Effect of estrous cycle phases on gene expression in bovine oviduct epithelial cells

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Abstract

Background and Aim: The oviduct environment is of particular importance because it is the site of fertilization and early embryo development. The oviduct, as a component of the reproductive system, responds to ovarian hormone (estradiol [E2] and progesterone [P4]) stimuli depending on the estrous cycle phase. This study aimed to elucidate the effect of estrous cycle phases (follicular and early and late luteal phases) on gene expression patterns in bovine oviduct epithelial cells (BOECs).

Materials and Methods: Oviducts were obtained from healthy slaughterhouse animals, corresponding to ipsilateral ovaries with dominant follicles or corpus luteum during early and late luteal phases. BOECs were recovered from the isthmus (IST) and ampulla (AMP), and the expression patterns of genes related to cytokinesis and mitosis mechanisms (rho-associated coiled-coil containing protein kinase and cellular communication network factor 2 [*CCN2*]), growth factors (insulin-like growth factor-binding protein 3, epidermal growth factor receptor [*EGFR*], vascular endothelial growth factor A, and *EGFR*), antioxidant mechanisms (glutathione peroxidase 4 [*GPX4*]), apoptosis (B-cell lymphoma 2), complement component (*C3*), energy metabolism (aldose reductase gene family 1-member b1 [*AKRIB1*] and solute carrier family 2), hormone receptors (estrogen receptor 1 and luteinizing hormone/choriogonadotropin receptor), and specific glycoproteins (oviductal glycoprotein 1) were analyzed.

Results: High P4 levels (late luteal phase) affected the expression of important genes related to antioxidant mechanisms (*GPX4*), energy metabolism (*AKRIB1*), growth factors (*IGBP3* and *EGFR*), and cell growth regulation (*CCN2*) in the AMP. Low P4 levels (early luteal phase) affected the expression of *AKR1B1*, *IGBP3*, and *CCN2*. In addition, estrogen likely had an effect on *OVPGP* expression in the cattle oviduct.

Conclusion: Differential gene expression patterns of BOECs in the AMP during the luteal phase (antioxidant mechanisms, energy metabolism, growth factors, and immunological regulators) and in the IST during the follicular phase (glycoproteins) may influence their renewal and population proportions, modulating the oviduct environment as well as gamete and embryo physiology.

Keywords: bovine oviduct epithelial cells, estrus phase, follicular, gene expression, luteal, oviduct.

Introduction

The bovine oviduct is the part of the female reproductive system where fertilization and early embryo development occur, providing an optimum environment for final oocyte maturation, sperm capacitation, sperm storage, and fertilization as well as gamete and embryo transport [1–3]. The oviduct environment is primarily regulated by oviductal epithelial cell (OEC) secretion and plasma components [4]. OECs are crucial for gamete transport, maintenance, preparation, and early embryo development [5]. OECs can be ciliated or secretory (non-ciliated), and they are distributed in the oviduct ampulla (AMP) and isthmus (IST) [6], with the infundibulum and AMP containing the largest proportion of ciliated OECs [7].

Physiologically, hormones in the circulation reach OECs from the basolateral surface and regulate various cell functions [8]. During the estrous cycle, the oviduct is affected by ovarian sex hormones, mainly estrogens and progesterone, which result in morphological and biochemical changes for maintaining an appropriate environment for gamete and embryonic development [9]. The morphological development and ratio of ciliated and secretory OECs are completed during the estrous cycle. During the luteal phase, goat OEC cell height decreases, mainly in the ciliated cells (CCs) of the entire oviduct [10]. The number of CCs increases during the late luteal phase in the infundibulum and AMP and decreases as it approaches the IST [11]. In particular, protein synthesis in the oviduct peaks during the follicular phase due to estrogens [12],

including the expression of oviductal glycoprotein 1 (*OVGP1*) and progesterone receptor [13].

The environment of the bovine oviduct has been studied *in vitro* and *in vivo*, enabling the characterization of secreted proteins and their roles in the oviduct environment [14]. Regulatory mechanisms for genomic pathways [15] affect capacitation, fertilization capacity, and early embryo development and competence. Schmaltz-Panneau *et al*. [16] found variations in OEC gene transcription in developing embryos. Therefore, studying the oviduct environment is crucial for understanding the underlying regulatory mechanisms that influence embryo development [17].

The use of bovine oviduct epithelial cells (BOECs) provides significant advances in the *in vitro* culture (IVC) systems of bovine embryos [18], with implications for embryo development and competence [19]. These advancements enable the development of further technologies, particularly those related to early embryo development and IVC, and establish the BOEC coculture as an appropriate model for studying the pathways for embryo-maternal communication in bovines [20].

Changes in the activity or abundance of several molecules during the estrous cycle may be crucial for the oviduct environment [21]. These molecules positively affect sperm motility, capacitation, and gamete interaction [22]. Transcriptomic studies detected a relationship between estrous cycle-dependent changes in gene expression levels and morphological changes in BOECs [23, 24]. The gene transcript upregulated in the luteal phase (under progesterone [P4] influence) is associated with the regulation of cell proliferation. In contrast, those upregulated in the follicular phase (under estradiol [E2] influence) are involved in protein modulation [25].

This study aimed to elucidate the differential expression of various BOEC genes during the estrous cycle.

Materials and Methods

Ethical approval

No ethical approval was required for this study because oviduct samples were recovered from bovine females from the slaughterhouse.

Study period and location

The study was carried out from January to August 2021. Molecular experiments were done in the Laboratory of Immunology and Molecular Biology – LIBM of the University of Tolima.

BOEC isolation and preparation

Following slaughter, the female reproductive tract was recovered from healthy animals, and oviducts (ipsilateral) were collected based on ovarian morphology using the criteria described by Ireland *et al*. [26]. Oviducts were separated from connective tissue, ligated, dissected, and washed in phosphate-buffered saline (PBS) before being transported on ice

to the laboratory in PBS with 2% penicillin-streptomycin solution (Sigma®, Germany). Before removing the closure in a laminar flow hood, the oviducts were briefly soaked in ethanol and rinsed in PBS. The oviduct was divided into two equal segments (AMP and IST), and each segment was gently squeezed in a stripping motion using a sterile glass slide. The oviduct content (yellowish paste) was washed twice with PBS by centrifugation at 300× g for 10 min [27] and then collected in a vial for RNA extraction. The ovarian structures identified for oviduct classification were as follows: (a) Dominant follicle at the follicular phase; (b) hemorrhagic corpus luteum at the early luteal phase; and (c) total corpus luteum at the mid-luteal phase of the estrous cycle [26].

Experimental design

Gene expression analyses were performed in the oviduct AMP and IST during the early luteal phase on days 2–6 (hemorrhagic corpus luteum, early), the mid-luteal phase on days 6–17 (functional corpus luteum, late), and the follicular phase on days 17–21 (follicular).

RNA extraction and cDNA synthesis

For each gene, a set of primers was designed usingGeneious Prime v2022.1 (https://www.geneious.com/prime/), according to Kearse *et al*. [28] (Table-1). Total RNA was extracted using the TRIzol method (Thermo Fisher Scientific, USALife Technologies; Cat# 15596018) and cDNA was synthesized using the SuperScript® IV First-Strand Synthesis System (Thermo Fisher Scientific, USA). The relative expression of genes of interest was normalized using glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as a reference gene and calculated using the $2^{-\Delta\Delta Ct}$ method [29]. Quantitative real-time polymerase chain reaction (qPCR) was performed using QuantStudio 3 (Thermo Fisher Scientific); the Luna Universal qPCR Master Mix (New England Biolabs, USA) was used. Melting curve analysis was performed to rule out gDNA contamination and non-specific primer annealing.

