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Differential gene expression and immunolocalization of platelet-derived growth factors and their receptors in caprine ovaries

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ABSTRACT

This study evaluated the messenger RNA (mRNA) expression and immunolocalization of all members of the platelet-derived growth factor (PDGF) family in caprine ovaries by quantitative PCR and immunohistochemistry, respectively. Detectable levels of PDGF-A mRNA were not observed in primordial follicles. Higher levels of PDGF-B mRNA were observed in primary follicles than in primordial follicles ($P < 0.05$). PDGF-D mRNA levels were higher in secondary follicles than in the other preantral follicle categories ($P < 0.05$). PDGF-B mRNA expression was higher than PDGF-C mRNA expression in primary follicles ($P < 0.05$). In antral follicles, PDGF-A mRNA expression was higher in cumulus-oocyte complexes (COCs) from small antral follicles than in those from large antral follicles and their respective granulosa/theca (GT) cells ($P < 0.05$). Furthermore, in COCs from small and large antral follicles, PDGF-A mRNA expression was higher than that of the other PDGF isoforms ($P < 0.05$). The mRNA levels of PDGF-B and PDGF-D and PDGFR-α and PDGFR-β were higher in GT cells from large antral follicles than in GT cells from small antral follicles and in their respective COCs ($P < 0.05$). In COCs and GT cells from small antral follicles, the mRNA levels of PDGFR-α were higher than those of PDGFR-β ($P < 0.05$). All proteins were observed in the cytoplasm of oocytes from all follicular categories. In granulosa cells, all PDGFs and PDGFR-β were detected from starting at the secondary stage, and in theca cells, all proteins, except PDGF-C, were detected starting at the antral stage. In conclusion, PDGF and its receptors are differentially expressed in the oocytes and ovarian cells according to the stage of follicular development, suggesting their role in the regulation of folliculogenesis in goats.

1. Introduction

Members of the platelet-derived growth factor (PDGF) family are dimeric glycoproteins composed of 4 different polypeptide chains (A, B, C, and D) that are encoded by 4 different genes. These polypeptide chains are synthesized as inactive precursors. After proteolytic processing, the 4 PDGF chains assemble into disulfide-bonded dimers via homodimerization or heterodimerization, which generates 5 different dimeric isoforms: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD. These PDGFs isoforms function by binding, with distinct specificities, to 2 α- and β-protein tyrosine kinase receptors (PDGFR-α and PDGFR-β) [1].

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PDGF was originally purified from platelets [2], and was subsequently shown to be produced by a variety of cell types, such as fibroblasts, endothelial cells, and smooth muscle cells [3–5]. The functions of PDGFs are mainly related to cellular proliferation, migration, and differentiation and angiogenesis [6].

A few studies have analyzed the presence of PDGFs and their receptors in the ovaries of mammals, including rats [7,8], mice [9], humans [10], and pigs [11]. In rats, PDGF-B, PDGF-C, and PDGF-D messenger RNA (mRNA) expression was detected in oocytes from preantral follicles and in the theca cells of secondary follicles. Yet, in this species, PDGF-A and PDGF-C and PDGFR-α and PDGFR-β proteins were detected in oocytes from primordial and primary follicles. In granulosa cells from primordial, secondary, and antral follicles, only PDGF-A and PDGFR-α proteins were detected, whereas in theca cells, all PDGF ligands and receptors were detected in secondary follicles and onward [7]. Unlike what was observed in rats, in mice, all PDGFs and receptors were detected in preantral follicles [9]. Pinkas et al [10] evaluated the mRNA and protein expression of PDGF-A and PDGF-B and the receptors PDGFR-α and PDGFR-β in human fetal and adult ovaries. All isoforms were expressed in oocytes and granulosa cells; however, in contrast to the findings in rodents [7,9], PDGFR-α protein was not detected in granulosa cells [10]. Similarly, in pigs, PDGFR-α was detected in all follicular compartments, except in granulosa cells, independent of follicular stage. In addition, PDGF-A was detected in oocytes and granulosa cells from porcine preantral follicles [11]. In goats, just 1 study analyzed the levels of PDGFR-α and PDGFR-β mRNA, and both were detected in all follicular categories [12].

