çapı 10 mm'nin altında olan foliküllerden elde edilen GV oositlerden oluştu. Tüm oositler, gentamisin ve insan serum albümini içeren Continuous Single Culture NX Complete ortamında 24 saat ayrı ayrı kültüre edildi. Grup 2'deki KOK'lar inkübasyon sonunda denude edilerek nükleer maturasyon açısından değerlendirildi. Metafaz II (MII) evresine ulaşan oositlere intrasitoplazmik sperm enjeksiyonu uygulandı. Birincil sonuç ölçütü MII'ye ulaşma oranı, ikincil sonuç ölçütleri ise 2PN oranı, klivaj evresi embriyo oranıydı.

Bulgular: Çalışmaya dahil edilen 52 hastadan toplam 885 oosit elde edildi. Grup 1'de 84 denüde edilmiş GV oositi, grup 2'de ise 141 denüde edilmemiş GV oositi değerlendirildi. Yirmi dört saatlik kültür sonrası MII oranları sırasıyla 3/84 (%2,37) ve 52/141 (%36,9) olarak bulundu. Grup 1'de yalnızca bir oosit fertilize oldu ve embriyo 3. günde gelişimini durdurdu. Grup 2'de fertilizasyon ve klivaj oranları sırasıyla 23/48 (%47,9) ve 14/23 (%73,4) olarak saptandı.

Sonuç: Denüde edilmemiş immatür oositlerde maturasyon, fertilizasyon ve embriyo gelişim oranları denüde edilmiş oositlere kıyasla istatistiksel olarak anlamlı derecede daha yüksekti.

Anahtar Kelimeler: İnvert mikroskop, germinal vezikül, immatür oosit, in vitro maturasyon

Introduction

In vitro maturation (IVM) is defined as the maturation of immature oocytes, either germinal vesicle (GV) or metaphase 1 (MI) stage oocytes in a laboratory culture system to obtain a metaphase 2 (MII) stage oocyte[1]. Several IVM types have been defined across various classification systems; the most commonly used are categorized into three main groups: classical IVM, biphasic IVM, and rescue IVM[1-3]. In classical and biphasic IVM, oocytes are matured *ex vivo* without prior denudation; however, in rescue-IVM (r-IVM), oocytes are collected following a conventional in vitro fertilization (IVF) cycle, denuded, and cultured in IVM medium[1-3]. r-IVM has been particularly used in patients with diminished ovarian reserve (DOR) and poor ovarian response, as well as in those yielding a limited number of mature oocytes, to increase the total number of MII oocytes available[1-3]. Although r-IVM allows the retrieval of additional MII oocytes, the procedure has several important limitations that hinder its widespread adoption. The most notable limitations include low maturation rates and significantly lower blastocyst formation rates compared with those observed in *in vivo*-matured oocytes[2].

In the literature, maturation and blastocyst development rates in IVM cycles vary considerably, with reported blastocyst formation rates falling below 20%[2,3]. It has been suggested that even when nuclear maturation occurs and an MII oocyte is formed, blastocyst development rates remain low owing to insufficient cytoplasmic maturation. Disruption of communication between the cumulus cells and the oocyte, following denudation, is considered one of the most important causes of deficient cytoplasmic maturation. In addition, aberrant mitochondrial distribution and reduced ATP generation may further compromise embryo competence[4,5].

Materials and Methods

We conducted a single-center, prospective, observational study from 15 November to 15 December 2025. The study was reviewed and approved by the Acıbadem University Ethics Committee and was performed in accordance with the principles of the 1975 Declaration of Helsinki as revised in 2000 (approval no: 2025-17/627, date: 30.10.2025). Informed consent was obtained from all participants before study enrollment.

Cumulus-oocyte complexes (COCs) obtained from follicles <10 mm in diameter were included into the study group (group 2). At oocyte retrieval, follicle diameters were measured by transvaginal ultrasonography; COCs from follicles <10 mm in diameter were allocated to a separate well of a 4-well dish. Immediately thereafter, the COCs were examined under an inverted microscope to assess oocyte nuclear maturation. Those identified as MII were isolated and, approximately 2-3 hours later, denuded and subjected to intracytoplasmic sperm injection (ICSI). COCs containing GV oocytes were separated and were not denuded; instead, they were transferred to the same culture medium as group 1 and incubated for 24 hours, after which they were denuded and assessed for nuclear maturation. The control group (Group 1) was composed of GV oocytes obtained from COCs collected from follicles with diameter larger than 10 mm during the OPU procedure. These denuded GV oocytes were then cultured in culture medium for 24 hours, after which their nuclear maturation status was re-evaluated. Subsequently, oocytes from both groups that had progressed to MII were evaluated and subjected to ICSI. In both groups, 2PN formation, cleavage-stage embryo development were monitored.

