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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.

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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

Table 1
Oligonucleotide primers used for the analysis of caprine follicles by the polymerase chain reaction (S sense and AS antisense).

Target gene	Primer sequence (5′→3′)	Sense (S) and anti-sense (AS)	Position	Primer-product size	NCBI reference sequence
GAPDH	ATGCCTCCTGCACCACCA	S	479–496	76	GI:298676424
	AGTCCCTCCACGATGCCAA	AS	536–554		
PDGF-A	GGAAGGACTGACGTCTCAGC	S	1064–1083	162	GI: 426254913
	CTACGGCAAGGAGATGTGGT	AS	1206–1225		
PDGF-B	GGAAGGTGCAGGTGAAAAAG	S	446–465	248	GI: 57164176
	GTCATGCGTGTGCTTGAACT	AS	674–693		
PDGF-C	GCCACTGGACCTCCTGAATA	S	677–696	175	GI: 297461511
	CAGGTCCACCACTCTGGATT	AS	832–851		
PDGF-D	CGTCAACTTGAGGGAAGAGC	S	957–916	192	GI: 139948854
	CCCCTCCTCTTGAAATAGCC	AS	1129–1148		
PDGFR-α	AGATTCCTTGGTCATCGTGGAGGA	S	35–59	203	GI: 194318547
	TGACCGTGGCCTCACAGATATAGA	AS	213–237		
PDGFR-β	ACATCATGCGGGACTCGAACTACA	S	2941–2965	165	GI: 282721145
	AGATCTCCCAGAGCAAGATGCCAA	AS	3081–3105		

Abbreviations: PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor.

and mounted on glass slides. Immunohistochemistry was performed according to a previously described protocol [16]. Briefly, the epitopes were activated by incubation in citric acid at 98°C to 100°C for 30 min, and then nonspecific binding was blocked by incubation with 1% normal goat serum diluted in phosphate-buffered saline. Then, the sections were incubated with monoclonal anti-PDGF-A, PDGF-B, PDGF-C (Santa Cruz Biotechnology, Inc, USA), and PDGF-D (Proteintech Group, Inc, USA) antibodies and anti-PDGFR-α and PDGFR-β (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies produced in rabbit (1:50) for 18 h at 4°C. Subsequently, the sections were incubated with a secondary biotinylated anti-rabbit IgG antibody (Santa Cruz Biotechnology), diluted 1:200 in phosphate-buffered saline containing 1% normal goat serum for 45 min. The sections were incubated with avidin-biotin-HRP complex (1:600, Vectastain Elite ABC kits; Vector Laboratories, Burlingame, CA, USA) for 45 min. The location of the protein was visualized with diaminobenzidine (0.05% diaminobenzidine and 0.03% H_2O_2 in Tris-HCl, pH 7.6; Sigma Chemicals, Poole, Dorset, UK). Finally, the sections were counterstained with hematoxylin and dehydrated and mounted in Canada balsam. In the negative controls, the primary antibody was replaced with nonspecific IgG from the same species.

The ovarian follicles were classified as primordial, primary, secondary, and antral follicles according to diameter and the morphology of the oocyte and granulosa cells, as described by Lucci et al [17]. In the different follicular compartments (oocyte and granulosa cells), the immunostaining was classified as absent (−), weak (+), moderate (++), or strong (+++).

2.4. Statistical analysis

The gene expression profiles were generated from 3 independent biological replicates, which were performed in triplicate. The relative gene expression was estimated using the 2-ΔΔCt-method [15]. Threshold and Ct values were automatically determined by Bio-Rad iQ5 software, using internal parameters. The Ct was expressed as the mean of 3 measurements ± SEM and was then subjected to a Shapiro-Wilk normality test using UNIVARIATE in the SAS 9.0 software package. The statistical significance of the differences among groups was assessed with the Mann-Whitney test ($P < 0.05$).

