Open Access

Check for updates

Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2025.56.2.261-267

Molecular investigations on exosome enriched *in vitro* maturation of bovine cumulus cells: Insights from cumulus cell dynamics#

P. Parvathi[1], R.S. Abhilash[1*], C. Jayakumar[1], H.M. Harshan[1], K.P. Magnus[1], G. Radhika[2], E. Kurian[3], P. Prathima[1] and S.M. Saifudeen[3]

[1]Department of Animal Reproduction, Gynaecology and Obstetrics, [2]Department of Animal Genetics and Breeding, [3]Centre for Advanced Studies in Animal Genetics and Breeding College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala- 680 651, [4]Department of Agronomy, Vanavarayar Institute of Agriculture, Pollachi, Tamil Nadu Agricultural University- 642 103, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, India- 673 576

Citation: Parvathi, P., Abhilash, R.S., Jayakumar, C., Harshan, H.M., Magnus, K.P., Radhika, G., Kurian, E., Prathima, P., and Saifudeen, S.M. 2024.Molecular investigations on exosome-enriched *in vitro* maturation of bovine cumulus cells: Insights from cumulus cell dynamics. *J. Vet. Anim. Sci.* **56** (2):261-267

Received: 25.11.2024 Accepted: 13.02.2025 Published: 30.06.2025

Abstract

Cumulus cells, derived from granulosa cells, play a vital role in supporting the maturation and development of oocytes. Exosomes are nanoparticles encapsulating bioactive molecules such as proteins, nucleic acids, enzymes, and metabolites, which are known to modulate cellular signaling pathways. The exosomes present in follicular fluid enhance the maturation of cumulus-oocyte complexes (COCs). In the current study, a total of 292 culture grade COCs collected from slaughterhouse ovaries were subjected to in vitro maturation (IVM) using two protocols: Group I (control), with 146 COCs matured at 38.5ºC for 24 h (physiological IVM) and Group II (EXO) with 146 COCs matured under identical conditions but supplemented with 1µL of exosomes per 100 µL of maturation medium. Both groups were incubated in a controlled environment of 5 per cent CO_2 and 95 per cent relative humidity. Following maturation, cumulus expansion rates (Grade A 62.10 ± 3.99 vs 50.00 ± 2.24 %) and IVM rates (91.33 ± 1.69 vs 75.93 ± 2.85 %) were noted to be significantly higher ($p \leq 0.01$) in the exosome supplemented group (Group II) compared to the control (Group I). The relative expression of hyaluronan synthase 2 (HAS2), a key enzyme in cumulus expansion, depicted a significant upregulation of 2-fold (fold change FC= 2.10; $p \leq 0.05$) correlating with enhanced morphological cumulus expansion. Additionally, the expression of caveolin 1 (CAV1), a gap junctional protein, exhibited a 1.4-fold increase (FC= 1.41; $p > 0.05$), though not statistically significant. These findings suggest a beneficial role of exosome supplementation during IVM in promoting cumulus cell functionality and developmental competence of bovine oocytes. The study underscores the potential of exosomes as a valuable supplement for improving the success rate of bovine in vitro embryo production.

Keywords: *Cumulus cell, cumulus oocyte complex, exosomes, cumulus expansion, hyaluronan synthase 2, caveolin 1, in vitro maturation, bovine, real-time PCR*

Granulosa cells are ovarian somatic cells in the Graafian follicle, differentiated into three populations, *i.e.* mural granulosa cells beneath the basement membrane and in contact with the thecal layer, antral granulosa cells lining the antral cavity, and cumulus cells (CCs) surrounding the oocytes (Aaron *et al*., 1984). The CCs maintain an

**Corresponding author: abhilash@kvasu.ac.in, Ph.9447265844*
#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

intimate relationship with oocytes through gap junctions established via cytoplasmic extensions called transzonal projections (TZPs) that traverse the zona pellucida (Baena and Terasaki, 2019).