The primary outcome was the MII maturation rate. The secondary outcomes were the 2PN rate and the cleavage-stage embryo rate.

Ovarian Hyperstimulation Protocol

All patients received a GnRH antagonist protocol and final oocyte maturation was triggered using human chorionic gonadotropin, gonadotropin-releasing hormone agonist, or both. Oocyte retrieval was performed 36 hours after triggering, using a 17-G needle under sedation.

Evaluation of COCs Under Inverted Microscope

Follicular fluid was poured into a 90-mm Petri dish, which was then tilted at a 30-45 °C angle to spread the fluid and visualize the COCs. COCs were subsequently examined under an inverted microscope, using the same dish, to

assess nuclear maturation status. Just before observation, the cumulus was gently spread laterally with a pipette to improve visualization of the oocyte. Figure 1 illustrates a MII oocyte and a GV as viewed under an inverted microscope.

Immature Oocyte Maturation Culture

Immature oocytes in both groups were cultured separately in Continuous Single Culture-NX Complete medium supplemented with gentamicin and human serum albumin for 24 hours. Subsequently, COCs in Group 2 were denuded and assessed for nuclear maturation. Oocytes that had progressed to MII in both groups were subsequently subjected to ICSI.

Results

A total of 885 oocytes were retrieved from 52 patients. Group 1 included 84 denuded immature oocytes, whereas group 2 contained 141 nondenuded COCs. In both groups, the immature oocytes were incubated in culture medium for 24 hours and subsequently evaluated for nuclear maturation. Table 1 summarizes the numbers of MII oocytes, fertilization rates, day-3 embryo rates, for both groups. In group 1, only 3 of the 84 immature oocytes matured to the MII stage following denudation. Of these three MII oocytes, only one fertilized, forming a 2PN zygote; however, this embryo arrested at the cleavage stage. In group 2, 141 COCs were identified as containing immature oocytes. Following 24 hours of culture, COCs were denuded: 52 (36.9%) oocytes had progressed to MII, 27 (19.2%) had progressed to MI, and 62 (43.9%) remained immature. ICSI was not performed on the MI oocytes. Notably, in one patient with azoospermia, the limited number of available spermatozoa precluded ICSI on the MII oocytes (n=4) that matured from immature oocytes; therefore, the available sperm were reserved for ICSI on MII oocytes retrieved from dominant follicles. ICSI was performed on 48 of the 52 MII oocytes; 23 (47.9%) of these demonstrated 2PN formation. Of the 23 2PN zygotes, 14 developed into day-3 embryos.

Discussion

In the present study, nuclear maturation was assessed without COC denudation, using an inverted microscope, which enabled distinction between mature and immature oocytes. When COCs containing immature oocytes were maintained in culture medium for 24 hours without denudation, a 36.9%

