**Review Article**

# Role of Cumulus Cells and Follicular Fluid on Oocyte Maturation and Developmental Competence of Embryos: Intact and Reconstructed Oocytes

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**Abstract** | Ovarian follicles develop during fetal and postnatal periods through several distinct phases and they release their matured ova upon puberty. Oocytes surrounded by a single layer of pregranulosa follicular cells are called primordial follicles and they remain dormant in the meiotic prophase I stage. Activation of primordial follicles initiate through pregranulosa follicular cells. The primordial follicle takes several months to grow and develop to preovulatory follicles in mammals. Changes occur during follicular growth and development stages on the oocyte and the surrounding cumulus cells to prepare the oocyte for further embryo development upon ovulation and fertilization. Molecules transfer to oocytes through gap junctions of cumulus cells. Therefore, cumulus cells and their secretion, follicular fluid, effect on oocyte maturation and developmental potential of the resulting embryos *in vivo* and *in vitro*. Because of the importance of cumulus cells on follicular development, this review was made to collect and consolidate the current knowledge of cumulus cells effects on maturation of intact and reconstructed oocytes and further developmental competence of embryos *in vivo* and *in vitro*.

**Keywords** | Follicle, Cumulus cells, Follicular fluid, Cytoplast, Nucleus, Nucleolus, Maturation, Embryo

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## INTRODUCTION

Somatic cells present in ovary, which called pregranulosa cells, surround the oogonia to form primordial follicle (Figure 1). The formation of primordial follicles occurs when germ cell nests break apart and individual oocytes become surrounded by pregranulosa cells (O'Connell and Pepling 2021). It has been found that somatic cells initiate primordial follicle activation and govern the development of dormant oocytes (Zhang et al., 2014). Primordial follicles, upon activation, grow and develop to reach the preantral and antral follicle stages (Wu et al., 2021). During growth and development of the ovarian follicle from primordial follicles to antral stages, oocytes grow, granulosa cells proliferate and theca cells differentiate (Richards and Pangas 2010). Growth of preantral follicles is gonadotropin-independent whereas growth of antral follicles is gonadotropin-dependent follicles (Figure 2).

Most of knowledge regarding factors affecting cumulus

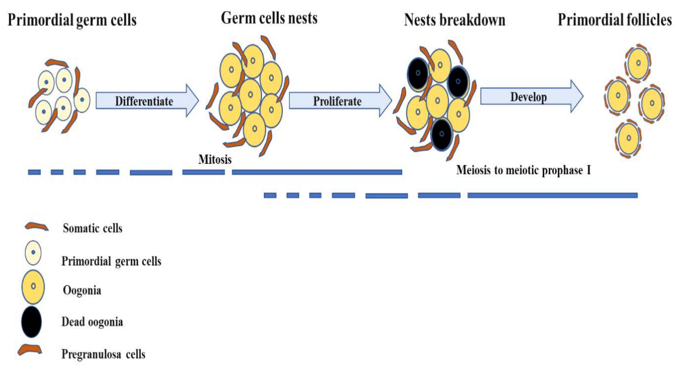


**Figure 1:** Differentiation, proliferation and development of ovarian structures during fetal life

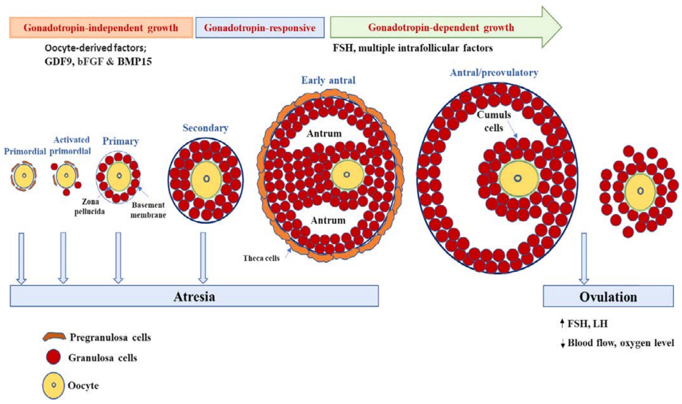


**Figure 2:** Growth of preantral gonadotropin-independent follicles and antral gonadotropin-dependent follicles after birth.
FSH, Follicle stimulating hormone
LH; Luteinizing hormone
GDF9; Growth differentiation factor 9
bFGF; basic fibroblast growth factor
BMP15; bone morphogenetic protein 15

cells gene expressions and follicular fluid stems from studies in ruminant, swine and rodent ovarian follicles (Mohammed 2008; Mohammed and Kassab 2014; Mohammed et al., 2005, 2011, 2012, 2019a,b; Bunel et al., 2020; Pournaghi et al., 2021; Ko et al., 2021). The roles of cumulus cells and follicular fluid on maturation of intact and reconstructed oocytes and further developmental competence of embryos will be compiled and discussed in this review. Furthermore, a more detailed understanding the factors affecting role of cumulus cells and their gene expression is still required.

