

# The role of perivitelline space abnormalities of oocytes in the developmental potential of embryos

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## Abstract

**Objective:** In assisted reproductive technology (ART), high embryo quality is closely related to high-quality oocytes. Cytoplasmic maturation and extracytoplasmic maturation are the most important components in determining oocyte quality. One of the most important components of extracytoplasmic maturation is perivitelline abnormalities. The aim of this study is to determine the effect of perivitelline abnormalities on the development of high-quality embryos.

**Material and Methods:** The study material consisted of 217 of 1154 oocytes from 98 intracytoplasmic sperm injection (ICSI) cycles undertaken due to male factor infertility. Only cycles with long gonadotropin-releasing hormone analogs combined with recombinant Follicle-stimulating hormone (rec-FSH) were included in study. We compared 105 metaphase-II oocytes that had dominantly perivitelline space abnormalities (large perivitelline space with or without granules) with 112 normal metaphase-II oocytes, based on the embryo grade determined by Alpha Scientists in Reproductive Medicine and the European Society of Human Reproduction and Embryology (ESHRE) Special Interest Group of Embryology. Normal metaphase-II oocytes were characterized by a round, clear zona pellucida; a small perivitelline space containing a single unfragmented first polar body; and a pale, moderately granular cytoplasm with no inclusions.

**Results:** The development rates of Grade I, II, and III embryos were 68.5%, 23.8%, and 7.7%, respectively, in the 105 oocytes with perivitelline abnormalities. The development rates of Grade I, II, and III embryos were 82.1%, 17.9%, and 0%, respectively, in the 112 morphologically normal oocytes. When compared with normal oocytes, Grade I (68.5% vs. 82.1%, *p* value; 0.019) and Grade III (7.7% vs. 0%, *p* value; 0.003) embryo development rates were significantly lower in oocytes that had perivitelline abnormalities.

**Conclusion:** It is important to analyze oocyte quality using multiple parameters, including the perivitelline space. Perivitelline space abnormalities might negatively affect embryo development in male factor-infertile couples that are stimulated with rec-FSH. Therefore, when choosing embryos for transfer, we must take into consideration the historical oocyte data. (J Turk Ger Gynecol Assoc 2014; 15: 161-3)

**Key words:** Embryo, oocyte, perivitelline space

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## Introduction

Assisted reproductive technology (ART) treatment success almost always depends on multiple factors. Every step of the ART affects pregnancy development. Thus, from folliculogenesis to embryo transfer, all steps of ART should be investigated to improve pregnancy rates.

Oocyte quality, which is dependent on oocyte maturity, also plays a major role in the development potential of the embryo (1). There are two components of maturity-nuclear maturity and cytoplasmic maturity-and both components must occur in a coordinated and well-synchronized manner. It is generally recognized that a high-quality oocyte must complete nuclear maturity (M-II oocyte) and should have a round-clear zona pellucida, a small perivitelline space containing a single-non fragmented normal-sized first polar body, and a pale, moderately granular cytoplasm with no vacuoles, and a smooth endoplasmic reticulum (2, 3). However, in addition to the factors that affect oocyte quality, controlled ovarian stimulation protocols are also important.

Even though oocyte morphological scoring is not yet uniform, ESHRE offers an evaluation of every oocyte with uniform parameters to facilitate the delivery of optimal embryos on day 3 or day 5. The most important criteria for oocyte morphology are the following: cumulus-oocyte complex scoring; zona pellucida scoring; perivitelline space abnormalities, such as dilatation or granularity; polar body scoring; cytoplasmic scoring; and vacuolization (4).

Perivitelline space abnormalities are among the most important dysmorphisms of the extracytoplasmic component. It has been reported that a large perivitelline space may be associated with increased oocyte degeneration (5) and lower fertilization rates (6). However, studies have failed to find a correlation between the size and shape of the perivitelline space and fertilization rate or embryo development (1, 7).

In the present study, we evaluated the differences in the development of grade I embryos in normal oocytes and in oocytes with a large perivitelline space.



## Material and Methods

The study material consisted of 217 of 1154 oocytes from 98 ICSI cycles undertaken due to male factor infertility in the period from May 2010 to May 2013. Institutional Review Board (IRB) approval was obtained for the study and informed consent form obtained from all couples. Only cycles with long gonadotropin-releasing hormone (GnRH) analogs combined with recombinant Follicle-stimulating hormone (rec-FSH) were included in the study. Oocyte retrieval was performed 36 hours after recombinant human chorionic gonadotropin injection. A total of 250 mcg of recombinant-Human chorionic gonadotropin (rec-hCG) was administered when at least 2-3 follicles with a diameter >18 mm developed. Approximately 2-4 hours after retrieval, oocytes were incubated with 80 IU/mL hyaluronidase for 20-30 sec. Then, cumulus-corona cells were stripped of the follicle with gentle pipetting. The morphology of the oocyte was examined with an inverted microscope at 200x or 400x magnification. The specifications of the first polar body, the perivitelline space, characteristics of the zona pellucida, and the cytoplasmic texture were assessed. A total of 105 oocytes with a perivitelline space abnormality (large perivitelline space with or without granules) (Figure 1) were compared with 112 oocytes that appeared normal (Figure 2) according to the day 3 Grade I, Grade II, and Grade III embryo development potentials. Embryology grading was performed according to the Alpha Scientists in Reproductive Medicine and European Society of Human Reproduction and Embryology (ESHRE) Special Interest Group of Embryology (4) as follows: Grade I embryos were defined as embryos with <10% fragmentation, stage-specific cell size and no multinucleation; Grade II embryos were defined as embryos with 10%-25% fragmentation, stage-specific cell size for the majority of cells, and no multinucleation; and Grade III embryos were defined as embryos with >25% fragmentation, cell size not stage-specific, and presence of multinucleation. From 1154 oocytes, 937 oocytes were excluded from the study, and the exclusion criteria were: i) presence of extracytoplasmic abnormalities in addition to or other than perivitelline abnormality, ii) presence of cytoplasmic abnormalities, iii) oocytes obtained from cycles other than GnRH analogs combined with rec-FSH, and iv) oocytes that were unable to develop day 3 embryos. So, the primary aim was to evaluate the effect of perivitelline abnormalities exclusively on embryo development potential.