3. Results

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

The mRNA levels of PDGF family members were quantified by quantitative RT-PCR. The results showed that PDGF-A mRNA was not expressed at appreciable levels in primordial follicles, whereas PDGF-A mRNA was expressed in primary and secondary follicles at similar levels ($P > 0.05$) (Fig. 1A). PDGF-B mRNA was expressed at higher levels in primary follicles than in primordial follicles ($P < 0.05$); however, its expression did not differ from that in secondary follicles ($P > 0.05$; Fig. 1B). In contrast, the expression of PDGF-D mRNA was higher in secondary follicles than in primordial and primary follicles ($P < 0.05$; Fig. 1D). In all the preantral follicles examined, the relative mRNA expression levels of PDGF-C and PDGFR-α and PDGFR-β were similar ($P > 0.05$; Figs. 1C and 2A and B). When the mRNA levels of all PDGF isoforms in each follicular category were compared, no significant differences were observed in primordial and secondary follicles ($P > 0.05$; Fig. 3A and C). However, in primary follicles, the mRNA levels of PDGF-B were higher than those of PDGF-C ($P < 0.05$), but were similar to those of PDGF-A and PDGF-D ($P > 0.05$; Fig. 3B). In preantral follicles, no differences were found in the mRNA expression of the 2 receptors ($P > 0.05$; Fig. 3D–F).

In antral follicles, PDGF-A mRNA expression in COCs from small antral follicles (≤3 mm) was higher than that in COCs from large antral follicles (>3 mm) ($P < 0.05$; Fig. 1E). In contrast, no differences were observed in the mRNA expression levels of PDGF-B, PDGF-C, and PDGF-D and PDGFR-α and PDGFR-β in COCs from small and large antral follicles ($P > 0.05$; Figs. 1F–H and 2C and D). Higher mRNA levels of PDGF-B and PDGF-D and both receptors was observed in granulosa/theca (GT) cells from large antral follicles than in GT cells from small antral follicles ($P < 0.05$; Figs. 1F, H and 2C, D). Conversely, PDGF-C mRNA expression

was higher in GT cells from small antral follicles than in those from large antral follicles ($P < 0.05$; Fig. 1G). No difference was observed in the PDGF-A mRNA levels in GT cells from small and large antral follicles ($P > 0.05$; Fig. 1E). Higher mRNA expression of PDGF-B and PDGF-D and PDGFR-α and PDGFR-β mRNA was observed in GT cells collected from large antral follicles than in COCs from large antral follicles ($P < 0.05$; Figs. 1F, H and 2C, D). In contrast, the levels of PDGF-A mRNA were higher in COCs than in GT cells from small antral follicles ($P < 0.05$; Fig. 1E). Furthermore, in COCs from both small and large antral follicles, the expression of PDGF-A mRNA was higher than that of the