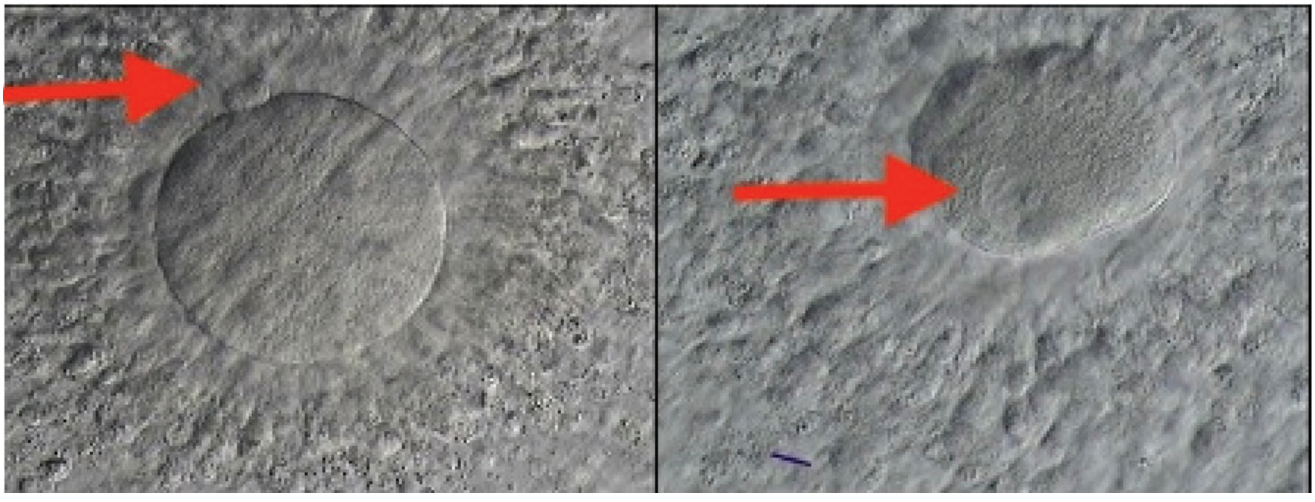

Figure 1. Visualization of the nuclear maturation of the oocytes under an inverted microscope. The red arrow in the left panel indicates a polar body, consistent with an metaphase II oocyte. The red arrow on the right panel indicates a germinal vesicle, consistent with an immature oocyte

Table 1. Comparison of group 1 and group 2 with respect to MII oocyte, 2PN, day-3 embryo, and blastocyst numbers and rates

Variable	Group 1 (denuded)	Group 2 (non-denuded)
Immature oocyte number	84	141
MII oocyte number (maturation rate)	3/84 (2.37%)	52/141 (36.9%)
2PN number/rate	1/3 (33.3%)	23/52 (47.9%)
Day-3 embryo number/rate	0	14/23 (73.4%)

MII: Metaphase II

maturation rate (GV to MII), a 47.9% fertilization rate, and a 73.4% day-3 embryo rate were observed. Although these rates are lower than those reported in the literature for r-IVM, no IVM-specific maturation medium was used in our protocol. To the best of our knowledge, this is the first study to assess oocyte nuclear maturation and to allow immature oocytes to undergo IVM without COC denudation.

The study conducted at our clinic was not designed to investigate the IVM of immature oocytes or to evaluate the outcomes of standard IVM protocols. Instead, our objective was to identify immature oocytes within COCs using an inverted microscope without denudation, to keep these COCs in culture for 24 hours, and to assess their maturation outcomes. Notably, IVM is not routinely performed at our center. Our routine protocol involves maintaining oocytes in culture medium for 2-3 hours following retrieval, after which they are denuded. ICSI is then performed on MII-stage oocytes, whereas immature oocytes undergo an additional 24-hour incubation, after which nuclear maturation is re-evaluated and ICSI is performed on those that have progressed to MII.

Tracing its historical development, rescue IVM entered clinical use in the late 1990s; however, it did not become a routine clinical practice, as adequate numbers of mature oocytes could not be consistently obtained and prolonged culture durations accelerated oocyte aging[6,7]. From the 2020s onward, rescue IVM continued to be employed in patients with DOR or in those yielding only a limited number of MII oocytes, with the aim of deriving mature oocytes from immature ones and thereby improving IVF outcomes[1,6,7]. Nevertheless, the lack of complete cytoplasmic maturation led to lower fertilization rates, reduced blastocyst formation, and lower euploidy rates[8]. Furthermore, the absence of standardized IVM culture media further contributed to these low success rates[9]. In a study conducted by Ahmad et al.[2], the effectiveness of r-IVM between women with DOR and those with normal ovarian response was compared, reporting higher rates of oocyte maturity, fertilization, and embryo quality in the DOR group. Although the study populations and comparators differ, these findings collectively underscore that r-IVM outcomes may be influenced by multiple factors beyond ovarian reserve, including oocyte handling prior to culture. Qin et al.[10] compared patients who underwent conventional IVF alone with those who additionally received r-IVM. The latter group demonstrated significantly improved IVF outcomes, including higher numbers of MII oocytes, 2PN rates, and day-3 embryo rates. In a systematic review and meta-analysis of 27 r-IVM trials, oocyte maturation rates were reported as 57% for MI-to-MII and 68% for GV-to-MII progression[11]. The blastulation rates were 16% for oocytes that matured from GV to MII. Additionally, that review demonstrated that clinical pregnancy and live birth rates were significantly higher in r-IVM cycles[11]. Similarly, Wei et al.[12] reported that r-IVM improved clinical pregnancy and live birth rates in patients with fewer than 9 MII oocytes. In a 2024 meta-analysis, 24 studies were evaluated, comprising a total of 74,136 oocytes. Among these oocytes, 59,144 were MII, 11,326 were MI, and 3,666 were GV oocytes. When maturation rates were assessed, 38.8% of MI oocytes and 58.2% of GV oocytes reached maturity[13]. In that meta-analysis, fertilization, cleavage, and blastocyst development rates, and consequently clinical pregnancy and live-birth rates, of GV-derived oocytes were significantly lower than those of *in vivo*-matured MII oocytes collected within the same cycle[13]. Although a direct comparison is limited by differences in culture conditions and by the absence of a dedicated IVM medium in our protocol, the maturation rates observed in the non-denuded group suggest that preservation of COC integrity may contribute to oocyte developmental competence during *in vitro* culture. A common feature of all these studies is that r-IVM was applied to immature oocytes following COC denudation. However, studies have demonstrated bidirectional communication between cumulus cells and the oocyte, in which cumulus cells transmit cytokines and growth factors to the oocyte through gap junctions. It has been shown that cAMP, cGMP, amino acids, pyruvate, ions, and various cytokines maintain the oocyte in meiotic arrest, prevent premature maturation, and provide the energy required for proper maturation. Following the early denudation of COCs, the bidirectional communication between cumulus cells and the oocyte is disrupted; as a result, while nuclear maturation occurs prematurely, cytoplasmic maturation is delayed, compromising the developmental potential of the oocyte[14,15].