Cumulus cells play a crucial role in supporting oocyte nutrition, metabolism, protection, maturation and developmental competency (Turathum *et al*., 2021). Exosomes, a subset of extracellular vesicles within the size range of 30 – 200 nm, are derived from endosomes through exocytosis (Chen *et al.*, 2023). These nanoparticles in various body fluids participate in signal transduction and modulate cellular bioactivity by delivering molecular cargo such as proteins, nucleic acids, microRNAs and enzymes (Gabrys *et al*., 2024). The extracellular vesicles from follicular fluid were identified as exosomes (<300 nm) and had a concentration of 1×10^{-11} to 5×10^{-11} particles/mL as per nanoparticle tracking analysis (Rodrigues *et al*., 2019).

Exosome isolation from body fluids can be performed using techniques such as differential ultracentrifugation (gold standard), size exclusion chromatography, immunoaffinity capture, microfluidics, and ultrafiltration (Gao *et al.,* 2023). The follicular fluid-derived exosomes were involved in intrafollicular signal transduction and regulation of gene expression, exerting beneficial effects on cumulus expansion and the maturation of bovine cumulus oocyte complexes (COCs) (Hung *et al*., 2015).

Cumulus expansion, a process of CC proliferation and extracellular matrix mucification, occurs during oocyte maturation and manifests morphologically as an enhanced volume of cumulus covering of the oocytes (Nevoral *et al*., 2014). Extracellular matrix of bovine cumulus cells predominantly comprises of hyaluronic acid, a glycosaminoglycan crucial for the resumption of meiotic arrest and potentiation of fertilisation (Parkes *et al.,* 2021). Hyaluronic acid is synthesised at the CC membrane via the hexosamine biosynthesis pathway, catalysed by the enzyme hyaluronan synthase (HAS). Among the three isomers HAS1, HAS2 and HAS3, HAS2 is primarily responsible for the production of large hyaluronic acid molecules necessary for cumulus expansion (Schoenfelder and Einspanier, 2003). Relative gene expression of *HAS2* provides a better understanding of the molecular basis of cumulus expansion and *in vitro* maturation rates.

Caveolins are cholesterol binding proteins in caveolae (lipid raft domain invaginations of plasma membrane), are present in CCs and play roles in signal transduction, material exchange and gap junction-mediated cell homeostasis (Defamie and Mesnil, 2012). Relative gene expression of *HAS2* and *Caveolin 1* (*CAV1)* could provide insights into the molecular dynamics of cumulus expansion, *in vitro* maturation (IVM) and gap junctional communication (Rispoli *et al.,* 2013; Wei *et al.,* 2022). The current study validated the applicability of exosome-enriched bovine IVM systems in achieving superior maturation outcomes, supported by conclusive morphological and molecular evidence, with a focus on cumulus cell dynamics.

Materials and methods

Procurement and processing of ovaries

Bovine ovaries sourced from local slaughterhouses were transported in thermos flasks containing preheated normal saline supplemented with antibiotics to maintain a physiological temperature of 38.5ºC. Upon arrival at the laboratory within two hours of collection, extra ovarian tissues were trimmed, and the ovaries were rinsed multiple times with 0.9 per cent sodium chloride solution to minimise contaminants.

Isolation of exosomes

Exosomes were isolated from follicular fluid using differential ultracentrifugation (Arya *et al.,* 2023). Follicular fluid was collected and centrifuged at 500 × g for one minute to remove debris before being stored at -80°C until sufficient volume was obtained for further processing. A total of 15 mL of fluid was thawed and diluted with PBS, then subjected to a series of centrifugations at 800 × g for 10 min and 2000 × g for 20 min to remove residual cells. The supernatant was then centrifuged at 12000 × g for 45 min to remove debris, followed by filtration through a 0.22 µm PVDF syringe filter (Sigma-Aldrich, USA). Ultracentrifugation at 110000 × g for 3 h at 4˚C was performed to pellet extracellular vesicles. The supernatant was discarded, and the pellets were resuspended in 200 µL of filtered PBS, stored overnight, aliquoted, and maintained at -80°C until use.

