



Aquaporin Gene Expression in Cultured Buffalo Cumulus and Fibroblast Cells

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ABSTRACT

Background: Aquaporins (AQPs) are essential membrane proteins that facilitate the movement of water and small molecules within cells. It also facilitates the passage of volatile substances, such as ammonia (NH₃) and carbon dioxide (CO₂), etc. through membranes. Cumulus cells support the metabolic needs of the developing oocyte and aquaporins play a crucial role in facilitating the transport of water and solutes necessary for these processes.

Methods: In the present study, cumulus and fibroblast cells were cultured *in vitro* and compared for the expression level of aquaporin-related genes between these cells. Total RNA was isolated and cDNA was synthesized; relative expression of reproductive tissue-related aquaporin genes, specifically *AQP3*, *AQP4*, *AQP7* and *AQP9* was studied in both cell types.

Result: The relative mRNA expression of *AQP3*, *AQP4* and *AQP7* was significantly higher ($P < 0.05$), whereas *AQP9* expression was lower, in cumulus cells compared to fibroblasts. Based on this study, it can be concluded that cumulus cells exhibit significantly higher levels of aquaporins than fibroblast cells.

Key words: Aquaporins, Cumulus cell, Fibroblast cell.

INTRODUCTION

Aquaporins (AQPs) constitute a family of transmembrane proteins that facilitate the transport of water and small solutes across cellular membranes. These proteins are evolutionarily conserved and are found in a diverse range of organisms, from bacteria to mammals. AQPs play a crucial role in various physiological processes, particularly in maintaining water homeostasis. In humans, 13 AQP isoforms have been identified and classified into three subfamilies based on their permeability characteristics: classical aquaporins (*AQP1*-*AQP4* and *AQP6*), aquaglyceroporins (*AQP7* and *AQP9*) and unorthodox aquaporins (*AQP11* and *AQP12*) (Benga and Gheorghe, 2009). The expression and functional roles of aquaporins have been extensively studied across various tissues and organs, i.e., *AQP1* is abundantly expressed in endothelial cells of blood vessels, where it plays a crucial role in regulating blood pressure and fluid homeostasis (Shangzu *et al.*, 2022). *AQP2* is essential for water reabsorption and urine concentration in the renal collecting ducts. In the skin, *AQP3* and *AQP4* play a crucial role in maintaining hydration, promoting wound healing and supporting the proliferation of skin cells. Cumulus cells, specialized somatic cells that surround developing oocytes in the ovary, play a crucial role in providing metabolic support and nutrients to the oocyte. These cells express several aquaporins (AQPs), including *AQP1*, *AQP3*, *AQP7* and *AQP11*, which contribute to water transport and cellular homeostasis. Petano-Duque *et al.* (2022) reported that *AQP1*, *AQP4* and *AQP9* are highly expressed in cumulus oocyte complexes of cattle and are essential for maintaining proper water balance within these cells. The study further demonstrated that the suppression of *AQP1* and *AQP3* expression impaired oocyte growth and

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fertilization, highlighting the importance of these channels in reproductive processes in humans. Fibroblasts, a type of connective tissue cells, are integral to tissue repair and wound healing. These cells express multiple AQPs, including *AQP1*, *AQP3*, *AQP4* and *AQP5*. *AQP1* functions as a key water channel protein, regulating water transport in fibroblasts (Agre, 2004). Studies have shown that overexpression of *AQP1* enhances fibroblast migration and proliferation, suggesting its importance in tissue regeneration and wound healing (Ma *et al.*, 2016). In addition to *AQP1*, fibroblasts also express *AQP3*, which has been implicated in regulating cell volume and promoting proliferation. Hara-Chikuma and Verkman, (2008) demonstrated that *AQP3*-deficient animals exhibited reduced skin hydration and impaired wound healing, highlighting the essential role of *AQP3* in maintaining fibroblast water homeostasis. Similarly, *AQP4* and *AQP5* play a role in water transport in fibroblasts. Pan *et al.* (2022) reported that *AQP4* expression increased in fibroblasts following thermal injury, suggesting a potential role in tissue repair and recovery.