In the present study, the maturation rate in group 1 was significantly lower than that in group 2. We attribute this outcome primarily to early cumulus cell removal and to the absence of a dedicated IVM medium. When discussing the effects of cumulus cells on oocyte maturation, it is important to emphasize that these cells possess significant antioxidant and cytoprotective functions[16]. Cumulus cells rapidly eliminate reactive oxygen species (ROS) present in the environment, thereby reducing or completely preventing the damaging effects that ROS may exert on the oocyte cytoplasm, meiotic spindle apparatus, mitochondrial DNA, and other cytoplasmic organelles[17]. In addition, cumulus cells help maintain stable glutathione (GSH) levels in the microenvironment, providing further antioxidant protection. Since the oocyte has a limited capacity to synthesize GSH, this support is crucial for achieving high-quality maturation[17,18]. Furthermore, bidirectional and dynamic communication exists between cumulus cells and the oocyte. Through gap-junctional signaling, this communication ensures that nuclear maturation and cytoplasmic maturation proceed in a coordinated and well-regulated manner[19,20]. When this communication

is disrupted, premature nuclear maturation occurs while cytoplasmic maturation remains insufficient, ultimately preventing the oocyte from reaching full developmental competence[19,20]. The markedly lower maturation rates observed in denuded oocytes are largely explained by the loss of this antioxidant protection and intercellular communication. We attribute the higher oocyte maturation rates in the non-denuded COC group to the antioxidant effects of the cumulus cells surrounding the oocyte and to the preservation of intact bidirectional communication that prevents premature nuclear maturation.

The absence of an appropriate IVM medium is one of the major contributing factors to the low maturation rates. IVM media are culture systems formulated to support maturation of immature oocytes under controlled laboratory conditions. They differ significantly from standard IVF media because immature oocytes have far more complex biological requirements, necessitating a microenvironment that mimics the natural follicular milieu by incorporating growth factors, hormones, and antioxidants[19]. Although patient characteristics, age, and related clinical variables can influence oocyte maturation rates, the use of sibling oocytes makes it unlikely that individual patient variables alone fully explain the magnitude of the observed difference between groups. Therefore, we consider the culture conditions and the degree of cumulus cell preservation to be the primary determinants of the observed discrepancy.

Study Limitations

The present study has several limitations. The first limitation is the relatively small sample size. The secondand most critical limitation is the absence of a dedicated IVM medium. Third, the study does not include pregnancy outcomes, which precludes a complete assessment of the clinical potential of non-denuded r-IVM. Nevertheless, our work represents a novel and potentially significant contribution to the field of r-IVM.

Conclusion

With the adoption of optimized and standardized IVM media, r-IVM protocols performed without prior COC denudation may yield higher maturation rates, improved blastocyst development rates, and ultimately increased live birth rates.

Ethics

Ethics Committee Approval: The study was reviewed and approved by the Acıbadem University Ethics Committee and was performed in accordance with the principles of the 1975 Declaration of Helsinki as revised in 2000 (approval no: 2025-17/627, date: 30.10.2025).

Informed Consent: Informed consent was obtained from all participants before study enrollment.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.P., A.Y., Concept: N.P., A.Y., E.T., B.T., Design: N.P., A.Y., Ö.K., E.T., B.T., Data Collection or Processing: N.P., A.Y., Ö.K., B.E., İ.Ö.A., B.A.T., Analysis or Interpretation: N.P., B.A.T., B.T., Literature Search: N.P., E.T., Writing: N.P., E.T., B.T.

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