Maturation of cumulus oocyte complexes

The COCs were retrieved via aspiration from 2–8 mm follicles and graded according to Loos *et al.* (1989). A total of 292 culture grade COCs (Grades A and B, defined by a homogeneous ooplasm and at least three layers of cumulus cells) were randomly divided into two groups for IVM: Group I (control), cultured under standard IVM conditions, 38.5°C for 24 h and Group II (EXO) cultured under the same conditions as Group I, but supplemented with exosomes at the rate of 1 µL/100 µL of maturation medium. Each group contained 146 COCs, cultured in modified Tissue Culture Medium-199 (mTCM 199) maturation medium. Following 24 h of maturation, the degree of cumulus expansion was assessed and graded based on Kobayashi *et al.* (1992) as Grade A with homogeneous spread of cumulus cells without clustering, Grade B with non-homogeneous restricted expansion with slight clustering and Grade C with slight or no expansion of cumulus cells with tight adherence to the zona pellucida (Fig. 1). Grades A and B were considered as expanded

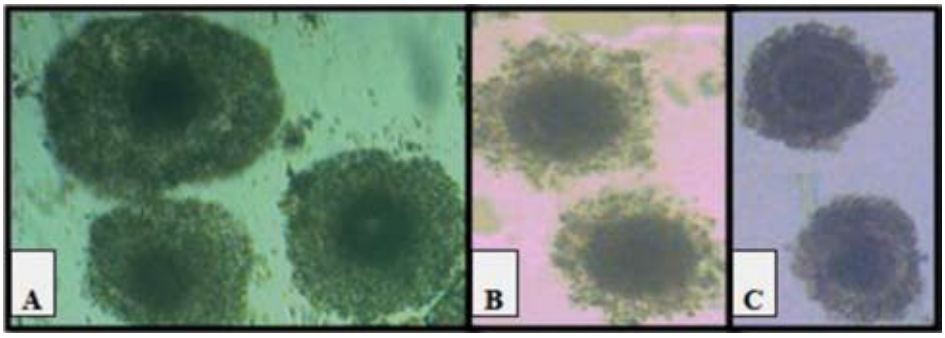

Fig: 1. Different degrees of cumulus expansion; A. Grade A- homogenous, good expansion; **B.** Grade B- clustered, restricted expansion; **C.** Grade C- slight or no expansion

COCs. The mean IVM rate (per cent) was determined by counting the number of oocytes exhibiting Grade A and B cumulus expansion relative to the total number of oocytes cultured for IVM.

Relative gene expression studies

Following 24 h of maturation, cumulus cells were separated from the oocytes by vortexing for 4 min and stored at -80°C in RNAlater (Sigma Lifesciences, Canada) until RNA isolation. Total RNA was extracted using the RNeasy Mini Plus kit (QIAGEN, Germany) following the manufacturer's protocol. The RNA samples from 40 A and B grade COCs were pooled, and RNA was eluted in 30 µL of RNase-free water. The concentration of eluted RNA was measured using a Nanodrop spectrophotometer, with most samples yielding approximately 10 ng/µL. The concentration of RNA was normalized to 100 ng per cDNA synthesis by utilising 10 µL of eluted RNA. Complementary DNA was synthesised using cDNA synthesis kit with RNase inhibitor (Origin diagnostics, Kerala). The synthesised cDNA had an average concentration of 1000 ng/µL. Primers for the target genes *HAS2* and *CAV1* were designed, with *GAPDH* serving as the reference gene (Table 1). Gene expression levels were quantified using real-time PCR (qPCR) with the comparative C_T method. The qPCR conditions were optimised by gradient PCR, and the annealing temperatures for each gene were standardised (Table 1). For qPCR, 1000 ng of cDNA was used per reaction (1 µL of synthesised cDNA), with reactions performed in triplicate across three independent biological replicates.

Statistical analysis

Statistical analysis was performed using one-way ANOVA, with a significance threshold set at $p < 0.05$ using the software Statistical Product and Service Solutions (SPSS), version 24.0 (Snedecor and Cochran, 1994).

This methodology aimed to elucidate the protective effects of exosomes on cumulus cells, providing insights into potential interventions for improving *in vitro* embryo production.

