



Preimplantation Embryo Quality Following Partial Denudation of Cumulus Oocyte Complex



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Abstract

The oocyte and the granulosa cells surrounding the oocyte in the graffian follicle are together called as the Cumulus oophorous complex. The granulosa cells synthesize estrogen from the androgens by aromatization under the influence of follicle stimulating hormone. The androgens are derived from theca cells under the influence of Luteinizing hormone from anterior pituitary. This makes the follicular milieu estrogenic. This is the paracrine influence of cumulus cells on oocyte. There are also trans zonal cytoplasmic extensions of oocyte that communicate with granulosa cells by gap junctions. This narrative review aims to highlight the cumulus oophorous interdependence and role of partial cumulus denudation in improving the success of intracytoplasmic sperm injection.

Keywords: Partial Denudation; Embryo Quality; Complete Denudation

Introduction

Cumulus-oocyte signaling interactions during oocyte maturation and fertilization are very characteristic. Two types of interactions take place-paracrine and gap junctions. A network of trans zona pellucida gap junctions within the cumulus-oocyte complex enables cumulus cells to communicate with the oocyte. Cumulus-oocyte communications have been found to be essential in substrate transfer (ions, nucleotides, amino acids, metabolites, and regulatory molecules) during intrafollicular oocyte growth and for final nuclear and cytoplasmic maturation.

Cumulus Oocyte Interdependence

Several key molecules are produced by oocytes (growth differentiation factor 9 or bone morphogenetic protein 15) that help in granulosa cell function (synthesis of estradiol by aromatization) and differentiation. The intracellular pH of growing oocyte is controlled by pH regulatory mechanisms that lie in cumulus cells. Oocyte also promotes its own development by metabolically cooperating with cumulus cells and inducing amino acid uptake and glycolysis.

Effects of Removing Cumulus Cells

On removal of cumulus cells or disruptions of cumulus oocyte communications, cyclic adenosine 3', 5' monophosphate level does not drop, and oocyte spontaneous meiotic resumption is prevented. Denuded oocytes may also have accrued less intracellular glutathione. When cumulus oophorus (Latin cumulus=heap, Greek oo=egg + phor=carrier; Latinized ending "-us") matures in vivo in physiological cycles and resumes meiosis the trans zona pellucida cytoplasmic processes are withdrawn

and gap junctional connection is lost. Paracrine communications compensate for this loss and become more active. This is evident

by many studies showings that results are much better in those embryos when cumulus cells are removed post IVF than when the cumulus cells are removed pre-IVF. It is physiological to leave most cumulus oophorous cells intact 24 hours past syngamy in order to increase the embryo quality and pregnancy rates.

Cumulus cells ensure complete nuclear and cytoplasmic maturation of oocyte, promote gamete interaction and embryogenesis. There may be an additional benefit of enhanced *in vitro* maturation in immature germinal vesicle stage and MI stage oocytes if homologous cumulus cells are left intact. Additionally, cumulus cells may have direct influence on embryonic metabolism by increased gene expression or help indirectly by reducing the oxidative stress.

When cumulus oophorous complex physical integrity is intact during *in vitro* maturation (IVM) and IVF, the developmental competence of such oocytes is better as compared with oocytes that are inseminated by intracytoplasmic sperm injection after oocyte denudation. Intracytoplasmic sperm injection is routinely done after denuding the oocytes of cumulus cells, assessing the maturity, grading and identifying the position of polar body. Oocyte denudation allows for correct positioning of polar body at 12 o'clock position while injecting and thereby minimizing the damage to the mitotic spindle.

However, denudation may compromise oocyte because a non physiologic, spontaneous resumption in meiosis that occurs *in vitro* when Cumulus oophorous cells are prematurely removed

from the meiotic- inhibiting environment of the follicle. Complete oocyte denudation can also lead to poor sperm-oocyte interaction. Interestingly, denuded oocytes cocultured with isolated cumulus cells partially can recover meiotic and developmental competence as revealed in human [1], mouse [2,3] and cow studies [4-7].

Numerous studies provide the data on cumulus oocyte interactions, and role of cumulus cells *in vitro* maturation of oocytes [7-10]. The most physiological way of improving blastomere quality will be by identifying, rectifying and minimizing oocyte and embryo damage. This *in vitro* analysis also helps in understanding the exact paracrine cumulus -oocyte interactions as the growth of oocyte till post fertilization Day 5 is evaluated.



Figure 1: Cumulus Oophoricus Complex after the Graffian Follicle is aspirated.

Complete Oocyte Denudation

In the currently practiced Conventional ICSI cumulus cells are completely removed from zona pellucida using micropipette of appropriate diameter. Because special care is taken not to harm the oocyte mechanically (displacement of polar body, distortion, dislocation), the final denudation step can take up to 1 minute. In case several cumulus cells remained attached to zona, they are removed immediately before ICSI using the holding and the injecting pipettes [11-13].

Partial Denudation

In the new technique of partial denudation micro pipetting is done with large diameter pipettes. Partial denudation is done until cumulus complex tissue no longer interfere with ICSI or hinder further evaluation of oocyte morphology [14]. An average of 300-500 cumulus cells per oocyte are not removed. Morphology check is done for all MII oocytes (clear, moderately granular cytoplasm, a small perivitelline space, an intact first polar body and a colorless zona pellucida).

All ICSI zygotes (2 pronucleus stage) are scored 18-20 hours after insemination and incubation in 80µl of Blast assist System Medium 1(Medicult). Zygote morphology and cleavage rate (Day 2-Day 3) are evaluated in all embryos (number and shape of blastomeres, degree of fragmentation, multinucleation and any signs of compaction). Day 5 blastocyst is evaluated according to the work of Gardener and Schoolcraft. The blastocyst is scored for degree of expansion of blastocoel [Grade I (blastocoel less

than half of the volume of embryo) to Grade IV (completely hatched blastocyst)]. Trophectoderm and inner cell mass is graded separately. Documentation is performed using Good Laboratory Practice guidelines. Clinical pregnancy Rate and mean number of embryos transferred are recorded.

Conclusion

Many factors have been associated with impaired blastocyst development. The socioeconomic burden of failed preimplantation embryo development is also high. The proportion of infertile couples has also increased in past 20 years. The care of an infertile couple necessitates judicious resource utilization. Functional cumulus oocyte interactions appear important during embryo development and *in vitro* maturation of oocytes; however, the cellular and molecular basis of this phenomenon remains unclear. A number of prospective *in vitro* studies in animal and human models has analyzed of impact of cumulus oophorous complex on preimplantation embryo development and pregnancy outcome and found better embryo development and clinical pregnancy rates with partial denudation.

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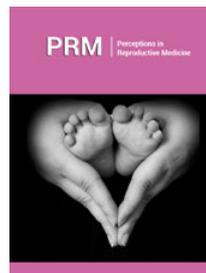
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