Statistical analysis

Data were analyzed using descriptive statistics and normality tests. Gene expression levels were compared among early, late, and follicular phases of the estrous cycle in AMP and IST using a one-way analysis of variance with Tukey's test as *post hoc*. Statistical analyses were performed using the GraphPad Prism software v8.0 for MacOS (Dotmatics; La Jolla, CA, USA). Differences with $p < 0.05$ were considered statistically significant.

Results

The transcript levels of glutathione peroxidase 4 (*GPX4*), aldose reductase gene family 1-member b1 (*AKRIB1*), cellular communication network factor 2 (*CCN2*) were significantly higher ($p < 0.01$), same as insulin-like growth factor-binding protein 3 (*IGFBP3*), and epidermal growth factor receptor

Table-1: Sequence of primers used for qPCR.

Gene name	Primer sequence (5´ - 3´)	Amplicon size (bp)	GenBank accession no.
ROCK2			
F	GCCTTGGAAGAAACGGGGTA	191	NM_174452.2
R	AGCCTTGGGAATTGGGAAGG		
GPX4			
F	CAAGCCTGTTGCCTGTGTTC	173	NM_001346430.1
R	TATTCCCACAAGGCAGCCAG		
IGFBP3			
F	GTCGGAAGAAGACCACAGCA	178	NM_174556.1
R	CTGGGTGTCTGTGCTCTGAG		
ESR1			
F	AGGCAGAGAATTTCCCCAGC	175	NM_001001443.1
R	GCGAATGAATGGCCATCCAC		
VEGFA			
F	CAAACCTCACCAAAGCCAGC	186	NM_001316955.1
R	CGCGAGTCTGTGTTTTTGCA		
OVGP1			
F	GCTGTCCACGTTTTCCAACC	192	NM_001080216.1
R	GTGAGCTGGGCCTCATTCTT		
C3			
F	CACTGTTGACCACAAGCTGC	95	NM_001040469.2
R	AAGACTTGGAGTCCCGCTTG		
EGFR			
F	ACTCTGATGCTGGGAGGGAT	126	XM_002696890.5
R	CCTGTCCATCACCAACTCCC		
LHCGR			
F	GAGAAGATGCACAACGACGC	193	NM_174381.1
R	AAGTCAGTGTGGCATCCAGG		
SLC2A1			
F	TGGGAGGCATGATTGGTTCC	157	NM_174602.2
R	CGACCCAGGATCAGCATCTC		
BCL2L2			
F	CGTGTGAGGGTGACGTGTAA	155	NM_001076533.1
R	CACCCATGCAAACAGTGTGG		
AKR1B1			
F	TAATTTGGCCCGTGTCCCTC	163	NM_001012519.1
R	TTGCATGTTCCCCAGATCCC		
CCN2			
F	TCTTCGGAGTGTGCACAGAC	134	NM_174030.2
R	GTAATGGCAGGCACAGGTCT		
GAPDH			
F	GGGATGAGGCTCAGAGCAAGAGA	118	NM_173979.3
R	AGCTCGTTGTAGAAGGTGTGGTGCC		

qPCR=Quantitative real-time polymerase chain reaction

(*EGFR*) levels which were higher in the AMP (p < 0.05) in the late luteal phase (late) than in the follicular phase (follicular), similar to transcript levels patterns of *GPX4* and *EGFR* that were higher in late compared to the early luteal phase (early) (p < 0.05). Oviductal glycoprotein 1 (*OVGP1*) and complement C3 (C3) were significantly higher (p < 0.01) in the follicular phase than in the late and early phases. The early phase showed a higher *AKR1B1* (p < 0.05), and significantly higher *IGFBP3*, and *CCN2* (p < 0.01) expression than the follicular phase, whereas no significant differences in Rho-associated coiled-coil containing protein kinase (*ROCK*), B-cell lymphoma 2 like 2 (*BCL2L2*), luteinizing hormone/choriogonadotropin receptor (*LHCGR*), solute carrier family 2 (*SLC2A1*), vascular endothelial growth factor A (*VEGFA*), and estrogen receptor 1 (*ESR1*) expression (Figure-1) were observed. In the IST, *OVGP1* expression was higher in the late phase than in the early and follicular phases (p < 0.05). However, *ROCK*, *GPX4*, *BCL2L2*, *AKRIB1*, *LHCGR*, *SLC2A1*, *IGFBP3*, *VEGFA*, *EGFR*, *C3*, and *CCN2* expression did not differ significantly between phases (Figure-2).