## Development Of Ovarian Follicles

Multiple extrafollicular and intrafollicular factors affecting survival, activation, and growth of ovarian follicles from the smallest primordial follicles to the largest preovulatory follicles. They are stage-specific growth and hormonal factors. Tang et al. (2012) found that growth factors (GDF-9, bFGF known also as FGF or FGF-β) enhance *in vitro* FSH effects on the survival, activation, and growth of cattle primordial follicles. The follicle lasts from primordial follicle to preovulatory follicle stage about 6 months or longer in cattle and human (van den Hurk and Santos, 2009; Baerwald and Pierson 2020).

Follicle development occur through *initial* and *cyclic* recruitments (McGee and Hsueh 2000). *Initial* recruitment describes dormant primordial follicles recruitment continuously into the growing follicle pool whereas *cyclic* recruitment describes antral follicles recruitment each reproductive cycle. Multiple waves of antral ovarian follicular development were reported in several studies during estrous cycle (2, 3 & 4 waves) (Gordon 2003; Cavalieri et al., 2018; Baerwald and Pierson, 2020). There is a pool of early antral follicles at the onset of follicular phase from which the ovulatory follicle(s) is selected continuously. In recent years, interest has grown in the use of aspirated oocytes from ovarian follicles during prepubertal and first stage of pregnancy for *in vitro* embryo production (Mohammed 2014 a,b, Ferré et al., 2020; Tian et al., 2021) to take advantage of genetically superior species and to shorten the interval between generations in different species.

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) regulate follicular wave development where FSH is preceded follicular wave emergence (Webb et al., 2003; Baby and Bartlewski, 2011) whereas granulosa cells acquire LH receptors around the time of follicular selection in both theca and granulosa cells ) and increase as follicle grow (Campbell et al., 1995, 2003; Webb et al., 2003; Baird and Mitchell, 2013). Diameter of gonadotrophin dependent follicle is 3-4 mm whereas diameter of follicles which their granulosa cells acquire LH receptors is 9-10 mm (Campbell et al., 1995; Gordon, 2003; Miller et al., 1999; Webb et al., 2003). Follicle sizes and their containing follicular fluid composition are affected by follicles development and/or nutritional level during estrous cycle (Mohammed and Kassab 2014; Mohammed et al., 2011, 2012a; Senosy et al., 2017, 2018).

## Cumulus Cells

Cumulus cells are a cluster of cells that surround and communicate with the oocyte through gap and intermediate junctions. It has been thought that the communication between the oocytes and cumulus cells through gap junctions is absolutely necessary for oocytes' growth and maturation and further embryonic development (Fig. 3 & 4). Salhab et al. (2011) investigated kinetics of gene expression and signaling in bovine cumulus cells during in vitro maturation in different media in relation to oocyte developmental competence, cumulus apoptosis and progesterone secretion. They found in cumulus cells that the factors and signaling pathways are potentially involved in controlling different stages of oocyte nuclear maturation and their de

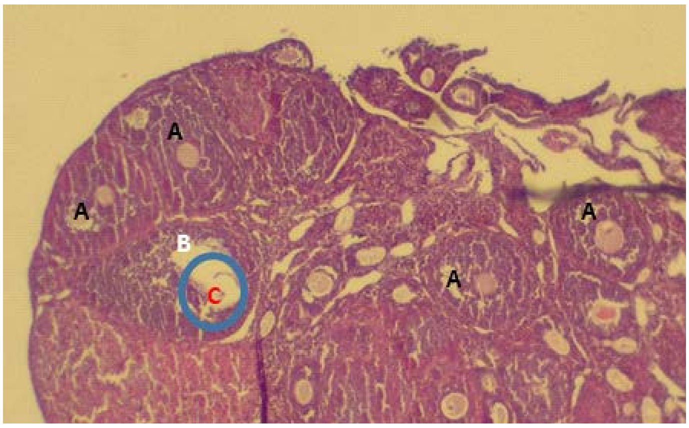


**Figure 3:** Ovarian follicle structures of mice; secondary follicles (A), graaffian follicles (B) and cumulus-enclosed germinal vesicle oocytes (Mohammed AA)