Results and discussion

The percentage of cumulus expansion in Group I (control, under physiological IVM conditions) for Grades A, B, and C (Fig. 1) was recorded as 50.00 ± 2.24, 25.92 ± 2.82 and 24.07± 2.85 per cent, respectively, suggesting predominance of Grade A expansion (Fig. 2). Cumulus expansion is fundamental to oocyte maturation (Allworth and Albertini, 1993), fertilisation by activating the acrosomal reactions of spermatozoa (Fukui, 1990) and determination of the developmental potential of oocytes to blastocyst and embryonic stage (Furnus *et al.,* 1998). Factors influencing cumulus expansion in bovines have been classified into endocrine and paracrine origin. Endocrine factors include hormonal alterations, while paracrine factors comprise

Table: 1. Sequence and properties of primers designed to amplify *hyaluronan synthase 2* and *caveolin 1* genes in *Bos taurus* species

Gene	Gene Accession No (NCBI):	Primer name	Primer Sequence (5'-------3')	Primer length (bp)	PCR product size (bp)	Annealing temp (°C)
GAPDH	NM_001034034.2	GAPDH F	TGGAGAAACCTGCCAAGTATG	21	127	61.6
		GAPDH R	TGAGTGTCGCTG TTGAAGTC	20		
HAS2	NC_037341.1	HAS2 F	TGTTGGAACGTTGCTGTATGC	21	86	61.7
		HAS2 R	TCTTCCGCCTGC CACATTTAT	21		
CAV1	NC_037331.1	CAV1 F	TGAACGAGAACAAGTGTACGA	22	85	64.5
		CAV1 R	ACCACGTCGTCGTTGAGATG	20		

growth factors used, constituents of follicular fluid and blood plasma, molecular pathways like mitogen-activated protein kinase pathway (MAPK), and the expression of genes like *HAS2, Pentraxin 3 (PTX3)* and *Tumour necrosis factor alpha-induced protein (TGFAIP)* (Nevoral *et al.*, 2014).

When follicular fluid-derived exosome supplementation was done during physiological IVM (Group II), a significant improvement in cumulus expansion was observed, with Grades A, B, and C recorded as 62.10 ± 3.99, 29.23 ± 3.03 and 8.67 ± 1.69 per cent, respectively. The percentage of Grade A (homogenous good quality) expansion was significantly higher ($p \leq 0.01$) in Group II (EXO) compared to Group I (control) group, while the percentage of occurrence of Grade C (low-quality) expansion was significantly lower ($p \leq 0.01$) in Group II (EXO) than in Group I (control) (Fig. 2). The higher occurrence of high-quality expansion (Grade A) and reduced occurrence of low-quality expansion (Grade C) in exosome-supplemented COC (Group II) compared to physiologically *in vitro* matured COC (Group I) indicated enhanced cumulus expansion with follicular fluid-derived exosome supplementation. These findings align with studies by Arya *et al.* (2023) and Revathy *et al*. (2023).

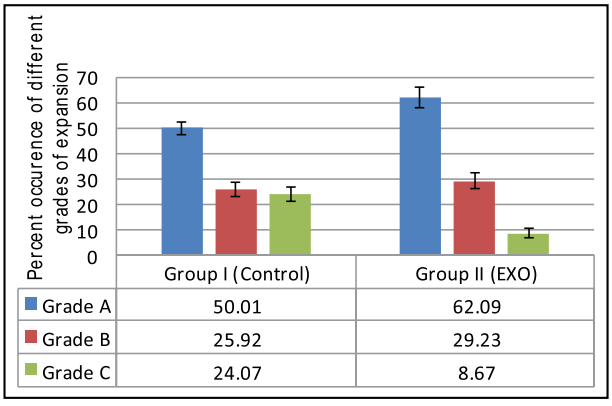

Fig: 2. Distribution of matured COCs based on morphological grading of cumulus expansion in bovine COCs matured in physiological and exosome-enriched IVM

The beneficial effects of follicular fluid-derived exosomes on cumulus expansion might be attributed towards the upregulation of cumulus expansion-enabling genes like *PTX3, Prostaglandin-endoperoxide synthase 2 (PTGS2)* and *TNFAIP* (Hung *et al*., 2015) and activation of the MAPK pathway, which plays an important role in initiating cumulus expansion (Shimada *et al.*, 2006; Matsuno *et al*., 2017).