Overall, the expression of AQPs in cumulus and fibroblast cells plays a critical role in regulating water transport and maintaining cellular water balance. The identification and characterization of these channels have provided valuable insights into the mechanisms governing cellular water homeostasis and have opened new avenues for research into their physiological and pathological significance. A deeper understanding of AQP expression and function in cumulus and fibroblast cells, particularly in bovine models, could enhance our knowledge of cellular hydration dynamics and their implications for reproductive and regenerative medicine. In the future, elucidating the role of AQPs in these cells may offer novel therapeutic strategies for a range of health conditions, including fertility disorders and impaired wound healing. Future studies focusing on the regulatory mechanisms and potential modulation of AQPs could provide significant advancements in both basic science and clinical applications.

Beyond their primary role in water transport, aquaporins have been implicated in various pathological conditions, including cancer, inflammation and neurological disorders. In recent years, increasing attention has been directed toward investigating AQP expression and function in buffalo cumulus and fibroblast cells, given their critical roles in ovarian follicular development and skin tissue maintenance, respectively. These cells contribute to tissue integrity, support the growth and development of neighbouring cells and regulate key cellular processes, including proliferation, differentiation and apoptosis. Studies have demonstrated AQPs expression in buffalo cumulus and fibroblast cells, suggesting their involvement in cellular water balance and other physiological functions. Among these, *AQP3*, a membrane protein widely expressed in tissues such as the skin, kidney and colon, facilitates the transport of water, glycerol and urea. *AQP3* is essential for maintaining skin hydration, promoting wound healing and facilitating cell proliferation (Jo *et al.*, 2011). Aquaporin 4, primarily located in the brain and spinal cord, plays a vital role in regulating cerebral oedema, synaptic plasticity and astrocyte migration. *AQP4* expression in buffalo cumulus cells, suggesting its involvement in water homeostasis and follicular growth within bovine ovaries (Sun *et al.*, 2009). Similarly, *AQP7* plays a crucial role in spermatogenesis, lipid homeostasis and energy metabolism. Its expression in buffalo fibroblast cells, suggesting that this channel may contribute to water and glycerol transport, as well as energy metabolism, in buffalo skin tissues (Iena and Lebeck, 2018). Another key member, *AQP9*, is primarily expressed in the liver, kidney and testis and is involved in sperm motility, renal urine concentration and hepatic urea metabolism. *AQP9* expression in buffalo cumulus cells suggests a potential role in water and urea transport, as well as oocyte maturation, in buffalo ovaries (Cai *et al.*, 2014). These findings highlight the diverse roles of AQPs in reproduction and tissue homeostasis, emphasizing their significance in cellular function and potential therapeutic applications.

MATERIALS AND METHODS

All the media and chemicals utilized in this study were procured from Sigma Chemical Co. (USA) and the plasticware was obtained from Nunc (Denmark), unless otherwise mentioned. Fetal bovine serum (FBS) was acquired from Gibco Life Technologies (USA).

In vitro culture of cumulus cells

Bovine cumulus cells were established from cumulus-oocyte complexes (COCs) of ovarian follicles, cultured in DMEM+Ham's F-12 nutrient mixture media supplemented with 10% fetal bovine serum at 37°C (Shah *et al.*, 2009). For the culture of cumulus cells, oocytes with a good number of cumulus layers were selected and cumulus-oocyte complexes were isolated. When the culture reached 70-80% confluency in monolayer within 8-10 days, it was harvested by trypsin treatment. Cumulus cell proliferation and morphology were monitored through microscopic observations. By day 5, cumulus cells demonstrated robust proliferation, forming interconnected networks. The culture reached approximately 80% confluency, indicating successful cell expansion. The resulting cumulus cells maintained their characteristic stellate morphology, indicative of a healthy and actively proliferating population. Thereafter, the cells were harvested with 0.25% trypsin and stored in trizol after washing with DPBS.

In vitro culture of fibroblast cells

Fibroblast cells were successfully established from cryopreserved cells using a well-established protocol (Tripathi *et al.*, 2025). Cryovials containing fibroblasts were rapidly thawed and cells were resuspended in the growth medium, *i.e.*, DMEM+Ham's F-12 nutrient mixture supplemented with 10% FBS, 100 IU/ml penicillin, 100 µg/ml streptomycin and gentamicin 50 µg/ml (Tripathi *et al.*, 2024). After centrifugation and viability assessment, cells were plated in collagen-coated plastic dishes in a 95% humidified incubator with 5% CO₂, where temperature was maintained at 37°C. After achieving 70-80% confluency on Day 4, the cells were harvested with 0.25% trypsin and stored in trizol after washing with DPBS. The protocol, performed under standard aseptic conditions, yielded high cell viability and maintained fibroblast characteristics.