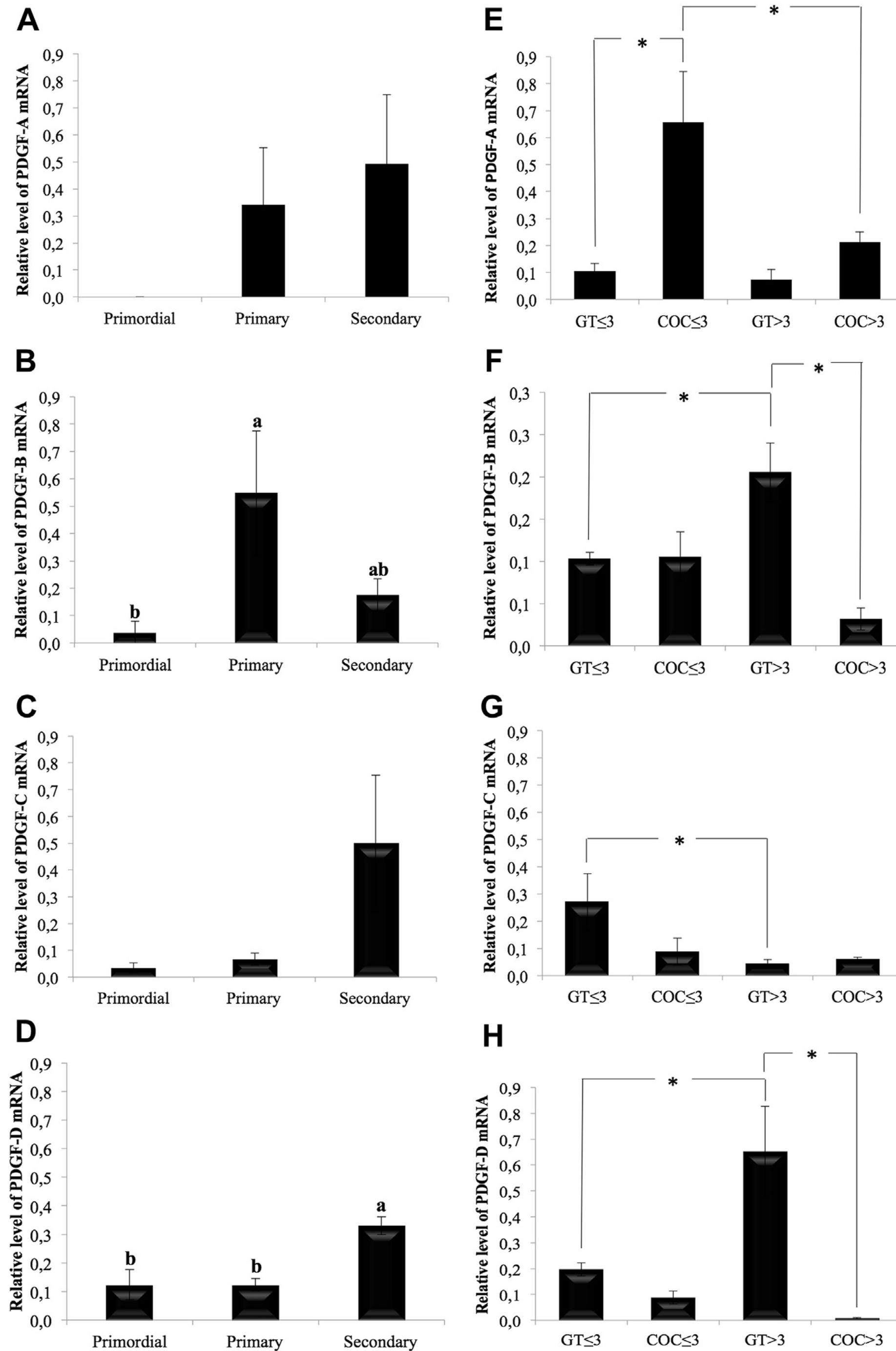

Fig. 1. Levels of PDGF mRNA in caprine ovarian follicles (mean ± SEM). Preantral follicles (A, B, C, and D), COCs, and granulosa/theca cells (GT) from small (1–3 mm) and large (>3–6 mm) antral follicles (E, F, G, and H). Different superscript letters (a and b) indicate significant differences between the categories ($P < 0.05$). *$P < 0.05$. COCs, cumulus-oocyte complexes; mRNA, messenger RNA; PDGF, platelet-derived growth factor; SEM, standard error of the mean.