Discussion

The oviduct is a dynamic organ that is anatomically divided into three different parts: The infundibulum, the AMP, and the IST, and it has the potential to provide a suitable microenvironment for gametes and embryo development [30]. The previous studies have demonstrated that genes are differentially expressed between oviductal regions and that it is related to the estrous cycle [23, 30, 31], including variations in oviduct epithelial transcription [32]. Our findings indicate that gene expression in BOECs differs between the AMP and IST as well as among the follicular, early, and late estrous phases. This differential gene expression may be related to BOEC functional differentiation in
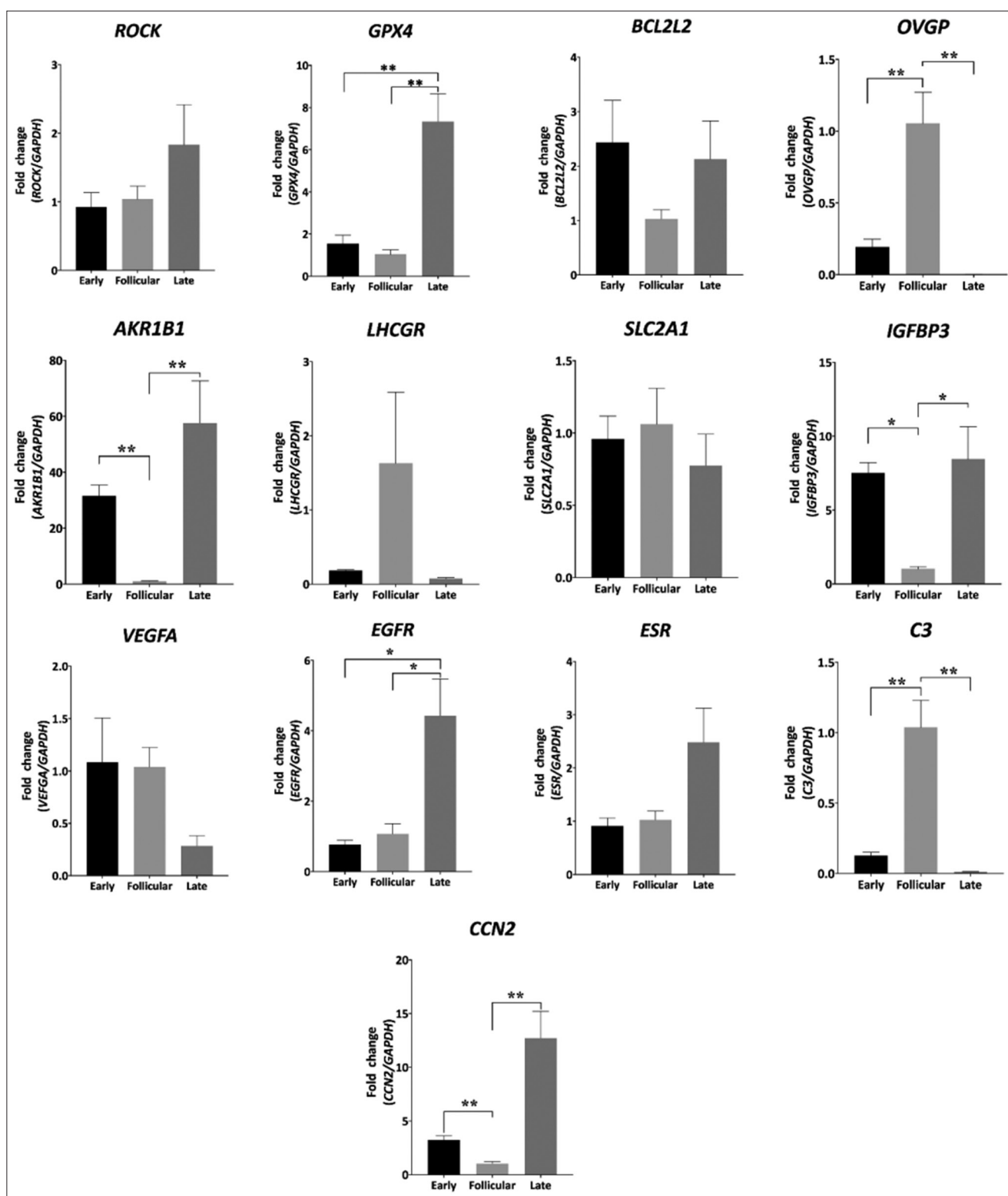
Figure-1: Gene expression patterns of different genes related to cytokinesis and mitosis mechanisms (*ROCK*, rho-associated coiled-coil containing protein kinase; *CCN*, Cellular communication network), growth factors (*IGFBP3*, insulin-like growth factor-binding protein 3; *EGFR,* epidermal growth factor receptor; *VEGFA*, vascular endothelial growth factor A), antioxidants mechanisms (*GPX4,* glutathione peroxidase 4), apoptosis (*BCL2,* B-cell lymphoma 2), complement component (*C3*), energetic metabolism (*AKRIB1*, aldose reductase gene family 1-member b1; *SLC2A1*, solute carrier family 2, facilitated glucose transporter member 1), hormones receptors (*ESR*, estrogen receptor; *LHCGR*, luteinizing hormone/choriogonadotropin receptor), and specific glycoproteins (*OVGP*, oviduct-specific glycoprotein) on oviduct ampulla, during different estrous cycle phases. Early: Early luteal phase, Late: Mid-luteal phase, Follicular: Follicular phase. Data are shown as mean ± SEM. *p < 0.05; **p < 0.01. SEM=Standard error of the mean.

each oviduct region. Thus, understanding specific regulatory mechanisms that are affected under the influence of E2 and P4 may help expand the knowledge of oviductal physiology, including signaling pathways and their modulation in the oviductal environment, to enhance the *in vitro* embryo culture environment.
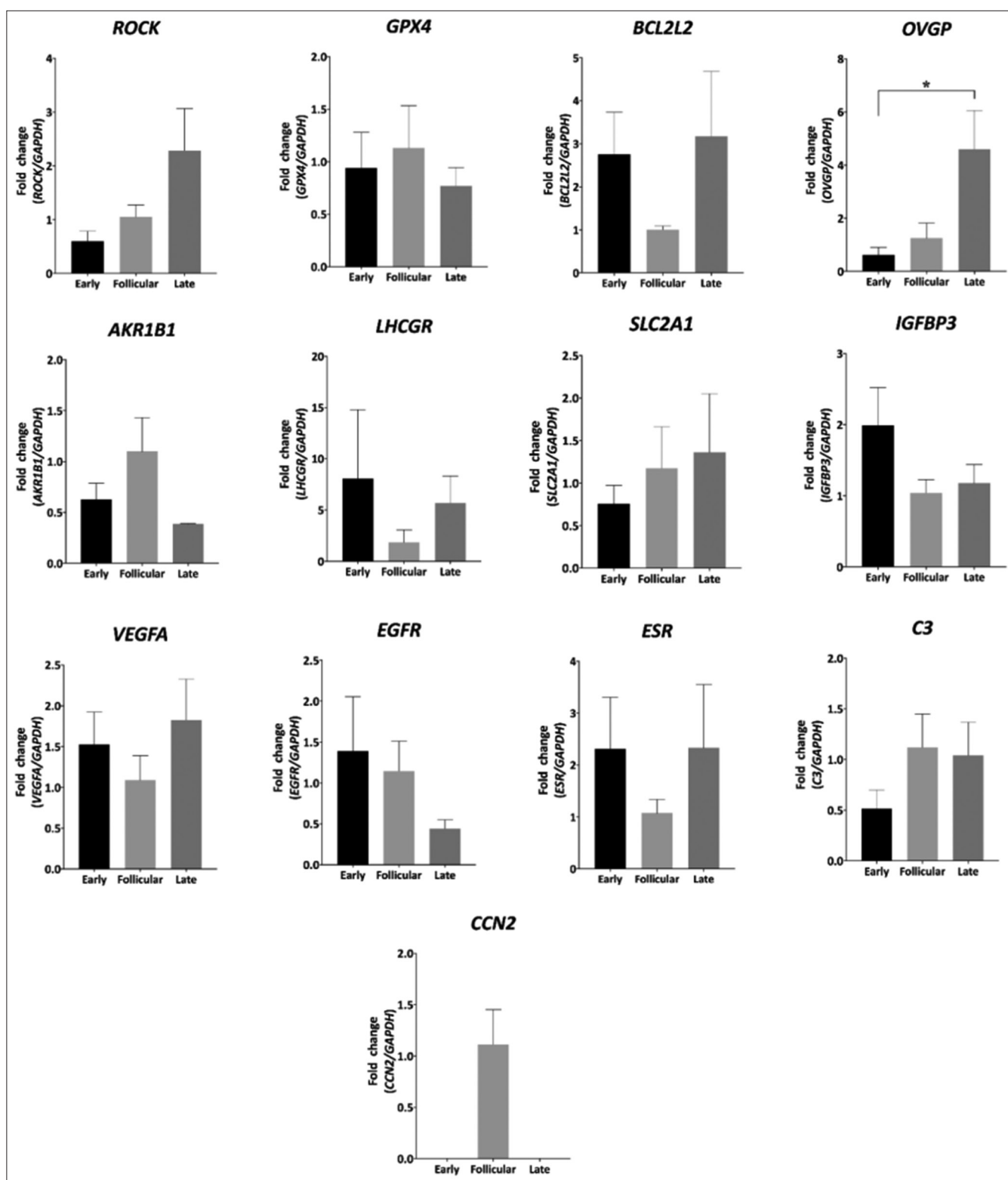
Figure-2: Gene expression patterns of different genes related to cytokinesis and mitosis mechanisms (*ROCK*, rho-associated coiled-coil containing protein kinase; *CCN*, Cellular communication network), growth factors (*IGFBP3*, insulin-like growth factor-binding protein 3; *EGFR,* epidermal growth factor receptor; *VEGFA*, vascular endothelial growth factor A), antioxidants mechanisms (*GPX4,* glutathione peroxidase 4), apoptosis (*BCL2,* B-cell lymphoma 2), complement component (*C3*), energetic metabolism (*AKRIB1*, aldose reductase gene family 1-member b1; *SLC2A1*, solute carrier family 2, facilitated glucose transporter member 1), hormones receptors (*ESR,* estrogen receptor; *LHCGR*, luteinizing hormone/choriogonadotropin receptor), and specific glycoproteins (*OVGP*, oviduct-specific glycoprotein) on oviduct isthmus, during different estrous cycle phases. Early: Early luteal phase, Late: Mid-luteal phase, Follicular: Follicular phase. Data are shown as mean ± SEM. *p < 0.05; **p < 0.01. SEM=Standard error of the mean.