Quantitative expression of genes

Total RNA was isolated from cumulus and fibroblast cells using trizol. After that, chloroform and isopropanol were added to the tube to precipitate nucleic acids and protein (Tripathi *et al.*, 2024). The final wash was performed using 70% ethyl alcohol, followed by centrifugation to remove all alcohol residues. The pellet was then dried for 15-20 minutes and subsequently eluted in 15-20 µl of nuclease-free water. For cDNA synthesis, the concentration of RNA was measured and adjusted to 100 ng/µl for foetal fibroblast and cumulus cells. The cDNA was prepared by RevertAid™ First Strand cDNA synthesis kit (Fermentas, Life Sciences, USA) according to the manufacturer's

instructions. For cDNA synthesis, 1 µl of total RNA (100 ng), 2 µl dNTP mix (10 mM), 1 µl oligodT (10 µM), 4 µl 5× Reaction Buffer, 1 µl Ribolock™ RNase inhibitor (20 u/µl), 1 µl RevertAid™ M-MuLV Reverse Transcriptase (200 u/µl) and nuclease-free water were added to make volume 20 µl in 200 µl tube. First, the reaction mixture containing RNA, primer and nuclease-free water was mixed and incubated at 65°C for 5 min in a thermal cycler. After that, the reaction mixture was chilled on ice for 5 min and the remaining components were added and incubated at 42°C for 60 min. The synthesized cDNA was stored at -20°C until use for qPCR.

The relative quantification of mRNA of many genes was done by using CFX96 real-time system (Bio-Rad, Hercules, USA). GAPDH was used as a reference gene for all experiments. The qPCR reactions were done using Maxima SYBR Green qPCR Master Mix (2X), with separate ROX vial (#K0251, Thermo Fisher Scientific Inc. USA), each run was performed in duplicate in a 10 µl reaction volume which contained 5 µl fluorescence dye, 2 µl of gene-specific primers (forward and reverse) from 10 µM stock and 1 µl template. The final volume was made up with nuclease-free water. The PCR condition used for all genes was as follows: Initial denaturation at 95°C for 3 min, 40 cycles (denaturation 95°C for 30 s, annealing 60°C for 30 s and extension at 72°C for 30 s), melting cycle starting from 65°C up to 95°C with a 0.5°C/s transition rate. The annealing temperature of all genes is mentioned in Table 1. The qPCR specificity was confirmed by analyzing the melting

curve generated by the machine using CFX Manager Software. The relative gene abundance for the target genes was calculated using the equation $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). To detect gene expression, specific primers are required for amplifying the target gene of interest. The gene-specific primers were designed to amplify a fragment of approximately 150-200 bp, preferably from the end of the cDNA. The primers were designed with highly conserved regions of either bovine or buffalo sequences using Primer3 Software. (<http://www-genome.wi.mit.edu/cgi-bin/prime/primer3-www.cgi>).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7 software. Analysis of variance (ANOVA) was conducted and a student's t-test was employed to compare the means of different groups.

RESULTS AND DISCUSSION

Culture of cumulus and fibroblast cells

At the initial stage, cumulus-oocyte complexes (COCs) were isolated from buffalo ovarian follicles of 6-8 mm in diameter. These COCs exhibited well-defined cumulus cell layers surrounding the oocyte, marking the onset of the *in vitro* culture process (Fig 1A). This phase represents the early moments of cell adhesion and the initiation of cellular interactions essential for establishing the culture. After five days of culture, cumulus cells demonstrated significant

Table 1: Primer sequence of aquaporin genes and qPCR conditions.