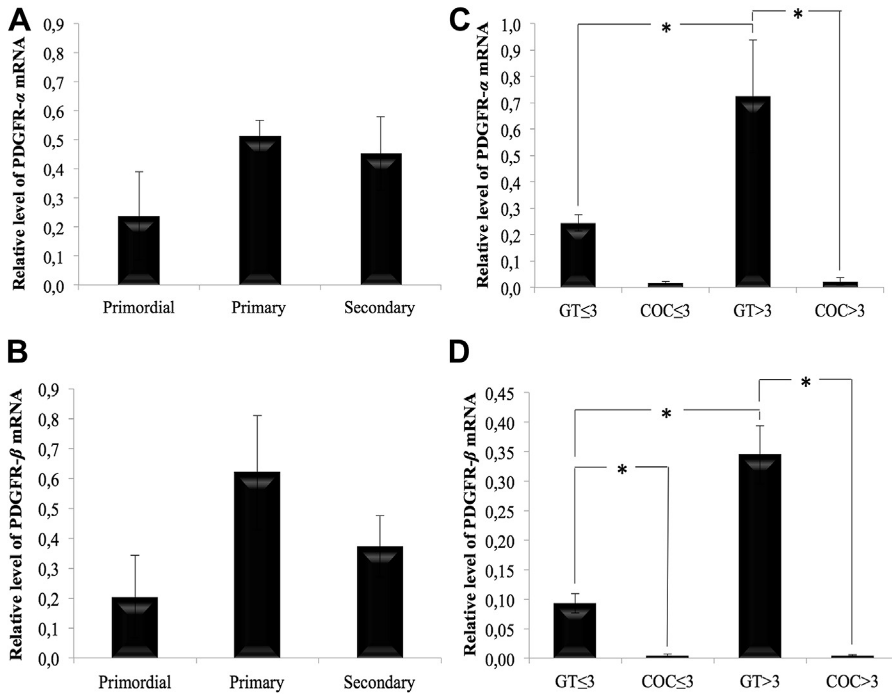

Fig. 2. Levels of PDGFR mRNA in caprine ovarian follicles (mean ± SEM). Preantral follicles (A and B); COCs and granulosa/theca cells (GT) from small (1–3 mm) and large (>3–6 mm) antral follicles (C, D). There was no significant differences among preantral follicles ($P > 0.05$). *$P < 0.05$. COCs, cumulus-oocyte complexes; mRNA, messenger RNA; PDGFR, platelet-derived growth factor receptor; SEM, standard error of the mean.