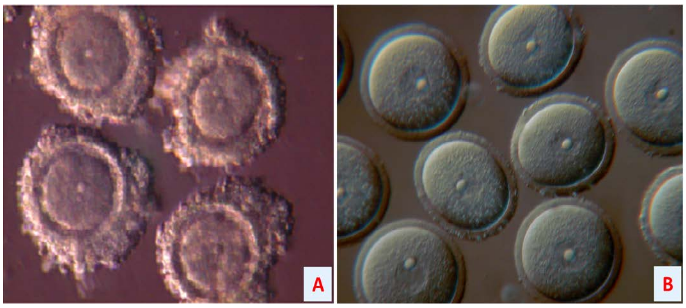


**Figure 4:** Mice denuded germinal vesicle oocytes (A) and cumulus-enclosed germinal vesicle oocytes (B)

velopmental competence to embryos.

Communication of cumulus cells with the germinal vesicle oocytes or addition of cumulus cells to maturation media during oocytes maturation *in vitro* effects on maturation rates and developmental competence of the resulting embryos (Mohammed 2006, 2008; Mohammed et al., 2005, 2008, 2010, 2019b, 2020; Lee et al., 2018). Cumulus cells is essential for transfer of some nutrients as amino acids to the oocytes. Amino acids (AAs) are uptake first by cumulus cells and transfer thereafter via gap junctions to the oocytes. There are several roles of AAs in cytoplast of oocyte and the resulting embryo as energy sources and protein synthesis (Rieger et al., 1992), intracellular buffers (Edwards et al., 1998), antioxidant compounds (Guérin et al., 2001), heavy metal chelators (Bavister 1995) and osmolytes (Dawson et al. 1998).

The secretion of intra-follicular cells is called follicular fluid (FF), which is a semi-viscous and yellow liquid filled the follicular antrum and surround the oocyte. The components of FF are mainly synthesized from secretion of granulosa cells and from blood plasma transudate. Composition of FF changes during estrous or menstrual cycles upon follicular development (Mohammed et al., 2019b). The fluid is rich in a polysaccharide molecule called hyaluronic acid or hyaluronan (HA). Amino acids concentrations in follicular fluid were associated with morphological quality of cumulus-oocyte complexes (COCs) and with post-fertilization embryo development to the blastocyst stage (Sinclair et al., 2008). It has been found that addition of specific amino acids in culture media facilitates embryo hatching in some species (Liu and Foote 1995; Pinyopummintr et al., 1996), helping to alleviate cultured-induced arrest through maternal zygotic transition (Lee et al., 2014). Therefore, cumulus cells or their follicular fluid secretions can improve cytoplasmic maturation of oocytes (Mohammed et al., 2005; Ikeda and Yamada, 2014, Mohammed et al., 2019b).

## Factors Affecting Functions Of Cumulus Cells

There are several factors affecting functions of cumulus cells and consequently oocyte maturation and embryo development (Table 1) (Zabihi et al., 2021; Martínez-Quezada et al., 2021). Kumar et al. (2020) evaluated the effect of FGF2 on bubaline oocyte maturation and cumulus cell expansion and developmental potential of the resulting zygotes. They found that *FGF2* transcript was higher in good quality oocytes and cumulus cells than any other grades of oocytes. Gutiérrez-Añez et al. (2021) found enhancement the developmental competence of oocytes upon ovum pick-up in treated prepubertal and adult dairy cattle with melatonin. Saini et al. (2022) demonstrates that 50 μM folic acid supplementation to maturation medium *in vitro* resulted in improvement of oocyte maturation and blastocyst rate, reduction in intracellular ROS levels, in addition to upregulation of the expression of *FOLR1* and folate metabolism enzyme, *MTR*. Liu et al. (2021) studied the possibility of using mitochondrial DNA copy number of cumulus cells as a biomarker of the potential of embryo implantation. They found that mitochondrial DNA is not linked to embryo implantation in prognosis IVF patients.