Gene	Primer sequence	Size (bp)	Annealing temp. (°C)	Accession no.
AQP3	F- TGGATCAAGCTGCCTGTGTA R- ATGTCCAAGTGTCAGAGGG	179	60	NM_001079794.1
AQP4	F- GTTCCAAGTGCCTGTCACTT R- CAATGGGACTTCTGGGGACT	161	60	NM_001317792.2
AQP7	F- CGCAGACAAGGGGAACAAC R- ACCTGAAGACTTGTGAGCCC	179	60	KY067940.1
AQP9	F-TGTTACCATCGCCTCTCTC R-AAGGGCCCACTACAGGAATC	155	60	NM_001205833.3
GAPDH	F- CTGTTGTGGATCTGACCTGC R- CCCCAGCATCGAAGGTAGAA	174	60	GU324291.1

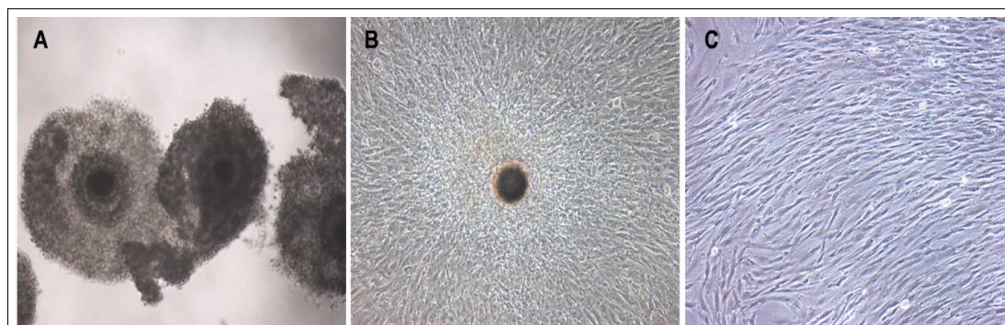


Fig 1: Buffalo cumulus-oocyte complexes (A); Primary culture of COCs at Day-5 (B) and Sub-cultured cumulus cells at confluency (C).

proliferation, forming small cellular clusters indicative of their ability to adhere, interact and proliferate within a controlled *in vitro* environment (Fig 1B). This transition marks the progression from isolated cumulus-oocyte complexes (COCs) to a more structured and integrated cellular network. At the advanced stage of cumulus cell culture, following the removal of the oocyte, the cumulus cells continued to proliferate, reaching 80-100% confluency (Fig 1C). This stage is characterized by robust cell division and extensive coverage of the substrate. The cultured cells were subsequently harvested and reseeded onto Petri dishes for further experimentation and analysis. The cryopreserved fibroblast stock exhibited high post-thaw viability, with over 90% of cells remaining viable (Fig 2A). During the initial culture phase, fibroblasts experienced a brief lag period before adhering to the culture substrate within 24 hours. The morphological assessment confirmed the characteristic spindle-shaped phenotype of fibroblasts. Microscopic examination on day 1 revealed initial cell attachment, with fibroblasts displaying a scattered distribution and minimal cell-cell contact (Fig 2B). At this stage, the average confluency was measured at 5-10%, marking the early phase of adhesion. By day 5, fibroblasts exhibited robust proliferation, forming an extensive interconnected network with abundant cell-cell interactions. Confluency reached approximately 80%, indicating near-complete coverage of the culture substrate (Fig 2C). The

fibroblasts maintained their characteristic spindle-shaped morphology, signifying a healthy and actively proliferating cell population (Tripathi *et al.*, 2024).

Quantitative relative expression of AQP genes

The relative fold change in mRNA expression of *AQP3*, *AQP4*, *AQP7* and *AQP9* in cumulus cells was 1.00 ± 0.00 , 0.76 ± 0.06 , 0.93 ± 0.08 and 0.78 ± 0.03 , respectively. Notably, *AQP4* expression was significantly lower ($P < 0.05$) compared to *AQP3*, which served as the control. However, *AQP4* expression differs significantly ($P > 0.05$) from *AQP3* and *AQP7*. Similarly, the mRNA abundance of *AQP7* shows significant variation ($P > 0.05$) compared to *AQP4* or *AQP9*, but not with *AQP3* in cumulus cells (Fig 3).

Similarly, the relative fold change in mRNA expression of *AQP3*, *AQP4*, *AQP7* and *AQP9* in fibroblast cells was 1.00 ± 0.00 , 0.46 ± 0.14 , 0.28 ± 0.11 and 1.27 ± 0.06 , respectively. The mRNA expression levels of *AQP4* and *AQP7* were significantly lower ($P < 0.05$) compared to *AQP3* and *AQP9*. Additionally, *AQP9* expression was significantly higher than *AQP4* and *AQP7* but did not differ significantly ($P > 0.05$) from *AQP3*, which was used as the control (Fig 4).

