Expression of receptors in cow’s oviductal epithelial cells to prostaglandins E2, D2 and F2α

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Abstract. The significance of studying a condition of the reproductive system in productive animals, especially in dairy cattle, is directly proportional to the population’s needs in dairy, lactic acid, and other livestock products. The article presents the results of studies conducted at the Agricultural University of Hohhot city, China. The aim of the study was to identify the expression of receptors for commercial prostaglandins. The object of research was the biological material obtained from slaughter cattle, namely, the section of the cow’s oviducts. The subject of research was isolated epithelial cells. Expression was assessed by the level of intracellular Ca2+ and cAMP when exposed to PGE2, PGD2 and PGF2α of various concentrations from 10^{-5} to 10^{-9}, also was using an agonist Butaprost. As a result, the concentration of PGE2 at 10^{-5} mol/L, PGF2α at 10^{-5} mol/L and 10^{-6} mol/L significantly contributed to an increase in the level of intracellular calcium (Ca2+) in the epithelial cells of the cow’s oviducts. The increase in the concentration of PGE2, PGD2, and Butaprost led to an increase in the level of cAMP concentration in epithelial cells of cow’s oviducts. EP1, EP2, EP4, FP, DP, TP receptors are available on cow’s oviductal epithelial cells. EP3 and IP receptors were not found on cow’s oviductal epithelial cells. Expression of the EP2, EP4, DP, FP receptor genes is well expressed, unlike EP1 and TP.

1. Introduction

In production, a decrease in milk productivity and the cattle birth rate brings economic losses of more than $10 billion a year both in our country and in China as well [1, 2]. Reducing the incidence of the animal reproductive system is one of the main issues in veterinary medicine. To solve this problem, first of all, it is necessary to analyze the role of reproductive-endocrine active substances in order to improve reproductive ability.

Prostaglandins and their receptors play an important physiological role in the regulation of smooth muscle of the oviducts. At the same time, uterine prostaglandins and their receptors, oviduct epithelial cells secreting cytokines are important in fertilization, implantation, and the process of early embryonic development [3, 4, 5].

Oviducts are a place for egg maturation, fertilization, and they also perform a transport function and play an important role in sperm cell capacitation. Oviducts create optimal conditions for the development of an early embryo. The microenvironment of the oviduct plays an important role for sperm, ova, and zygotes. The oviducts are well equipped with blood vessels, and the secretory cells of...
the epithelium produce the secret necessary to maintain the internal environment, the fluid in the lumen of the oviduct is rich in cytokines and other substances.

Epithelial cells of the cow’s oviducts play a role in the reproduction of both animals and humans. In this regard, it is great significance to study the mechanical cultivation method such as high purity cells, with high activity, for subsequent research.

A wide variety of prostaglandin functions, as well as the absence of a special biosynthesis organ in the body, make it possible to classify them as “local” or cellular hormones.

Prostaglandins perform an important function in the oviducts and the reproductive process as a whole. Studies have shown that prostaglandin F\(_2\alpha\) affects the activity of the oviducts, and prostaglandin E\(_2\) inhibits its activity. PGF\(_2\alpha\) acts more in the isthmus, and PGE\(_2\) in the ampule of the oviduct [6, 7].

Pathophysiological studies have shown that with inflammation of the oviducts, the contractile activity of their smooth muscles is disturbed, the secretory function of the epithelial cells of the oviducts decreases, which can lead to infertility in cows.

Abnormal smooth muscle movements in the oviducts can lead to abnormal secretion of epithelial cells, which can lead to infertility, and then to oviduct dysfunction, which affects the reproductive function of cows [8].

The human prostaglandin receptors in epithelial cells EP1, EP2, EP3, EP4, FP are the same, and COX-1, COX-2, and prostaglandin synthase are also the same [9]. Group E prostaglandin receptors include EP receptors, which are divided into EP1, EP2, EP3, and EP4. FP receptor refers to prostaglandin PGF\(_2\alpha\).

Prostaglandin receptors act through G-proteins by binding to them, thus they exhibit a biological effect, this effect is closely associated with changes in the content of the cAMP and Ca\(^{2+}\) secondary messenger.

Studies have shown that an increase in the concentration of Ca\(^{2+}\) can stimulate a decrease in the muscle layer of the oviducts, while an increase in the level of cAMP will produce the opposite effect [6, 7].

Objective: Study of cow oviduct epithelial cells using the fluorescence method and ELISA to determine the concentration of intracellular calcium and cAMP on the concentration of prostaglandins (PGE\(_2\), PGD\(_2\), and PGF\(_2\alpha\)) and a prostaglandin receptor agonist (Butaprost receptor agonist EP\(_2\)), as well as the use of the method PCR to detect the receptors of the prostaglandins understudy in cow’s oviduct epithelial cells.

2. Conditions, materials and methods

The fallopian tubes (oviducts) were taken from cows at the slaughterhouse of a meat processing enterprise in Hohhot. For transportation, sections of oviducts with ovaries and part of the uterine horn were immersed in 0.9% saline. A container with the material in 0.9% saline was placed in an icebox for further transportation. Oviducts with a mesentery, an ampoule and an isthmus about 1 cm long were placed in a Kreb’s solution.

In the work, was used prostaglandins (PGE\(_2\), PGD\(_2\), and PGF\(_2\alpha\)), an agonist of the EP\(_2\) Butaprost receptor, and a TP receptor agonist at appropriate concentrations.