The oviduct's function is dependent on its OECs, which are classified into CCs andsecretory cells (SCs). SCs have secretory activity and may be larger than CCs. They exude nutrients into the oviduct lumen for gamete and embryo development and form cellular protrusions that increase in size and number

as the estrous cycle progresses [33–35]. In particular, when oocytes are in the AMP after ovulation, SCs exhibit morphological changes indicative of increased activity [36], implying a link between SC morphology and endocrine levels during the estrous cycle [37]. On the other hand, CCs have hair-like projections (cilia) that generate a ciliary beating and sweep mucus and other debris off the epithelium and oviduct lumen. Changes in cilia orientation and beat frequency can modify oviduct fluid flow, which could affect gamete and embryo transport [38].

Ovarian steroids (E2 and P4) regulate oviductal function, particularly gamete and early embryo transport, by modulating SC and CC physiology [39]. E2 increases SC proliferation, secretory activity, and CC transport velocity, whereas P4 decreases SC secretory activity and CC transport velocity while initiating a process of self-renewal of the BOECs for the subsequent estrous cycle. This BOEC renewal, which is required for proper maternal receptivity, is regulated by proliferation, differentiation, and apoptosis [40].

This study analyzed ROCK and CCN2 gene transcripts, which are related to OEC renewal. Rho-associated coiled-coil containing protein kinase (ROCK) regulates cell division, cell-matrix adhesions, and the activation of the c-Fos serum response element [41]. Our findings indicate that the estrous phase and oviduct region have no effect on ROCK expression patterns, implying the absence of Rho-dependent kinase mechanisms during different estrous phases. On the other hand, CCN2 mRNA levels were upregulated in the AMP (late-early), with no differential expression in the IST, which may be related to P4 secretion. Cyclin B1 (CCN) is a regulatory protein that is involved in both the oocyte cell cycle and embryonic development. It assembles a complex with p34 (cyclin-dependent kinase 1) to form mitosis-promoting factor, which is required for the proper control of the G2/M transition phase of the cell cycle [42]. Genistein, which induces cell cycle arrest, downregulates CCN and BCL-2/BAX expression in BOEC primary cultures, resulting in reduced cellular proliferation and migration [43]. In mice, maternal factors and ovarian steroids regulate CCN2 (CTGF) and transforming growth factor-beta 1 expression in the uterus [44]. The differential expression of both genes reflects the dynamics of cytokinetic processes during the estrous cycle phases in oviduct regions.

Growth factors play a pivotal role in the oviduct microenvironment, modulating cell proliferation, wound healing, and cellular differentiation [45]. In this study, IGFBP3, EGFR, and VEGFA gene expression were analyzed. IGFBP3 is an IGF-binding protein [46]; in BOECs expressing IGFBP3, particularly in the IST, it may help create a synchronized IGF-I and IGF-II gradient along the oviduct as the embryo passes through it [47, 48]. BOEC IGFBP3 secretion increases in response to early embryo development in a conditioned medium [49]. Our findings suggest that this growth factor receptor in the AMP may be responsive to P4, exhibiting increased expression during the early and late luteal phases, corresponding to the early embryo development till day 4.

These patterns were similar to those of the EGFR, where P4 (late-early) had an effect on the AMP. EGFR is a transmembrane glycoprotein of the protein kinase superfamily [50] that binds to EGF, promoting receptor dimerization and tyrosine auto-phosphorylation, which promotes cell proliferation [51]. It may also be involved in the regulation of the bovine oviductal microenvironment [52] by modulating OEC renewal and oviduct lumen characteristics by controlling apoptotic gene expression (BAX and BCL-2) [53]. The mid-luteal stage exhibits enhanced EGF secretion and EGFR mRNA expression in the bovine endometrium, which may regulate uterine function [54].

Angiogenesis plays an important role in cyclical mechanisms during the estrous cycle, such as follicular growth and corpus luteum formation, which are dependent on the development of a dense capillary net [55], and transudation from plasma contributes to the defining characteristics of the luminal microenvironment in cattle, such as uterine and oviductal fluid [56]. VEGFA is a member of the platelet-derived VEGF family that encodes a heparin-binding protein, which promotes vascular endothelial cell proliferation and migration [57]. Our findings indicate that the estrous phase and oviduct region have no effect on the expression patterns of this gene. However, a previous study in heifers demonstrated that low follicle count downregulated VEGFA expression in ipsilateral oviducts compared to the contralateral IST [58]. VEGF and its receptors, flk-1 and flt-1a, are secreted in the bovine oviduct [59], where they have autocrine and paracrine effects on BOECs, influencing physiological functions through permeability modifications. VEGFs, which are angiogenic in nature, are associated with vascular support in the oviduct, being directly associated with OEC support and oviductal fluid perfusion.

The redox state of the bovine oviduct is pivotal for gamete physiology and embryo survival, and antioxidant expression has been reported [60, 61]. GPX4 catalyzes the reduction of hydrogen peroxide and organic and lipid hydroperoxides, thereby protecting cells from oxidative stress [62]. GPX4 is also essential for sperm development; consequently, it is classified as a "moonlighting" protein based on its ability to function as both a peroxidase and a structural protein in mature spermatozoa [63]. GPX4 transcript levels decreased in BOEC cultured embryos [49] but increased in the IST ipsilateral to the dominant follicle during the follicular phase and in the post-ovulatory period [64]. Our findings suggest that P4 levels (late) have an effect on GPX4 expression in the AMP. Previously, a significantly increased transcript

abundance of *GPX4* in ipsilateral BOECs was observed in the early luteal phase [27], which is consistent with our findings. On the other hand, apoptosis mechanisms are well known in gametes and early embryos and are directly linked to their viability. Therefore, the presence of these factors in the oviduct environment should be investigated. An apoptosis regulator, B-cell lymphoma 2 (*BCL2*) control outer mitochondrial membrane permeabilization (MOMP) protein that regulates apoptosis mitochondrial pathway [65]. In contrast to the findings of *GPX4*, the estrous phase and oviduct region had no effect on *BCL2* expression patterns in our study. Recently, researchers found that increased *BCL2* and *BAX* mRNA expression in BOEC conditioned media and exosomes influence apoptosis in embryos [5]. In other mammals, apoptosis levels are P4 dependent (caudal oviduct and uterotubal junction) and can affect spermatozoa bound to OECs as well as the fertilization process [66].