The expression levels of aquaporins were compared between cumulus and fibroblast cells, analysis revealed a differential expression pattern between the two cell types. The relative fold change of *AQP3*, *AQP4*, *AQP7* and *AQP9* was 1.00 ± 0.00 , 0.69 ± 0.02 , 0.43 ± 0.09 , 1.90 ± 0.04 and 2.92 ± 0.09 , 1.14 ± 0.12 , 1.39 ± 0.11 , 1.16 ± 0.06 in fibroblast

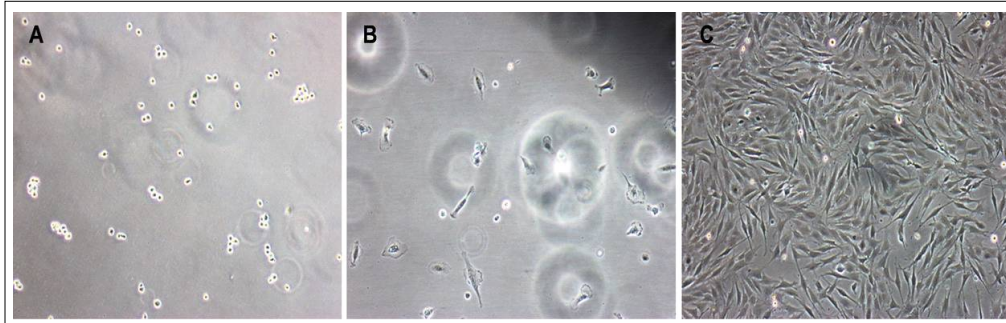


Fig 2: Initial seeding of buffalo fibroblast cells (A); Cultured cells on Day -1 (B) and Fibroblast cells at confluency (C).

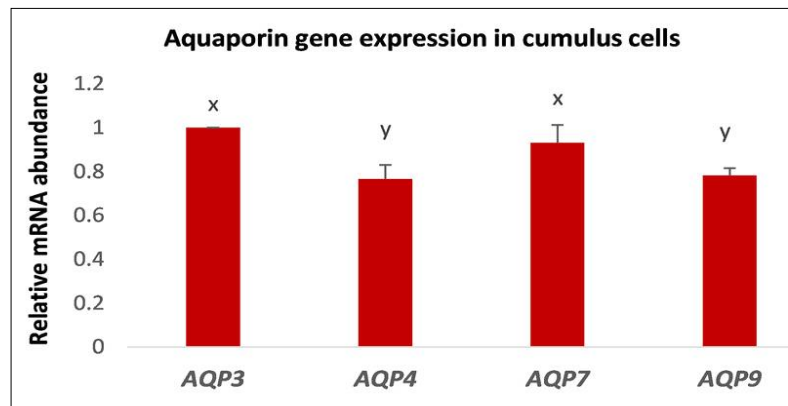


Fig 3: Relative mRNA abundance of aquaporin genes in cumulus cells. Columns with the same superscript do not differ significantly ($P < 0.05$).

and cumulus cells, respectively. Results showed that *AQP3*, *AQP4* and *AQP7* expression were significantly higher in cumulus cells compared to fibroblasts ($P < 0.05$), suggesting a potential role in facilitating water transport during oocyte maturation (Table 2). In contrast, *AQP9* showed no significant difference ($P > 0.05$) between the two cell types, indicating consistent baseline expression across these cell populations (Fig 5).

Aquaporins play a crucial role in regulating water flow and maintaining the structural integrity of cumulus cell

layers in buffaloes. As integral membrane proteins, aquaporins function as water channels, facilitating the efficient movement of water molecules across cell membranes. In this study, the expression levels of *AQP3*, *AQP4* and *AQP7* were significantly higher in buffalo cumulus cells compared to buffalo fetal fibroblast cells, except *AQP9*, which did not follow this pattern. This difference in expression is likely due to the distinct microenvironments in which these cells reside. Cumulus cells are surrounded by follicular fluid in the ovarian follicles, whereas fibroblasts

Table 2: Relative mRNA abundance of *AQP3*, *AQP4*, *AQP7* and *AQP9* genes in buffalo cumulus cells compared to buffalo fetal fibroblast cells.