Ko et al. (2021) suggested through *in vitro* cultured of human granulosa cells that insulin might gave a negative effect on regulation of GnRH and FSH by attenuating GnRH and FSH action in the phosphorylation of ERK1/2 versus a positive effect on LH regulation by potentiating LH action in the phosphorylation of ERK1/2. This indicates clearly the important role of insulin in human reproductive function through granulosa cells. Gene expression in cumulus cells were investigated in several studies. de Senna Costa et al. (2022) explored if leptin influences the caprine oocyte maturation of oocytes and gene expression in cumulus cells. The results demonstrate improvement of oocyte nuclear maturation and *PPARγ* and *BAX* gene expression in cumulus cells upon leptin treatment. Wolff et al. (2022) indicate that the mRNA expression in cumulus granulosa cells of LHCG, FSH and androgen receptors, aromatase and anti-Müllerian hormone (AMH) differ in normal matured compared to gonadotrophin stimulated follicles. These results confirm some gonadotrophin stimulation effects on ovarian follicle function. Bunel et al. (2020) investigated inhibition of LH secretion using cetrorelix on cumulus cell gene expression in cattle. They suggested the inhibition of LH secretion might decrease growth and survival of follicles, which confirm the hypothesis of LH importance for final follicle maturation stage. Pournaghi et al. (2021) investigated the effects of melatonin on the exosome release from bovine cumulus cells. The results indicated the increase of unsaturated fatty acids due to melatonin treatment showing exosomal dynamic changes of bovine cumulus cells.

## Oocyte maturation

Oocyte maturation either *in vivo* or *in vitro* is the most important step for further embryo development in mammals (Mohammed et al., 2005; Yousefian et al., 2021). The maturation phase of oocytes *in vitro* requires relatively less time where it lasts 24 hr. in human and ruminant oocytes. Nuclear and cytoplasmic changes occur during oocyte maturation, which are important for successful fertilization and further embryo development (Mohammed et al., 2008; 2010; 2019a, b; Moulavi and Hosseini 2019; Saini et al., 2022). Species and feeding, follicular wave, follicle size, follicular and luteal stages are some of the factors affecting the quality of oocytes and maturation rate *in vivo* and *in vitro* (Mohammed et al., 2005, 2012a, 2020, 2021). The maturation media and their supplementations (hormones, FF, BSA, glutamine, amino acids…etc.) and incubation culture conditions (oxygen, CO2, humidity, light) *in vitro* were indicated to affect maturation, fertilization and further embryo development (Mohammed et al., 2005; Yousefian et al., 2021; Kang et al., 2021; Zabihi et al., 2021; Chelenga et al., 2022).

Our study and others indicated that oocyte quality, follicular fluid supplementation, co-culture cumulus cells affected oocyte maturation rate and timing of embryo cleavages in addition to blastocyst rate and hatching (Mohammed et al., 2005, 2008; Ge et al., 2008). Furthermore, nutrition and feed additive has been indicated to influence on follicle and embryonic development (Mohammed 2018, 2019; Mohammed and Attaai 2011; Mohammed and Farghaly 2018; Mohammed and Al-Hozab 2020; Mohammed et al., 2012a,b, 2019a, 2020, 2021; Liang et al., 2012; Moulavi and Hosseini 2019; Ali et al., 2021; Pournaghi et al., 2021; Gutiérrez-Añez et al., 2021; Saini et al., 2022).

## Cumulus enclosed artificial oocytes "gametes"

There are our unique studies (Mohammed 2006; Mohammed et al., 2008, 2010, 2019a) concerning complete and selective enucleation of cumulus-enclosed germinal vesicle oocytes to confirm the role of cumulus cells on oocyte maturation and embryo development (Figure 5). Our interesting results indicated helpful and potential roles of cumulus cells in supporting the developmental competence of the resulting embryos over somatic or embryonic nuclear transfer.

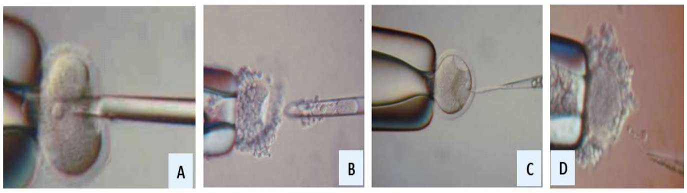


**Figure 5:** Complete and selective enucleation of denuded and cumulus-enclosed germinal vesicle oocytes; complete enucleation with removal the whole nucleus (A,B) and selective enucleation leaving the nuclear sap and nucleolus in the cytoplast (C,D).