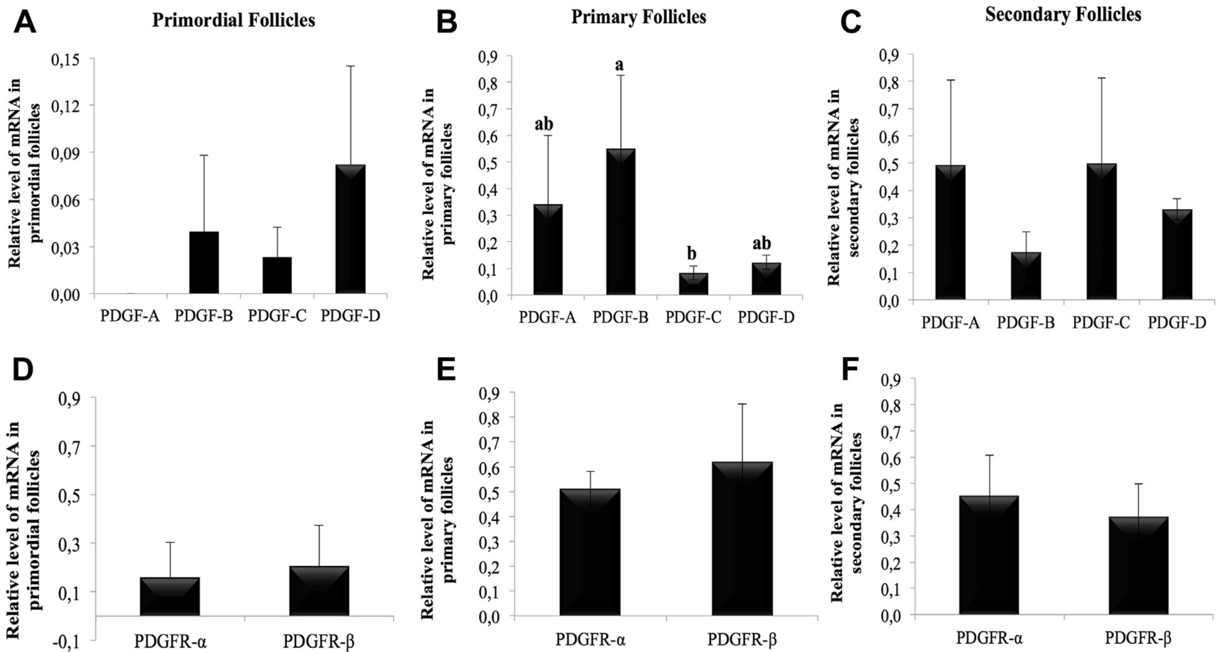

Fig. 3. Levels of PDGF and PDGFR mRNA in primordial (A and D), primary (B and E), and secondary (C and F) caprine ovarian follicles (mean ± SEM). Different superscript letters (a and b) indicate significant differences between the categories ($P < 0.05$). mRNA, messenger RNA; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; SEM, standard error of the mean.

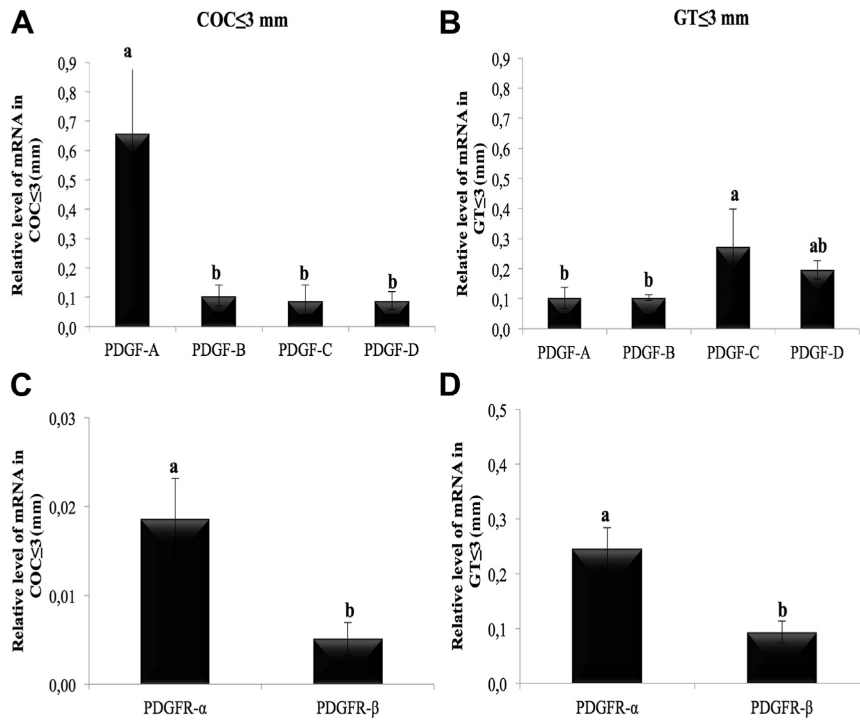

Fig. 4. Levels of PDGF and PDGFR mRNA in COCs (A and C) and granulosa/theca cells (GT) from small (1–3 mm) antral follicles (B, D) (mean ± SEM). Different superscript letters (a and b) indicate significant differences between the categories ($P < 0.05$). mRNA, messenger RNA; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; SEM, standard error of the mean.

other of PDGFs ($P < 0.05$; Figs. 4A and 5A). In GT cells from small antral follicles, the mRNAs levels of PDGF-C were higher than those of PDGF-A and PDGF-B ($P < 0.05$), but were similar to the levels of PDGF-D ($P > 0.05$; Fig. 4B). In GT cells from large antral follicles, no significant differences in the expression of all PDGF mRNAs were observed ($P > 0.05$; Fig. 5B). For the receptors, the levels of PDGFR-α mRNA were higher than those of PDGFR-β mRNA in COCs and GT cells