Under physiological IVM conditions (Group I), the average percentage of matured COCs was recorded as 75.93 ± 2.85 per cent (Fig. 3). This observation is consistent with those of Leibfried-Rutledge *et al.* (1989), who reported that the extend of cumulus covering positively correlates with the rate of oocyte maturation. Additionally, actin filaments in gap junctions are converted into microtubules during IVM, a key factor in maturation (Allworth and Albertini, 1993). The significantly enhanced percentage of IVM of COCs (91.33 ± 1.69 %) (Fig. 3), in the exosome-supplemented Group II, demonstrated that the efficacy of follicular fluid-derived exosomes was superior to the physiological conditions (Group I) in enhancing bovine COC maturation. Gap junctions play an irreplaceable role in resuming meiotic arrest in oocytes (Richard and Baltz, 2014). Exosomes were found to upregulate genes encoding gap junctional connexin proteins, such as *Connexin 43 (CX43)* and *Connexin 38 (CX38)* which code for gap junctional connexin proteins. Micro RNAs in the exosomal molecular cargo were attributed towards this stimulatory action on gene expression (Sidrat *et al*., 2020). Enhanced mitochondrial membrane potential, improved gap junction communication, reduced cumulus apoptosis, and superior cumulus expansion contribute to better maturation outcomes with exosome supplementation (Wei *et al*., 2022).

Cumulus expansion occurs shortly after the luteinizing hormone (LH) surge, primarily influenced by epidermal growth factor (EGF) and follicle-stimulating hormone (FSH). This process involves the proliferation of cumulus cells and the synthesis and mucification of the extracellular matrix (ECM), predominantly composed of hyaluronic acid, a disaccharide polymer of N-acetylglucosamine and D-glucuronate. Hyaluronic acid synthesis taking place at the cumulus cell membrane is catalysed by hyaluronan synthase, particularly the HAS2 isoform, which is crucial for producing large hyaluronic acid molecules that facilitate cumulus expansion (Schoenfelder and Einspanier, 2003). Notably, *HAS2* is the gene encoding this enzyme. This ECM remodelling enhances acrosome reaction and capacitation during fertilisation, influencing cleavage and blastocyst formation rates (Gutnisky *et al.,* 2007).

In the current study, *HAS2* expression in CCs matured with exosomes (Group II) was significantly upregulated by 2-fold (FC= 2.09; $p \leq 0.05$) compared to the control group (Group I) (Fig. 4). This finding suggests that follicular fluid-derived exosomes could produce an

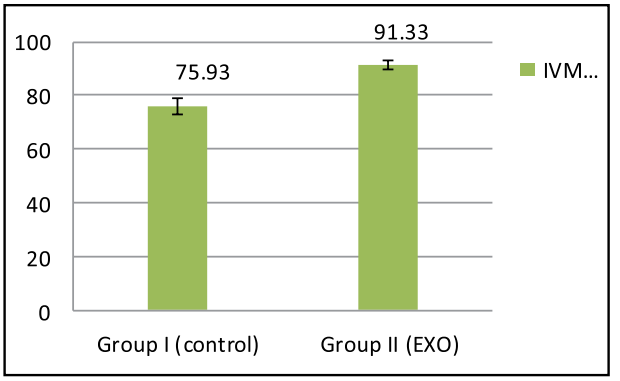

Fig: 3. *In vitro* maturation rate of bovine COCs matured in physiological and exosome-enriched IVM

outcome better than physiological IVM in terms of quality and rate of cumulus expansion. This finding is in line with Wei *et al.* (2022), who reported similar upregulation of *HAS2* in cumulus cells enriched with exosomes of oviductal epithelial cell origin. Exosomes activate the MAPK signalling pathway (Vincencio *et al.,* 2015), which regulates *HAS2* and *PTGS2* expression, which are genes involved in cumulus expansion (Javadi *et al*., 2022). The molecular findings are also correlating with the morphological enhancement of cumulus expansion by follicular fluid-derived exosomes in the current study.