IBM SPSS 20 program was used for the statistical analysis (Statistical Package for Social Science, IBM SPSS Inc., Chicago, IL, USA). The differences between the groups were investigated with the two-proportion test, and Pearson chi-square test was used. A p-value <0.05 was considered statistically significant.

## Results

A total of 105 oocytes with perivitelline abnormalities were compared to 112 normal oocytes according to the embryo development potential. According to the inclusion criteria, 105 oocytes had no additional cytoplasmic and extra-cytoplasmic abnormality besides perivitelline abnormality; 112 oocytes were totally normal according to the cytoplasmic and extra-cytoplasmic evaluation. The Grade I, II, and III embryo development rates were 68.5%, 23.8%, and 7.7%, respectively, in the 105 oocytes mainly with a



**Figure 1. A metaphase II oocyte with perivitelline space abnormalities (large perivitelline space with granules)**



**Figure 2. A normal metaphase II oocyte with round-clear zona pellucida, a small perivitelline space containing a single-non fragmented normal-sized first polar body, and a pale, moderately granular cytoplasm**

perivitelline abnormality. The Grade I, II, and III embryo development rates were 82.1%, 17.9%, and 0%, respectively, with the 112 morphologically normal oocytes. The Grade I (68.5% vs. 82.1%, p value; 0.019) embryo development rate was significantly lower, and the Grade III (7.7% vs. 0%, p=0.003) embryo development rate was significantly higher in oocytes that had a perivitelline abnormality compared to normal oocytes (Table 1).

## Discussion

The present study demonstrated a significant relationship between perivitelline space abnormalities and the embryo quality of day 3 embryos. The development rate of Grade I embryos was significantly higher in normal oocytes (no cytoplasmic and extracytoplasmic abnormalities) than in oocytes with perivitelline space abnormalities.

Perivitelline space abnormalities (large perivitelline space with or without granules) are among the most important extracy-

**Table 1. Grade I, II, and III embryo development rates in oocytes with perivitelline abnormality and normal oocytes**

	Oocytes with perivitelline abnormality (n=105)	Normal oocytes (n=112)	p value
Grade I embryo development, n (%)	72 (68.5)	92 (82.1)	0.019
Grade II embryo development, n (%)	25 (23.8)	20 (17.9)	0.280
Grade III embryo development, n (%)	8 (7.7)	0 (0)	0.003

toplasmic dysmorphisms of the oocyte, but we still we do not understand the exact mechanism of these abnormalities (8). Additionally, it is not clear if the abnormality is physiological or pathological in nature. Some perivitelline space granules are the remnants of coronal cell processes that usually withdraw as the oocyte undergoes resumption of meiosis (9).

The importance of perivitelline space abnormalities is more challenging in ART cycles. Hassan et al. (10) found an association between the granularity of the perivitelline space and lower numbers of MII oocytes; however, perivitelline space granularity was not associated with fertilization rate, cleavage rate, and embryo quality. Interestingly, they found that granularity was positively associated with the dosage of human menopausal gonadotropins administered during stimulation. However, in a study by Rienzi et al. (11), a large perivitelline space was found to be one of the most significant factors associated with lower fertilization rate and pronuclear morphology. In contrast to the study of Hassan et al. (10), Rienzi et al. (11) reported the results of patients who underwent ovarian stimulation with rec-FSH. We also demonstrated the negative effect of perivitelline space abnormality on embryo development in a patient that had stimulation with rec-FSH. Therefore, the association of perivitelline space abnormality and HMG, as proposed by Hassan et al. (10), is questionable. Probably, the development of perivitelline space abnormality may not be dependent on the type of gonadotropins, whether HMG or rec-FSH is used.

However, different studies have failed to find a correlation between perivitelline space abnormalities and ART treatment prognosis (2, 12). Moreover, Balaban et al. (2) reported that in couples undergoing ICSI, cytoplasmic abnormalities or extracytoplasmic abnormalities (including perivitelline space) were not associated with decreased fertilization rate or unfavorable embryo quality.

In our study, it was important to study oocytes that mainly had a perivitelline space abnormality. Therefore, the contribution of other cytoplasmic and extracytoplasmic abnormalities to the results was minimal. To the best of our knowledge, Hassan et al. (10) are the only researchers to investigate the role of perivitelline space abnormalities exclusively. However, their study population had undergone ART treatment with HMG. This point is also one of the most important limitations of our study-our results may only be limited to patients who had ovarian stimulation with rec-FSH. Additionally, future studies investigating the association of perivitelline space abnormality with clinical pregnancy rates would be more informative.

In conclusion, oocytes must be evaluated according to multiple parameters, including an analysis of the perivitelline space. Perivitelline space abnormalities might negatively affect embryo development in at least infertile couples with male factor infertility that are stimulated with rec-FSH. Therefore, when choosing embryos for transfer, we must take into consideration the historical oocyte data.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Institutional Review Board.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - F.T., Y.A.; Design - F.T.; Supervision - H.H., Y.A.; Resource - F.T.; Materials - F.T.; Data Collection&/or Processing - H.H., Y.A.; Analysis&/or Interpretation - H.H., Y.A., F.T.; Literature Search - Y.A.; Writing - Y.A.; Critical Reviews- H.H., Y.A., F.T.

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