As described previously, a few studies have assessed the presence of PDGF family members in different compartments and at different follicular stages. In these studies, variations in the expression patterns of PDGF isoforms and receptors were observed in different species. However, in farm animals, including goats, there is no information on the mRNA expression of PDGF family proteins (PDGF-A, PDGF-B, PDGF-C, and PDGF-D) or the protein localization of these ligands and their receptors in ovarian follicles. The goat is a very important agricultural species worldwide, and it can be used as an animal model for the study of folliculogenesis in other species, including humans. Therefore, the aims of this study were to investigate the mRNA and protein expression levels of all PDGFs and their receptors in caprine ovarian follicles at different stages of development.

2. Materials and methods

2.1. Source of ovaries

Ovaries (n = 36) from 18 non-pregnant, adult, mixed-breed goats (*Capra hircus*) between 1 and 3 yr of age were collected from a local abattoir (Fortaleza, CE, Brazil). Some of the ovaries (n = 26) were used for quantitative PCR (qPCR), and the remainder (n = 10) were used for immunohistochemistry. Immediately after slaughter, the ovaries were washed with 70% alcohol for 10 s. Then, the ovaries were washed twice with Minimum Essential Medium (MEM) buffered with HEPES (MEM-HEPES) and supplemented with penicillin (100 μg/mL) and streptomycin (100 μg/mL). Then, the ovaries were transported at 4°C to our laboratory within 1 h.

2.2. mRNA quantification of PDGF family members in caprine ovarian follicles

From a total of 26 ovaries, 10 were used to isolate primordial, primary, and secondary follicles, and 16 were used to collect cumulus-oocyte complexes (COCs) and mural cells (granulosa and theca cells) from small (1–3 mm) and large (>3–6 mm) antral follicles. Primordial and primary follicles were isolated using a tissue chopper, as previously described [13], and secondary follicles were microdissected from the ovarian cortex. After isolation, the follicles were washed with HEPES-buffered MEM, classified by category, and placed into separate Eppendorf tubes. This procedure was completed within 2 h, and then 3 groups of 20 follicles in each category were stored at −80°C until RNA extraction. COCs were recovered from small and large antral follicles from the second group of ovaries (n = 16). Compact COCs were selected as described by van Tol et al [14]. Thereafter, 3 groups of 20 COCs were stored at −80°C until RNA extraction. To collect mural granulosa and theca cell complexes, small (n = 10) and large antral follicles (n = 10) were isolated from the ovaries and dissected from the stromal tissue using 26-gauge needles. The follicles were then bisected, and groups (n = 3) of mural granulosa/theca cell were collected and stored at −80°C.

Total RNA was isolated with the TRIzol Plus Purification kit (Invitrogen, São Paulo, Brazil). The RNA preparations were treated with DNase I and processed with the RNeasy Micro Kit (Invitrogen). cDNA was synthesized from the RNA (0.15 μg from each sample) using Superscript II RNase H-Reverse Transcriptase (Invitrogen).

Each qPCR reaction (final volume, 20 μL) contained 1 μL of each cDNA, 10 μL of 1 × Power SYBR Green PCR Master Mix, 7.4 μL of ultrapure water, and 0.4 μM sense and antisense primers. The gene-specific primers used for the amplification of different transcripts are shown in Table 1. Best Keeper software highlighted GAPDH as the reference gene with the lowest overall variation. Primer specificity and amplification efficiency were verified for each gene. The PCR cycling conditions consisted of an initial denaturation and polymerase activation step at 94°C for 15 min, followed by 40 cycles of 15 s at 94°C, 30 s at 60°C, and 45 s at 72°C, and then a final extension for 10 min at 72°C. After amplification, melting curve analysis was performed between 60°C and 95°C for all genes. All amplifications were carried out in a Bio-Rad iQ5. The delta-delta-CT method was used to transform threshold cycle values into normalized relative expression levels [15].

2.3. Immunolocalization of PDGF family proteins in caprine ovarian follicles

In the laboratory, caprine ovaries (n = 10) were fixed in paraformaldehyde (4%) for 18 h, dehydrated, and embedded in paraffin. Then, 5-μm sections were obtained