Caveolae are flask-shaped invaginations in the cell membrane, house proteins known as caveolins (CAV1, CAV2, etc.), which are essential for endocytosis (Kiss and Botos, 2009). Caveolin proteins in cumulus cells play a crucial role in gap junction communication and signal transduction, with *CAV1* being the gene coding for the CAV1 protein in bovine cumulus cells (Sasseville *et al*., 2009).

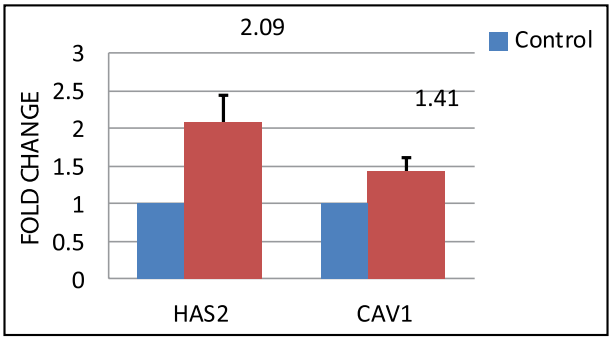

Fig: 4. Relative quantification of *HAS2* and *CAV1* genes **in** bovine cumulus cell matured by physiological and exosome-enriched IVM

In the present study, *CAV1* exhibited a fold change of 1.41 (p> 0.05) in CCs matured under physiological conditions supplemented with exosomes (Group II) compared to the control group (Group I) (Fig. 4). However, its expression pattern was not significantly different from the control, possibly due to the limited sample size. Exosomal microRNAs are known to stimulate gene expression in recipient cells, promoting the genes involved in gap junction communication (Sidrat *et al*., 2020; Wei *et al.,* 2022). FSH receptors co-immunoprecipitate with CAV1, suggesting that upregulation of *CAV1* enhances FSH receptor signal transduction (McKenize and Cohen, 2009). The critical role of FSH in the development of a competent CC can be attributed to its involvement in LH receptor formation on the CC membrane, production of HAS enzymes required for HA synthesis, and stimulation of cumulus expansion enabling factors like EGF and SMAD 2/3 (small mothers against decapentaplegic protein) pathway (Turathum *et al*., 2021). Since FSH is a key regulator of cumulus expansion (Nevoral *et al.,* 2014), *CAV1* upregulation could also contribute towards the morphological improvements in cumulus expansion. Additionally, Wang *et al.* (2024) highlighted the role of caveolin proteins in facilitating exosome uptake in granulosa cells via the phosphoinositide 3-kinase pathway.

Conclusion

This study highlighted the advantages of supplementing follicular fluid-derived exosomes in in vitro maturation (IVM) of bovine cumulus-oocyte complexes (COCs). The results revealed that exosome-enriched media significantly enhanced cumulus expansion and maturation rates, indicating their important role in modulating cumulus cell dynamics and improving oocyte developmental competence. The observed upregulation of *HAS2* expression strongly correlates with improved cumulus morphology, whereas the slight increase in *CAV1* expression, though not statistically significant, suggests the potential involvement of complex signalling pathways. These findings underscore the potential of follicular fluid-derived exosome supplementation as a promising approach to improve the efficiency of *in vitro* bovine embryo production. The study also emphasised the pivotal role of cumulus cells and exosomal molecular cargo in advancing reproductive biotechnology.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Aaron, J.W., Hsueh, T.E.Y., Phillip, A.B.C., Jones, S. and Thomas, H.J. 1984. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. *Endocrine Rev*. **5**: 76-127.

Allworth, A.E. and Albertini, D.F. 1993. Meiotic maturation in cultured bovine oocytes is accompanied by remodeling of the cumulus cell cytoskeleton. *Dev. Biol.* **158**: 101-112.

Arya, T., Abhilash, R.S., Jayakumar, C., Amritha, A., Naicy, T., Harshan, H.M. and Revathy, M.M. 2023. Developmental competence of bovine oocytes in maturation media supplemented with follicular fluid exosomes. *J. Vet. Anim. Sci.* **54**: 465-471.

Baena, V. and Terasaki, M. 2019. Three-dimensional organization of transzonal projections and other cytoplasmic extensions in the mouse ovarian follicle. *Sci Rep*. **9**: 1-13.