Gene	Relative mRNA abundance		
	Fibroblast cells	Cumulus cells	Fibroblast vs Cumulus cells
<i>AQP3</i>	1.00±0.00 ^e	1.00±0.00 ^x	1.00±0.00 ^b vs 2.92±0.09 ^a
<i>AQP4</i>	0.46±0.14 ^f	0.76±0.06 ^y	0.69±0.02 ^b vs 1.14±0.12 ^a
<i>AQP7</i>	0.28±0.11 ^f	0.93±0.08 ^x	0.43±0.09 ^b vs 1.39±0.11 ^a
<i>AQP9</i>	1.27±0.06 ^g	0.78±0.03 ^y	1.90±0.40 ^a vs 1.16±0.06 ^a

Means with the different superscript within the first and second columns differ significantly ($P < 0.05$) and means with the different superscript within a row in the third column differ significantly ($P < 0.05$).

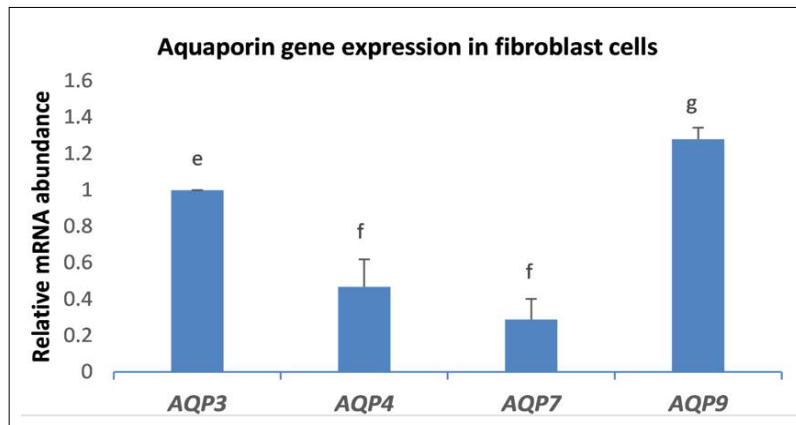


Fig 4: Relative mRNA abundance of aquaporin genes in fibroblast cells. Columns with same superscript do not differ significantly ($P < 0.05$).

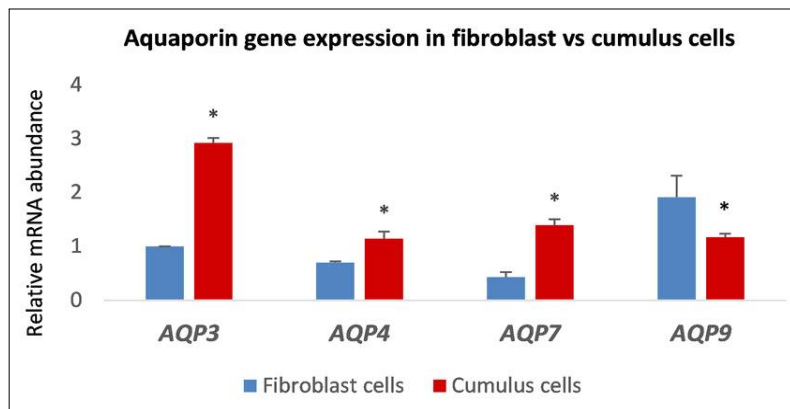


Fig 5: Relative mRNA abundance between fibroblast and cumulus cells within an aquaporin gene differs significantly (*denotes $P < 0.05$).

exist in a more structurally stable, non-fluid environment. Consequently, cumulus cells exhibited higher expression of aquaporin genes than fibroblast cells.

These findings further suggested that aquaporins play an essential role in regulating water transport in cumulus cells. Previous studies have linked increased water influx and elevated *AQP3* expression to the expansion and maintenance of the structural integrity of cumulus cell layers (Petano-Duque *et al.*, 2022). Similarly, the overexpression of *AQP4* and *AQP7* in bovine cumulus cells indicated their involvement in water transport processes within these cells. Additionally, *AQP3* has been identified as a hydrophilic membrane protein in mammalian ovarian cells (Im *et al.*, 2020). The enhanced expression of *AQP3* observed in this study further supports its role in the maturation of ovarian cumulus cells, reinforcing its importance in follicular development and reproductive physiology.