The C3a peptide, also known as C3a anaphylatoxin, is a complement component that regulates inflammation and has antimicrobial effects [67]. Complement proteins have been suggested to mediate functional homeostasis, which has been linked to physiological aspects of cattle oviduct and fertility [56], sperm-oocyte interaction [68], and embryo development [69]. In rodents, C3 is secreted by OECs and increases trophectoderm development, blastocyst size, and hatching rates [70, 71]. Our findings suggest that C3 transcription levels in the oviduct AMP may be influenced by E2 levels.

Glucose is an essential energy source for mammalian pre-implantation embryo development, and oviduct glucose and lactate concentrations have been linked to oviduct and epithelial differential regulation [72]. Glucose and lactate levels are important to gamete and early embryo processes, and multiple genes are involved in regulatory mechanisms. The *AKRIB1* is a key enzyme that catalyzes the reduction of several aldehydes as well as steroid metabolism [73], including P4 (for implantation) [74] and prostaglandin F2α synthesis (luteolysis and completing pregnancy). The increased *AKRIB1* expression in our study indicates a possible effect of P4 on the AMP during late and early phases, which was not evident on the IST, and can influence the oviductal environment, including early embryos in transit during the first stages of development. In bovines, *AKR1B1* upregulation in blastocyst biopsies influences the pregnancy absence and endometrial resorption during luteolysis, and it has been related to early embryo pro-apoptotic pathways [75]. Similarly, solute carrier family 2 (*SLC2A1*), facilitated glucose transporter member 1 (SLC2A1), or GLUT1, is a major glucose transporter in the mammalian blood–brain barrier, mammary gland, kidney, muscle [76], fetus [77], and ovary [78], where it is responsible for maintaining basal glucose [79]. In the present study, *SLC2A*1 mRNA level did not vary between the estrous phase and the oviduct region, contrasting with

previous studies where *SLC2*A showed differential expression in murine early embryos and laying hen oviducts based on glucose metabolism [80, 81].

E2 has an effect on several regions of the reproductive tract, including the oviduct, and its receptors are essential for sexual development and reproductive activity [82]. E2 concentrations during the estrous phase suggest that a large amount of E2 is delivered to the oviduct [83]. Estrogen receptor (*ESR*) is a ligand-activated transcription factor composed of several domains essential for E2 hormone-binding, DNA-binding, and transcription activation [84]. In particular, *ESR1* is highly expressed in the IST during the follicular phase [85]. In mice, disrupting the E2 signal in OECs causes defective embryo transport, implying that the E2 signal through *ESR1* is necessary for embryo transport in the oviduct. In humans, increased pre-ovulatory estradiol levels act through epithelial ERα, suppressing protease-mediated aspects of innate immunity in the oviduct, influencing the mucosal immunological barrier to support fertilization and embryo development [86]. However, the *ESR* mRNA levels in BOECs in our study were comparable between estrous phases in both oviduct regions, suggesting that receptor expression in BOECs is stable regardless of the estrous cycle phase.

The LHCGR belongs to the G-protein coupled receptor 1 family and its function is regulated by G proteins that activate adenylate cyclase [87]. Alterations in this gene influence the appearance of secondary sexual characteristics in males, such as familial male early puberty (testotoxicosis), secondary hypogonadism, Leydig cell adenoma (early puberty), and hypoplasia (male pseudohermaphroditism). However, BOEC luteinizing hormone: Human chorionic gonadotropin (LH: hCG) under exogenous LH: hCG promotes *OGP* expression by increasing transcript stability [88]. *LHCGR* expression levels on BOECs are reported for the first time in this study, and it is suggested that the estrous phase and oviduct region have no impact on its expression patterns.

Oviduct-specific glycoprotein (*OVGP*) is related to the mucin and glycosyl hydrolase 18 gene families. *OVGP* expression and protein secretion occur during final follicular development through cleavage-stage embryo development, suggesting that *OVGP* expression is E2 dependent. *OVGP* is secreted from SCs and associates with ovulated oocytes, blastomeres, and spermatozoan acrosome regions. *OVGP1* mRNA expression and its relationship with cell *in vivo* behavior constitute a marker for functionality [22], whereas it indicates OEC physiological phenotype under *in vitro* conditions [89]. In our study, *OVGP* expression was upregulated in the oviduct AMP and IST in response to E2 and P4. The effect of E2 on *OVGP* expression is comparable to an *in vivo* study finding, in which BOECs showed

increased *OVGP1* mRNA expression in response to E2 [89], although the effect of P4 on *OVGP* expression was not described. However, due to the lack of significant variations in *ESR* expression, a clear association between this type of interaction and *OVGP* expression cannot be established.

Understanding the physiological events of the female reproductive tract necessitates an understanding of the expression patterns of functions that elucidate the dynamics of the oviductal environment during the estrous cycle, gamete transport, and early embryo development.

Conclusion

The luteal phase (early and late) of the estrous cycle affected gene expression patterns, which were reflected in antioxidant mechanisms (*GPX4*), energy metabolism (*AKRIB1*), growth factors (*IGFBP3*, *EGFR*), and cell adhesion - communication (*CCN2*) in the oviductal AMP, while the follicular phase influenced the gene expression of an essential glycoprotein, *OVGP*, in the oviductal IST. The dynamics of specific mechanisms in OECs, both in the AMP and IST, influence oviductal epithelial renewal and population proportions, which further modify oviductal lumen conditions, as well as oviductal fluid secretions and volume, which influence gamete and embryo physiology during transit.

Authors' Contributions

RL, IR, and FU: Conceived and designed the study. RL, IR, and FU: Performed the experiments. IR, RL, and FU: Analyzed the data. RL and IR: Drafted the manuscript. All authors have read and approved the final manuscript.

Acknowledgments

Competing Interests

The authors declare that they have no competing interests.