Because of low embryo development, pregnancy rate and delivery over somatic/ embryonic nuclear transfer to oocytes, interest has been grown to use germinal vesicle (GV) cytoplast as recipient. It has been suggested that earlier somatic/embryonic nuclear transfer to GV cytoplast compared to MII cytoplast might be helpful for cell reprogramming of the introduced nuclei. The reconstructed oocytes seem to be an interesting model for studying the mechanisms of meiotic maturation, treatment of reproductive disorders or embryonic and somatic cloning.

Chang et al. (2005) reported that the developmental incompetency of denuded mouse oocytes undergoing maturation *in vitro* is ooplasmic in nature and is associated with aberrant Oct-4 expression. Thus, for better understanding, the background of difficulties in co-operation between foreign nucleus and cytoplasm in GV reconstructed oocytes, the development of new micromanipulation techniques and/or new culture systems of GV oocytes are required, which might also help to overcome the low efficiency of nuclear transfer through improvement of cellular reprogramming and developmental competence of resulting embryos. Therefore, the adapted micromanipulation techniques of complete and selective in addition to nucleolus transfer enabled to confirm the roles of cumulus cells, nuclear material and nucleolus on oocyte maturation and development of the resulting embryo (Fulka et al., 2004; Mohammed et al., 2008, 2010, 2019a; Benc et al., 2019).

Moreover, meiotic maturation of intact or enucleated GV oocytes reconstructed with male germ cells in cases of male infertility might enable creating the new type of oocytes carrying the male haploid genome of the introduced germ cells over maturation. So far, only a few trials concerning the meiotic maturation of enucleated GV oocytes reconstructed with embryonic/somatic nuclei were undertaken whereas *in vitro* fertilization of such matured oocytes has

**Table 1:** Factors affecting function of cumulus cells

| Treatments | Effect | References |
|---|---|---|
| Gonadotrophin | Gonadotrophin reduced concentrations of follicular fluid hormones and disrupts their quantitative association with mRNA cumulus cell | Wolff *et al.*, 2022 |
| LH | LH is important for final follicle maturation stage through its influence on the cumulus cells. | Bunel *et al.*, 2020 |
| Insulin | Insulin has a negative effect on regulation of GnRH and FSH versus a positive effect on LH regulation | Ko *et al.*, 2021 |
| Melatonin | Melatonin increased of unsaturated fatty acids due to melatonin treatment of bovine cumulus cells | Pournaghi *et al.*, 2021<br>Gutiérrez-Añez *et al.*, 2021 |
| Leptin | Improvement of *PPARγ* and *BAX* gene expression in cumulus cells over leptin treatment | de Senna Costa *et al.*, 2022 |
| 9-cis-retinoic acid | 9-cis-RA has beneficial effect on cytoplasmic and nuclear maturation | Liang *et al.*, 2012 |
| Astaxanthin | Astaxanthin supplementation promoted blastocyst yield of oocytes | Chelenga *et al.*, 2022 |
| Folate | Folate supplementation during oocyte maturation positively impacted the folate-methionine metabolism in pre-implantation embryos | Saini *et al.*, 2022 |
| Resveratrol | Resveratrol improved expansion of cumulus cells and developmental competence of ovine oocytes | Zabihi *et al.*, 2021 |
| Perfluorohexane sulfonate | Perfluorohexane sulfonate caused ytotoxicity and inhibition of oocyte maturation | Martínez-Quezada *et al.*, 2021 |

been studied in our study (Mohammed et al., 2008, 2010, 2019a).

## CONCLUSION

Regulating ovarian follicles development in mammals is considered the determinant of the reproductive performances. Regulation extends through *in vitro* culture systems including additives to media in addition to co-culture cumulus cells and their secretions; follicular fluid. The roles of cumulus cells clarify through gene expressions, oocyte maturation and development of the resulting embryo. The positive effects of cumulus cell on oocyte maturation and embryo development would be helpful in assisted reproductive techniques nowadays for treatment of infertility, enhancement of meat and milk production and saving endangered species through *in vitro* manipulations of follicles and oocytes. Further investigations are warranted to fully understand the factors maximize the potential roles of cumulus cells.

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## AUTHORS CONTRIBUTION

Mohammed shared in experimental design, wrote and submit MS and prepared figures. Rashid Al Zeidi, Haitham Al Masruri and Aiman Al Mufarji collected references and prepared figures.

## CONFLICT OF INTEREST

None of the authors has any conflict of interest to declare.

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