Aquaporin 4 is a crucial water channel protein responsible for regulating water movement across cell membranes. While *AQP4* is primarily expressed in the central nervous system (Benarroch, 2007; Lorente *et al.*, 2023), limited studies have explored its role in oocyte maturation. Notably, *AQP4*-deficient mice exhibited subfertility, characterized by reduced conception rates and a lower number of corpora lutea and antral follicles, indicating ovulation failure. These findings suggest that *AQP4* may play a role in antrum formation during follicular expansion, with its expression in granulosa cells temporarily decreasing during the early ovulatory phase (Sun *et al.*, 2009; Ferre *et al.*, 2023). In this study, *AQP4* expression was significantly higher in bovine cumulus cells compared to buffalo fetal fibroblast cells, further supporting its role in oocyte development. Understanding the regulatory mechanisms governing *AQP4* expression during oocyte maturation remains an important area of investigation. Additionally, *AQP7* expression in granulosa cells has been positively correlated with female fertility and successful folliculogenesis (Lee *et al.*, 2016; Pan *et al.*, 2024). The co-expression of *AQP7* and *AQP9* in mammalian ovarian cells (Ma *et al.*, 2016) suggests their potential involvement in transporting neutral solutes during follicle and oocyte development (Huang *et al.*, 2006). This aligns with the elevated expression of *AQP7* in cumulus cells, which may be necessary for maintaining proper hydration and cellular function during oocyte growth and maturation. The differential expression of *AQPs* in cumulus cells versus fetal fibroblast cells suggests that distinct regulatory mechanisms govern water transport in these cell types. Given their proximity to oocytes, cumulus cells require efficient water influx for optimal function (Jin *et al.*, 2011), whereas fetal fibroblast cells may not have the same demand for enhanced water transport.

CONCLUSION

This study demonstrated that buffalo cumulus cells significantly overexpress *AQP3*, *AQP4* and *AQP7* compared to buffalo fetal fibroblast cells, while *AQP9* expression is lower

in cumulus cells. These findings suggest that *AQPs* are integral to regulating water flow and maintaining the structural integrity of the cumulus cell layers in buffalo oocytes. Further research is needed to elucidate the precise mechanisms through which *AQPs* contribute to reproductive processes and to explore their potential as targets for improving assisted reproductive technologies (ART) in buffaloes and other livestock species. Investigating the functional impact of *AQPs* overexpression on oocyte maturation and developmental competence may provide deeper insights into their biological significance in reproduction.

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Conflict of interest

It is declared that there is no conflict of interest related to the authorship, research, or publication of this manuscript.

REFERENCES

- Agre, P. (2004). Aquaporin water channels. *Bioscience Reports*. **24(3)**: 127-163.
- Benarroch, E.E., (2007). Aquaporin-4, homeostasis and neurologic disease. *Neurology*. **69(24)**: 2266-2268.
- Benga, G. (2009). Water channel proteins (later called aquaporins) and relatives: past, present and future. *IUBMB Life*. **61(2)**: 112-133.
- Cai, L., Yang, B., Zhang, M. and Wang, Y. (2014). Aquaporins in the female reproductive system of mammals. *Frontiers in Biology*. **9(6)**: 422-431.
- Ferre, A., Chauvigne, F., Vlasova, A., Norberg, B., Bargelloni, L., Guigo, R., Finn, R.N. and Cerda, J. (2023). Functional evolution of clustered aquaporin genes reveals insights into the oceanic success of teleost eggs. *Mol Biol Evol*. **40(4)**: msad071.
- Hara-Chikuma, M. and Verkman, A.S. (2008). Aquaporin-3 facilitates epidermal cell migration and proliferation during wound healing. *Journal of Molecular Medicine*. **86**: 221-231.
- Huang, H.F., He, R.H., Sun, C.C., Zhang, Y., Meng, Q.X. and Ma, Y.Y. (2006). Function of aquaporins in female and male reproductive systems. *Human Reproduction Update*. **12(6)**: 785-795.
- Iena, F.M. and Lebeck, J. (2018). Implications of aquaglyceroporin 7 in energy metabolism. *International Journal of Molecular Sciences*. **19(1)**: 154.
- Im, J.W., Lee, C.Y., Kim, D.H. and Bae, H.R. (2020). Differential expression of aquaporin subtypes in the female reproductive tract of mice. *Development and Reproduction*. **24(3)**: 177-185.
- Jin, B., Kawai, Y., Hara, T., Takeda, S., Seki, S., Nakata, Y.I., Matsukawa, K., Koshimoto, C., Kasai, M. and Edashige, K. (2011). Pathway for the movement of water and cryoprotectants in bovine oocytes and embryos. *Biology of Reproduction*. **85(4)**: 834-847.
- Jo, J.W., Jee, B.C., Suh, C.S., Kim, S.H., Choi, Y.M., Kim, J.G. and Moon, S.Y. (2011). Effect of maturation on the expression of aquaporin 3 in mouse oocyte. *Zygote*. **19(1)**: 9-14.