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References

1. Brüssow, K.P., Rátky, J. and Rodriguez-Martinez, H. (2008) Fertilization and early embryonic development in the porcine fallopian tube. *Reprod. Domest. Anim.*, 43(Suppl 2): 245–251.
2. Holt, W.V. and Fazeli, A. (2016) Sperm selection in the female mammalian reproductive tract. Focus on the oviduct: Hypotheses, mechanisms, and new opportunities. *Theriogenology*, 85(1): 105–112.
3. Hunter, R, (2003) Reflections upon sperm-endosalpingeal and sperm-zona pellucida interactions *in vivo* and *in vitro*. *Reprod. Domest. Anim.*, 38(2): 147–154.
4. Leese, H.J., Tay, J.I., Reischl, J. and Downing, S.J. (2001) Formation of fallopian tubal fluid: Role of a neglected epithelium. *Reproduction*, 121(3): 339–346.
5. Sidrat, T., Khan, A.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.*, 21(20): 7589.
6. Sostaric, E., Dieleman, S.J., van de Lest, C.H.A., Colenbrander, B., Vos Nuria Garcia-Gil P.L.A. and Gadella, B.M. (2008) Sperm binding properties and secretory activity of the bovine oviduct immediately before and after ovulation. *Mol. Reprod. Dev.*, 75(1): 60–74.
7. Ardon, F., Markello, R.D., Hu, L., Deutsch, Z.I., Tung, C.K., Wu, M. and Suarez, S.S. (2016) Dynamics of bovine sperm interaction with epithelium differ between oviductal isthmus and ampulla1. *Biol. Reprod.*, 95(4): 90.
8. Hunter, R.H.F. (2012) Components of oviduct physiology in eutherian mammals. *Biol. Rev.*, 87(1): 244–255.
9. Barton, B., Herrera, G., Anamthathmakula, P., Rock, J., Willie, A., Harris, E., Takemaru, K.I. and Winuthayanon, W. (2020) Roles of steroid hormones in oviductal function. *Reproduction*, 159(3): R125–R137.
10. Abe, H., Onodera, M., Sugawara, S., Satoh, T. and Hoshi, H. (1999) Ultrastructural features of goat oviductal secretory cells at follicular and luteal phases of the oestrous cycle. *J. Anat.*, 195(Pt 4): 515–521.
11. Yániz, J.L., Lopez-Gatius, F., Santolaria, P. and Mullins, K.J. (2000) Study of the functional anatomy of bovine oviductal mucosa. *Anat. Rec.*, 260(3): 268–278.
12. Buhi, W.C., Alvarez, I.M. and Kouba, A.J. (2000) Secreted proteins of the oviduct. *Cells Tissues Organs*, 166(2): 165–179.
13. Ulbrich, S.E., Kettler, A. and Einspanier, R. (2003) Expression and localization of estrogen receptor α, estrogen receptor β and progesterone receptor in the bovine oviduct *in vivo* and *in vitro*. *J. Steroid Biochem. Mol. Biol.*, 84(2): 279–289.
14. Aviles, M., Coy, P. and Rizos, D. (2015) The oviduct: A key organ for the success of early reproductive events. *Anim. Front.*, 5(1): 25–31.
15. Maillo, V., Gaora, P.Ó., Forde, N., Besenfelder, U., Havlicek, V., Burns, G.W., Spencer, T.E., Gutierrez-Adan, A., Lonergan, P. and Rizos, D. (2015) Oviduct-embryo interactions in cattle: Two-way traffic or a one-way street? *Biol. Reprod.*, 92(6): 144.
16. Schmaltz-Panneau, B., Cordova, A., Dhorne-Pollet, S., Hennequet-Antier, C., Uzbekova, S., Martinot, E., Doret, S., Martin, P., Mermillod, P. and Locatelli, Y. (2014) Early bovine embryos regulate oviduct epithelial cell gene expression during *in vitro* co-culture. *Anim. Reprod. Sci.*, 149(3–4): 103–116.
17. Besenfelder, U., Brem, G. and Havlicek, V. (2020) Review: Environmental impact on early embryonic development in the bovine species. *Animal*, 14(S1): s103–s112.
18. Gandolfi, F. and Moor, R.M. (1987) Stimulation of early embryonic development in the sheep by coculture with oviduct epithelial cells. *J. Reprod. Fertil.*, 81(1): 23–28.
19. Cordova, A., Perreau, C., Uzbekova, S., Ponsart, C., Locatelli, Y. and Mermillod, P. (2014) Development rate and gene expression of IVP bovine embryos cocultured with bovine oviduct epithelial cells atearly or late stage of preimplantation development. *Theriogenology*, 81(9): 1163–1173.
20. Wolf, E., Arnold, G., Bauersachs, S., Beier, H., Blum, H., Einspanier, R., Fröhlich, T., Herrler, A., Hiendleder, S., Kölle, S., Prelle, K., Reichenbach, H.D., Stojkovic, M., Wenigerkind, H. and Sinowatz, F. (2003) Embryo-maternal communication in bovine strategies for deciphering

a complex cross-talk. *Reprod. Domest. Anim.*, 38(4): 276–289.

21. Boice, M.L., McCarthy, T.J., Mavrogianis, P.A., Fazlebas, A.T. and Verhage, H.G. (1990) Localization of oviductal glycoproteins within the zona pellucida and perivitelline space of ovulated ova and early embryos in baboons (*Papio anubis*). *Biol. Reprod.*, 43(2): 340–346.

22. Buhi, W.C. (2002) Characterization and biological roles of oviduct-specific, estrogen-dependent glycoprotein. *Reproduction*, 123(3): 355–362.

23. Cerny, K.L., Garrett, E., Walton, A.J., Anderson, L.H. and Bridges, P.J. (2015) A transcriptome analysis of bovine oviductal epithelial cells collected during the follicular phase versus the luteal phase of the estrous cycle. *Reprod. Biol. Endocrinol.*, 13: 84.

24. Gonella-Diaza, A.M., da Andrade, S.C.S., Sponchiado, M., Pugliesi, G., Mesquita, F.S., Van Hoeck, V., de Francisco Strefezzi, R., Gasparin, G.R., Coutinho, L.L. and Binelli, M. (2015) Size of the ovulatory follicle dictates spatial differences in the oviductal transcriptome in cattle. *PLoS One*, 10(12): e0145321.

25. Bauersachs, S., Blum, H., Mallok, S., Wenigerkind, H., Rief, S., Prelle, K. and Wolf, E. (2003) Regulation of ipsilateral and contralateral bovine oviduct epithelial cell function in the postovulation period: A transcriptomics approach. *Biol Reprod.*, 68(4): 1170–1177.

26. Ireland, J.J., Murphee, R.L. and Coulson, P.B. (1980) Accuracy of predicting stages of bovine estrous cycle by gross appearance of the corpus luteum1. *J. Dairy Sci.*, 63(1): 155–160.

27. Rottmayer, R., Ulbrich, S.E., Kölle, S., Prelle, K., Neumueller, C., Sinowatz, F., Meyer, H.H.D., Wolf, E. and Hiendleder, S. (2006) A bovine oviduct epithelial cell suspension culture system suitable for studying embryo-maternal interactions: Morphological and functional characterization. *Reproduction*, 132(4): 637–648.

28. Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. and Drummond, A. (2012) Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12): 1647–1649.

29. Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods*, 25(4): 402–408.

30. Maillo, V., de Frutos, C., O'Gaora, P., Forde, N., Burns, G.W., Spencer, T.E., Gutierrez-Adan, A., Lonergan, P. and Rizos, D. (2016) Spatial differences in gene expression in the bovine oviduct. *Reprod. Camb. Engl.*, 152(1): 37–46.

31. Swangchan-Uthai, T., Walsh, S.W., Alexander, S.L.H., Cheng, Z., Crowe, M.A., Evans, A.C.O. and Wathes, D.C. (2011) Comparison of mRNA for IGFs and their binding proteins in the oviduct during the peri-oestrous period between dairy heifers and lactating cows. *Reprod. Camb. Engl.*, 142(3): 457–465.

32. Bauersachs, S., Rehfeld, S., Ulbrich, S.E., Mallok, S., Prelle, K., Wenigerkind, H., Einspanier, R., Blum, H. and Wolf, E. (2004) Monitoring gene expression changes in bovine oviduct epithelial cells during the oestrous cycle. *J. Mol. Endocrinol.*, 32(2): 449–466.

33. Eriksen, T., Terkelsen, O., Hyttel, P. and Greve, T. (1994) Ultrastructural features of secretory cells in the bovine oviduct epithelium. *Anat. Embryol. (Berl)*, 190(6): 583–590.

34. Abe, H. and Hoshi, H. (1997) Bovine oviductal epithelial cells: Their cell culture and applications in studies for reproductive biology. *Cytotechnology*, 23(1–3): 171–183.

35. Hunter, R.H. and Wilmut, I. (1984) Sperm transport in the cow: Peri-ovulatory redistribution of viable cells within the oviduct. *Reprod. Nutr. Dev.*, 24(5A): 597–608.

36. Shirley, B. and Reeder, R.L. (1996) Cyclic changes in the ampulla of the rat oviduct. *J. Exp. Zool.*, 276(2): 164–173.