Prostaglandin E\(_2\) (PGE\(_2\), batch number: 14010), prostaglandin F\(_2\alpha\) (PGF\(_2\alpha\), batch number: 16010), prostaglandin D\(_2\) (PGD\(_2\), batch number: 12010), an EP\(_2\) receptor agonist (Butaprost, batch number: 13740) were purchased in pure form from America Cayman (USA). Other reagents were analytical grade domestic or imported.

Multifunctional reader for microplate photometer (Synergy 4, American Bio-Tek); automatic microplate photometer (Bio-Tek, ELX800); Real-time PCR apparatus (Life ViiA7); 611UF water purifier (Biotech); XB70 ice machine (Ningbo Grant); - 80 °C freezer (Thermo); freezer - 20 (Mailin); XK96-B mixing machine; water bath with a lid (DK-8D, Husenlaboratory Glassware Co., Ltd.); electronic analytical balance; mini centrifuge (LX-200, Kylin-Bell, automatic cell counter (CYTORECON, CYT-1000), electronic analytical scales, baths, dispensers, etc.
The statistical significance of the difference in the results was calculated using the 'Student' parametric criterion [10].

3. Results and discussion

Cow’s oviduct epithelial cells (BOECs) were isolated mechanically and cultured using trypsin by enzymatic digestion; the use of fluorescent probes makes it possible to detect PGE$_2$ and PGF$_{2\alpha}$, which affect the level of Ca$^{2+}$ concentration in epithelial cells of bovine oviducts; using the ELISA method allowed to detect prostaglandins PGE$_2$, PGF$_{2\alpha}$ and an agonist of the EP2 Butaprost receptor; for this, the level of cAMP was measured using the ELISA method in the epithelial cells of cow’s oviducts; prostaglandin receptor species were detected in real-time by RT-PCR.

Analyzing this diagram (figure 1), it can be said that when the concentration of prostaglandin E$_2$ is $10^{-5}$ mol/L, the concentration of intracellular calcium increases to 225 nmol/L.

![Figure 1](image1.png)

**Figure 1.** The impact of prostaglandin E$_2$ on the calcium ions concentration in the oviduct’s epithelial cells.

In figure 2, we see a sharp jump and further growth of intracellular calcium at a concentration of prostaglandin F$_{2\alpha}$ of $10^{-5}$ mol/L. At a concentration of $10^{-6}$ mol/L, an increase in calcium content is also noted.

![Figure 2](image2.png)

**Figure 2.** The impact of prostaglandin F$_{2\alpha}$ on the calcium ions concentration in the oviduct’s epithelial cells.
Analyzing figure 3, we can say that with an increase in the concentration of prostaglandin D$_2$, prostaglandin E$_2$, and butaprost, the concentration of intracellular cAMP also increases, but there is a dependence of the concentrations. The concentration of prostaglandin E$_2$ 10$^{-6}$ mol/l can contribute to a constant level of cAMP concentration in the epithelial cells of cow’s oviducts. Butaprost in the range (10$^{-9}$ - 10$^{-6}$ mol/L) has no effect on the level of cAMP, but at a concentration of Butaprost 10$^{-5}$ mol/L, there is a significant increase in the content of cAMP in the epithelial cells of the cow’s oviducts. With an increase in the concentration of prostaglandin D$_2$ from 10$^{-9}$ to 10$^{-5}$ mol/L, the concentration of cAMP in the epithelial cells of cow’s oviducts increases significantly.

![Figure 3](image-url)

**Figure 3.** The impact of prostaglandin D$_2$, prostaglandin E$_2$, and butaprost on cAMP concentration in cow’s oviduct epithelial cells.

Figure 4 shows that the EP1, EP2, EP4, FP, DP, TP receptor exists on cow’s oviduct epithelial cells. EP3 and IP receptors do not exist. Expression of the EP2, EP4, FP, DP receptor genes is well expressed, unlike EP1, TP.

![Figure 4](image-url)

**Figure 4.** Types of receptors found during PCR studies of cow’s oviduct epithelial cells in real-time.

4. Conclusions
The effect of prostaglandins on cow’s oviductal epithelial cells depends on the concentration of the studied prostaglandins. The concentration of PGE$_2$ in 10$^{-5}$ mol / L markedly contributed to an increase in the level of intracellular calcium (Ca$^{2+}$) in the epithelial cells of cow’s oviducts. The concentration of PGF$_{2\alpha}$ in 10$^{-5}$ mol/L and 10$^{-6}$ mol/L also contributed to an increase in the level of Ca$^{2+}$ in epithelial cells of cow’s oviducts. An increase in the concentration of PGE$_2$, PGD$_2$, and Butaprost significantly
contributed to an increase in the concentration of cAMP in epithelial cells of cow’s oviducts. An increase in intracellular cAMP promotes fluid secretion into the lumen of the oviduct. The listed effects are most likely to have an impact on the advancement of sperm, the fertilization process and the early development of the embryo, as well as on other reproductive processes.

An increase in the concentration of Ca\(^{2+}\) and cAMP in the epithelial cells of cow’s oviducts under the action of the studied prostaglandins indicates the presence of specific receptors, such as EP1, EP2, EP4, FP, DP. Application of the PCR method revealed a high level of expression of EP2, EP4, DP receptors, therefore, the studied prostaglandins have an effect on cow’s oviducts epithelial cells, to a greater extent, precisely through these receptors.

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