- Lee, H.J., Jee, B.C., Kim, S.K., Kim, H., Lee, J.R., Suh, C.S. and Kim, S.H. (2016). Expressions of aquaporin family in human luteinized granulosa cells and their correlations with IVF outcomes. *Human Reproduction*. **31(4)**: 822-831.
- Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods*. **25(4)**: 402-408.
- Lorente-Martínez, H., Agorreta, A., Irisarri, I., Zardoya, R., Edwards, S.V. and San Mauro, D. (2023). Multiple instances of adaptive evolution in aquaporins of amphibious fishes. *Biology*. **12(6)**: 846.
- Ma, J., Zhou, C., Yang, J., Ding, X., Zhu, Y. and Chen, X. (2016). Expression of AQP6 and AQP8 in epithelial ovarian tumor. *Journal of Molecular Histology*. **47**: 129-134.
- Pan, Q.L., Lin, F.X., Liu, N. and Chen, R.C. (2022). The role of aquaporin 4 (AQP4) in spinal cord injury. *Biomedicine and Pharmacotherapy*. **145**: 112384.
- Pan, Y., Pan, C. and Zhang, C. (2024). Unraveling the complexity of follicular fluid: Insights into its composition, function and clinical implications. *Journal of Ovarian Research*. **17(1)**: 237.
- Petano-Duque, J.M., Castro-Vargas, R.E., Cruz-Mendez, J.S., Lozano-Villegas, K.J., Herrera-Sánchez, M.P., Uribe-García, H.F., Naranjo-Gómez, J.S., Otero-Arroyo, R.J. and Rondón-Barragán, I.S. (2022). Gene expression of aquaporins (AQPs) in cumulus oocytes complex and embryo of cattle. *Animals*. **13(1)**: 98.
- Shah, R.A., George, A., Singh, M.K., Kumar, D., Anand, T., Chauhan, M.S. and Singla, S.K. (2009). Pregnancies established from handmade cloned blastocysts reconstructed using skin fibroblasts in buffalo (*Bubalus bubalis*). *Theriogenology*. **71(8)**: 1215-1219.
- Shangzu, Z., Dingxiong, X., ChengJun, M., Yan, C., Yangyang, L., Zhiwei, L. and Yongqi, L. (2022). Aquaporins: Important players in the cardiovascular pathophysiology. *Pharmacological Research*. **183**: 106363.
- Sun, X.L., Zhang, J., Fan, Y., Ding, J.H., Sha, J.H. and Hu, G. (2009). Aquaporin-4 deficiency induces subfertility in female mice. *Fertility and Sterility*. **92(5)**: 1736-1743.
- Tripathi, G., Gupta, S., Rinka, K., Gupta, T., Selokar, N.L. and Singh, M.K. (2024). Exogenous miR-29b reduces DNA methylation and apoptosis in transgenic cells. *Indian Journal of Animal Research*. 1-7. doi: 10.18805/IJAR.B-5284.
- Tripathi, G., Gupta, S., Rinka, K., Gupta, T., Selokar, N.L. and Singh, M.K. (2025). MicroRNA-449b reduces methylation and apoptosis-related gene expression in fibroblast cells containing human insulin gene. *Indian Journal of Animal Research*. 1-7. doi10.18805/IJAR.B-5478.