37. Ayen, E., Shahrooz, R. and Kazemie, S. (2012) Histological and histomorphometrical changes of different regions of oviduct during follicular and luteal phases of estrus cycle in adult Azarbaijan buffalo. *Iran. J. Vet. Res.*, 13(1): 42–48.

38. Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D., Darnell, J. (2000) Molecular Cell Biology. 4th ed. WH Freeman, New York.

39. Olsen, S.L., Li, S. and Winuthayanon, W. (2018) Embryo transport. In: Skinner, M.K., editor. Encyclopedia of Reproduction. 2nd ed. Academic Press, Oxford. p357–363. Available from: https://www.sciencedirect.com/science/article/pii/B9780128012383644896. Retrieved on 23-06-2021.

40. Morris, D. and Diskin, M. (2008) Effect of progesterone on embryo survival. *Anim. Int. J. Anim. Biosci.*, 2(8): 1112–1119.

41. Julian, L. and Olson, M.F. (2014) Rho-associated coiled-coil containing kinases (ROCK). *Small GTPases*, 5(2): e29846.

42. Li, J., Tang, J.X., Cheng, J.M., Hu, B., Wang, Y.Q., Aalia, B., Li, X.U., Jin, C., Wang, X.X., Deng, S.l., Zhang, Y., Chen, S.R., Qian, W.P., Sun, Q.Y., Huang, X.X. and Liu, YX. (2018) Cyclin B2 can compensate for cyclin B1 in oocyte meiosis I. *J. Cell Biol.*, 217(11): 3901–3911.

43. García, D.C., Valdecantos, P.A., Miceli, D.C. and Roldán-Olarte, M. (2017) Genistein affects proliferation and migration of bovine oviductal epithelial cells. *Res. Vet. Sci.*, 114: 59–63.

44. Rageh, M.A., Moussad, E.E., Wilson, A.K. and Brigstock, D.R. (2001) Steroidal regulation of connective tissue growth factor (CCN2; CTGF) synthesis in the mouse uterus. *Mol. Pathol.*, 54(5): 338–346.

45. Stone, W.L., Leavitt, L. and Varacallo, M. (2021) Physiology, Growth Factor. StatPearls Publishing, Treasure Island, FL.

46. Winger, Q.A., de los Rios, P., Han, V.K., Armstrong, D.T., Hill, D.J. and Watson, A.J. (1997) Bovine oviductal and embryonic insulin-like growth factor binding proteins: Possible regulators of "embryotrophic" insulin-like growth factor circuits. *Biol. Reprod.*, 56(6): 1415–1423.

47. Pushpakumara, P.G., Robinson, R.S., Demmers, K.J., Mann, G.E., Sinclair, K.D., Webb, R. and Wathes, D.C. (2002) Expression of the insulin-like growth factor (IGF) system in the bovine oviduct at oestrus and during early pregnancy. *Reproduction*, 123(6): 859–868.

48. Stevenson, K.R. and Wathes, D.C. (1996) Insulin-like growth factors and their binding proteins in the ovine oviduct during the oestrous cycle. *J. Reprod. Fertil.*, 108(1): 31–40.

49. Hamdi, M., Sánchez-Calabuig, M.J., Rodríguez-Alonso, B., Arnal, S.B., Roussi, K., Sturmey, R., Gutiérrez-Adán, A., Lonergan, P. and Rizos, D. (2019) Gene expression and metabolic response of bovine oviduct epithelial cells to the early embryo. *Reproduction*, 158(1): 85–94.

50. Herbst, R.S. (2004) Review of epidermal growth factor receptor biology. *Int. J. Radiat. Oncol. Biol. Phys.*, 59(2 Suppl): 21–26.

51. Zeng, F. and Harris, R.C. (2014) Epidermal growth factor, from gene organization to bedside. *Semin. Cell Dev. Biol.*, 28: 2–11.

52. Wijayagunawardane, M.P.B., Hambruch, N., Haeger, J.D. and Pfarrer, C. (2015) Effect of epidermal growth factor (EGF) on the phosphorylation of mitogen-activated protein kinase (MAPK) in the bovine oviduct *in vitro*: Alteration by heat stress. *J. Reprod. Dev.*, 61(5): 383–389.

53. Knott, A.W., Juno, R.J., Jarboe, M.D., Zhang, Y., Profitt, S.A., Thoerner, J.C., Erwin, C.R. and Warner, B.W. (2003) EGF receptor signaling affects bcl-2 family gene

expression and apoptosis after massive small bowel resection. *J. Pediatr. Surg.*, 38(6): 875–880.

54. Takatsu, K., Kuse, M., Yoshioka, S. and Acosta, T.J. (2015) Expression of epidermal growth factor (EGF) and its receptor in bovine endometrium throughout the luteal phase: Effects of EGF on prostaglandin production in endometrial cells. *Anim. Reprod.*, 12(2): 328–335.

55. Suzuki, T., Sasano, H., Takaya, R., Fukaya, T., Yajima, A. and Nagura, H. (1998) Cyclic changes of vasculature and vascular phenotypes in normal human ovaries. *Hum. Reprod.*, 13(4): 953–959.

56. Pillai, V.V., Weber, D.M., Phinney, B.S. and Selvaraj, V. (2017) Profiling of proteins secreted in the bovine oviduct reveals diverse functions of this luminal microenvironment. *PLoS One*, 12(11): e0188105.

57. Melincovici, C.S., Boşca, A.B., Şuşman, S., Mărginean, M., Mihu, C., Istrate, M., Moldovan, I.M., Roman, A.L. and Mihu, C.M. (2018) Vascular endothelial growth factor (VEGF) key factor in normal and pathological angiogenesis. *Rom. J. Morphol. Embryol.*, 59(2): 455–467.

58. Fontes, P.K., Ereno, R.L., Peixoto, A.R., Carvalho, R.F., Scarano, W.R., Trinca, L.A., Barros, C.M. and de Souza Castilho, A.C. (2018) Can the antral follicular count modulate the gene expression of bovine oviducts in Aberdeen Angus and Nelore heifers? *PLoS One*, 13(8): e0202017.

59. Lam, P.M., Briton-Jones, C., Cheung, C.K., Lok, I.H., Yuen, P.M., Cheung, L.P. and Haines, C. (2003) Vascular endothelial growth factor in the human oviduct: Localization and regulation of messenger RNA expression *in vivo*. *Biol. Reprod.*, 68(5): 1870–1876.

60. Kürüm, A., Karahan, S., Kocamiş, H., Çinar, M. and Ergün, E. (2019) Determination of antioxidants in bovine oviduct epithelial cell culture isolated at different periods of the estrous cycle. *Turk. J. Vet. Anim. Sci.*, 43: 448–455.

61. Kurum, A., Deprem, T., Kocamis, H. and Karahan, S. (2016) Immunohistochemical expression of antioxidants in bovine oviduct epithelial cells of estral and luteal phases. *Ankara Üniv. Vet. Fak. Derg.*, 63(2): 103–110.

62. Margis, R., Dunand, C., Teixeira, F.K. and Margis-Pinheiro, M. (2008) Glutathione peroxidase family an evolutionary overview. *FEBS J.*, 275(15): 3959–3970.