Chen, C., Zhang, Z., Gu, X., Sheng, X., Xiao, L. and Wang, X. 2023. Exosomes: new regulators of reproductive development. *Matr. Today Bio.* **19**: 1-13.

Defamie, N. and Mesnil, M. 2012. The modulation of gap junctional intercellular communication by lipid rafts. *Biochem. Biophys. Acta*. **1818**: 1866-1869.

Fukui Y. 1990. Effect of follicle cells on the acrosome reaction, fertilization, and developmental competence of bovine oocytes matured *in vitro*. *Mol. Reprod. Dev.* **26**: 40–46.

Furnus, C.C., de Matos, D.G. and Moses D.F. 1998. Cumulus expansion during *in vitro* maturation of bovine oocytes: relationship with intracellular glutathione level and its role on subsequent embryo development. *Mol. Reprod. Dev.* **51**: 76–83.

Gabrys, J., Gurgul, A., Szmatoła, T., Mitka, K.B., Andronowska, A., Karnas, E., Kucharski, M., Puchałka, J.; Kochan, W.J. and Poniewierska, B.M. 2024. Follicular fluid-derived extracellular vesicles influence on *in vitro* maturation of equine oocyte: impact on cumulus cell viability, expansion and transcriptome. *Int. J. Mol. Sci.* **25**: 1-23.

Gao, J., Li, A., Hu, J., Feng, L., Liu, L.and Shen, Z. 2023. Recent developments in isolating methods for exosomes. *Front. Bioeng. Biotechnol*. **10**: 1-17.

Gutnisky, C., Dalvit, G.C., Pintos, L.N., Thompson, J.G., Beconi, M.T. and Cetica, P.D. 2007. Influence of hyaluronic acid synthesis and cumulus mucification on bovine oocyte *in vitro* maturation, fertilisation and embryo development. *Reprod. Fert. Dev*. **19**: 488-497.

Hung, W.T., Hong, X., Christenson, L.K., and McGinnis, L.K. 2015. Extracellular vesicles from bovine follicular fluid support cumulus expansion. *Biol. Reprod*. **93**: 1-9.

Javadi, M., Rad, J.S., Pashaiasl, M., Farashah, M.S.G. and Roshangar, L. 2022. The effects of plasma derived extracellular vesicles on cumulus expansion and oocyte maturation in mice. *Reprod. biol*. **22**: 1-8.

Kiss, A.L. and Botos, E. 2009. Endocytosis via caveolae: alternative pathway with distinct cellular compartments to avoid lysosomal degradation? *J. Cell. Mol. Med.* **13**: 1228–1237.

Kobayashi, K., Yamashita, S. and Hoshi, H. 1992. Influence of epidermal growth factor and transforming growth factor α on *in vitro* maturation of cumulus cell enclosed bovine oocytes in a defined medium. *J. Reprod. Fert.* **100**: 439- 446**.**

Leibfried-Rutledge, M.L., Critser, E.S., Parrish, J.J. and First, N.L. 1989. *In vitro* maturation and fertilization of bovine oocytes. *Theriogenology.* **31**: 61–74.

Loos, D.F., Van Vliet, C.P., Maurik, V. and Kruip, A.M.T. 1989. Morphology of immature bovine oocytes. *Gamete Res.* **24**: 197-204.

Matsuno, Y., Onuma, A., Fujioka, Y.A., Yasuhara, K., Fujii, W., Naito, K. and Sugiura, K. 2017. Effects of exosome like vesicles on cumulus expansion in pigs *in vitro*. *J. Reprod. Dev.***63**: 51-58.

McKenize, K.A. and Cohen, B.D. 2009. Investigation of human follicle stimulating hormone residency in membrane microdomains. *J. Fed. Am. Soc. Exp. Biol.* **23**: 1-7.

Nevoral, J., Orsak, M., Klein, P., Petr, J., Dvorakova, M., Weingartova, I., Vyskocilova, A., Zamostna, K., Krejcova, T. and Jílek, F. 2014. Cumulus cell expansion, its role in oocyte biology and perspectives of measurement: a review. *Sci. Agric. Bohemica*. **45**: 212–225.

Parkes, W.S., Amargant, F., Zhou, L.T., Villanueva, C.E., Duncan, F.E. and Pritchard, M.T. 2021. Hyaluronan and collagen are prominent extracellular matrix components in bovine and porcine ovaries. *Genes*. **12**: 1-23.

Revathy, M.M., Unnikrishnan, M.P., Jayakumar, C., Abhilash, R.S., Ramnath, V. and Sreeranjini, A.R. 2023. Assessment of cumulus cell expansion rate and first polar body extrusion rate in bovine oocytes supplemented with bovine follicular fluid exosomes. *Int. J. Vet.* **8**: 149-152.

Richard, S. and Baltz, J.M. 2014. Prophase I arrest of mouse oocytes mediated by natriuretic peptide precursor c requires GJA1 (connexin-43) and GJA4 (connexin-37) gap junctions in the antral follicle and cumulus oocyte complex. *Biol. Reprod.* **90**: 1-10.

Rispoli, L.A., Payton, R.R.,Gondro, C., Saxton, A.M., Nagle, K.A., Jenkins, B.W.,Schrick, F.N. and Edwards, J.L. 2013. Heat stress effects on the cumulus cells surrounding the bovine oocyte during maturation: altered matrix metallopeptidase 9 and progesterone production. *Reproduction.* **146**: 193–207.

Rodrigues, T.A., Tuna, K.M., Alli, A.A., Tribulo, P., Hansen, P.J., Koh, J. and Paula-Lopes, F.F. 2019. Follicular fluid exosomes act on the bovine oocyte to improve oocyte competence to support development and survival to heat shock. *Reprod. Fert. Dev.* **31**: 888-897.

Sasseville, M., Gagnon, M.C., Guillemette, C., Sullivan, R., Gilchrist, R.B. and Richard, F.J. 2009. Regulation of gap junctions in porcine cumulus oocyte complexes: contributions of granulosa cell contact, gonadotropins, and lipid rafts. *J. Mol. Endocrinology.* **23**: 700-710.

Schoenfelder, M. and Einspanier, R. 2003. Expression of hyaluronan synthases and corresponding

hyaluronan receptors is differentially regulated during oocyte maturation in cattle. *Biol. Reprod*. **69**: 269–277.

Shimada, M., Hernandez-Gonzalez, I., Gonzalez-Robayna, I. and Richards, J.S. 2006. Paracrine and autocrine regulation of epidermal growth factor-like factors in cumulus oocyte complexes and granulosa cells: key roles for prostaglandin synthase 2 and progesterone receptor. *Mol. Endocrinology.* **20**: 1352-1365.

Sidrat, T., Khan, A., Joo, M.D., Wei, Y., Lee, K.L., Xu, L. and Kong, I.K.2020. Bovine oviduct epithelial cell derived culture media and exosomes improve mitochondrial health by restoring metabolic flux during pre implantation development. *Int. J. Mol. Sci.* **2**: 1-20.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*. (8th Ed.). The Iowa State University Press, Iowa, 564p.

Turathum. B., Gao, E.M. and Chian, R.C. 2021. The function of cumulus cells in oocyte growth and maturation and in subsequent ovulation and fertilization*. Cells.* **10**: 2292-2298.

Vincencio, J.M., Yellon, D.M., Sivaraman, V., Das, D., Boi-Doku, C., Arjun, S., Zheng, Y., Riquelme, J.A., Kearney, J., Sharma, V., Multhoff, G., Hall A.R. and Davidson, S.M. 2015. Plasma exosomes protect the myocardium from ischemia reperfusion injury. *J. Am. Coll. Cardiology.* **65**: 1525-1536.

Wang, X., Zhang, Z., Qi, Y., Zhang, Z., Zhang, Y., Meng, K., Yuan, J. and Quan, F. 2024.Study of the uptake mechanism of two small extracellular vesicle subtypes by granulosa cells. *Anim. Reprod. Sci.* **270**: 1-13.

Wei, Y., Idrees, M., Sidrat, T., Joo, M., Xu, L., Ko, J. and Kong, I. 2022. BOEC–Exo addition promotes *in vitro* maturation of bovine oocyte and enhances the developmental competence of early embryos. *Animals.* **12**: 1-15. ■