63. Imai, H., Hakkaku, N., Iwamoto, R., Suzuki, J., Suzuki, T., Tajima, Y., Konishi, K., Minami, S., Ichinose, S., Ishizaka, K., Shioda, S., Arata, S., Nishimura, M., Naito, S. and Nakagawa, Y. (2009) Depletion of selenoprotein GPx4 in spermatocytes causes male infertility in mice. *J. Biol. Chem.*, 284(47): 32522–32532.

64. Lapointe, J., Kimmins, S., Maclaren, L.A. and Bilodeau, J.F. (2005) Estrogen selectively upregulates the phospholipid hydroperoxide glutathione peroxidase in the oviducts. *Endocrinology*, 146(6): 2583–2592.

65. Adams, C.M., Clark-Garvey, S., Porcu, P. and Eischen, C.M. (2019) Targeting the Bcl-2 family in B cell lymphoma. *Front. Oncol.*, 8: 636.

66. Urhausen, C., Beineke, A., Piechotta, M., Karre, I., Beyerbach, M. and Günzel-Apel, A.R. (2011) Apoptosis in the uterotubal junction and oviductal isthmus during the estrous cycle of the bitch. *Anat. Rec. (Hoboken)*, 294(2): 342–348.

67. Ricklin, D., Reis, E.S., Mastellos, D.C., Gros, P. and Lambris, J.D. (2016) Complement component C3 the "Swiss Army Knife" of innate immunity and host defense. *Immunol. Rev.*, 274(1): 33–58.

68. Anderson, D.J., Abbott, A.F. and Jack, R.M. (1993) The role of complement component C3b and its receptors in sperm-oocyte interaction. *Proc. Natl. Acad. Sci. U. S. A.*, 90(21): 10051–10055.

69. Lee, Y.L., Lee, K.F., Xu, J.S., He, Q.Y., Chiu, J.F., Lee, W.M., Luk, J.M. and Yeung, W.S.B. (2004) The embryotrophic activity of oviductal cell-derived complement C3b and iC3b, a novel function of complement protein in reproduction. *J. Biol. Chem.*, 279(13): 12763–12768.

70. Xu, J.S., Cheung, T.M., Chan, S.T.H., Ho, P.C. and Yeung, W.S.B. (2001) Temporal effect of human oviductal cell and its derived embryotrophic factors on mouse embryo development. *Biol. Reprod.*, 65(5): 1481–1488.

71. Tse, P.K., Lee, Y.L., Chow, W.N., Luk, J.M.C., Lee, K.F. and Yeung, W.S. (2008) Preimplantation embryos cooperate with oviductal cells to produce embryotrophic inactivated complement-3b. *Endocrinology*, 149(3): 1268–1276.

72. Hugentobler, S.A., Humpherson, P.G., Leese, H.J., Sreenan, J.M. and Morris, D.G. (2008) Energy substrates in bovine oviduct and uterine fluid and blood plasma during the oestrous cycle. *Mol. Reprod. Dev.*, 75(3): 496–503.

73. Penning, T.M. (2015) The Aldo-Keto reductases (AKRs): Overview. *Chem. Biol. Interact.*, 234: 236–246.

74. Jurisicova, A. and Acton, B.M. (2004) Deadly decisions: The role of genes regulating programmed cell death in human preimplantation embryo development. *Reprod. Camb. Engl.*, 128(3): 281–291.

75. El-Sayed, A., Hoelker, M., Rings, F., Salilew, D., Jennen, D., Tholen, E., Sirard, M.A., Schellander, K. and Tesfaye, D. (2007) Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. *Physiol. Genom.*, 28(1): 84–96.

76. Zhao, F.Q., Okine, E.K. and Kennelly, J.J. (1999) Glucose transporter gene expression in bovine mammary gland. *J. Anim. Sci.*, 77(9): 2517–2522.

77. Hocquette, J.F., Sauerwein, H., Higashiyama, Y., Picard, B. and Abe, H. (2006) Prenatal developmental changes in glucose transporters, intermediary metabolism and hormonal receptors related to the IGF/insulin-glucose axis in the heart and adipose tissue of bovines. *Reprod. Nutr. Dev.*, 46(3): 257–272.

78. Nishimoto, H., Matsutani, R., Yamamoto, S., Takahashi, T., Hayashi, K.G., Miyamoto, A., Hamano, S. and Tetsuka, M. (2006) Gene expression of glucose transporter (GLUT) 1, 3 and 4 in bovine follicle and corpus luteum. *J. Endocrinol.*, 188(1): 111–119.

79. Nishizaki, T. and Matsuoka, T. (1998) Low glucose enhances Na+/glucose transport in bovine brain artery endothelial cells. *Stroke*, 29(4): 844–849.

80. Arhin, S.K., Zhao, J., Ji, X., Shi, C., Tang, J., Gu, Y., Xi, H., Cheng, J., Qu, X., Shi, H., Jin, X. and Lv, J. (2019) Multiple facilitated glucose transporters SLC2As are required for normal mouse preimplantation embryo development. *Am. J. Transl. Res.*, 11(6): 3412–3425.

81. Lim, C.H., Jeong, W., Lim, W., Kim, J., Song, G. and Bazer, F.W. (2012) Differential expression of select members of the slc family of genes and regulation of expression by MicroRNAs in the chicken oviduct1. *Biol. Reprod.*, 87(6): 145.

82. Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., Tujague, M., Ström, A., Treuter, E., Warner, M. and Gustafsson, J.A. (2007) Estrogen receptors: How do they signal and what are their targets. *Physiol. Rev.*, 87(3): 905–931.

83. Wijayagunawardane, M.P.B., Miyamoto, A., Cerbito, W.A., Acosta, T.J., Takagi, M. and Sato, K. (1998) Local distributions of oviductal estradiol, progesterone, prostaglandins, oxytocin and endothelin-1 in the cyclic cow. *Theriogenology*, 49(3): 607–618.

84. Schürks, M., Rist, P.M. and Kurth, T. (2010) Sex hormone receptor gene polymorphisms and migraine: A systematic review and meta-analysis. *Cephalalgia*, 30(11): 1306–1328.

85. Kölle, S., Dubielzig, S., Reese, S., Wehrend, A., König, P. and Kummer, W. (2009) Ciliary transport, gamete interaction, and effects of the early embryo in the oviduct: *Ex vivo* analyses using a new digital videomicroscopic system in the cow. *Biol. Reprod.*, 81(2): 267–274.

86. Winuthayanon, W., Bernhardt, M.L., Padilla-Banks, E., Myers, P.H., Edin, M.L., Lih, F.B., Hewitt, S.C., Korach, K.S. and Williams, C.J. (2015) Oviductal estrogen

receptor α signaling prevents protease-mediated embryo death. *eLife*, 4: e10453.

87. Li, D.Y., Zhang, L., Yang, M.Y., Xu, H.L., Yin, H.D., Li, Y. and Zhu, Q. (2013) Effect of luteinizing hormone/choriogonadotropin receptor (LHCGR) gene on chicken reproductive traits. *Mol. Biol. Rep*., 40(12): 7111–7116.

88. Sun, T., Lei, Z.M. and Rao, C.V. (1997) A novel regulation of the oviductal glycoprotein gene expression by luteinizing hormone in bovine tubal epithelial cells. *Mol. Cell Endocrinol*., 131(1): 97–108.

89. Palma-Vera, S., Einspanier, R. and Schoen, J. (2014) Bovine oviductal epithelial cells: Long term culture characterization and impact of insulin on cell morphology. *Reprod. Biol*., 14(3): 206–212.

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