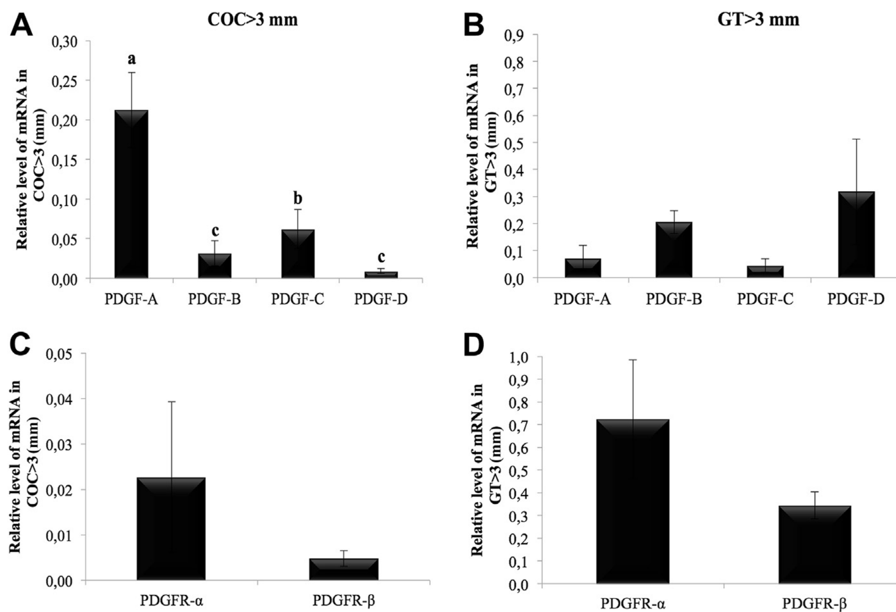

Fig. 5. Levels of PDGF and PDGFR mRNA in COCs (A and C) and granulosa/theca cells (GT) from large (>3–6 mm) antral follicles (B and D) (mean ± SEM). Different superscript letters (a, b, and c) indicate significant differences between the categories ($P < 0.05$). mRNA, messenger RNA; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; SEM, standard error of the mean.

Table 2
Relative intensity of immunohistochemical staining for PDGF-A, B, C, and D and PDGFR-α and β protein in caprine ovarian follicles.

Follicular compartment	Follicular category			
	Primordial	Primary	Secondary	Antral
PDGF-A				
Oocyte				
Nucleus	–	–	–	–
Cytoplasm	++	++	++	+++
Granulosa	–	–	++	+++
Theca	NA	NA	–	+++
PDGF-B				
Oocyte				
Nucleus	–	–	–	–
Cytoplasm	++	++	++	++
Granulosa	–	–	++	+++
Theca	NA	NA	–	++
PDGF-C				
Oocyte				
Nucleus	–	–	–	–
Cytoplasm	+	++	++	++
Granulosa	–	–	++	+++
Theca	NA	NA	–	–
PDGF-D				
Oocyte				
Nucleus	–	–	–	–
Cytoplasm	+	++	+	+
Granulosa	–	–	++	++
Theca	NA	NA	–	+
PDGFR-α				
Oocyte				
Nucleus	+++	+++	+++	+++
Cytoplasm	+	+	++	++
Granulosa	++	+++	+++	+++
Theca	NA	NA	–	+
PDGFR-β				
Oocyte				
Nucleus	–	–	–	–
Cytoplasm	++	++	++	+++
Granulosa	–	–	+	+++
Theca	NA	NA	–	+++

Abbreviations: NA, not applicable; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor.
Symbols indicate that (–) refers to absent; (+) refers to weak; (++) refers to moderate; and (+++) refers to strong immunoreaction.

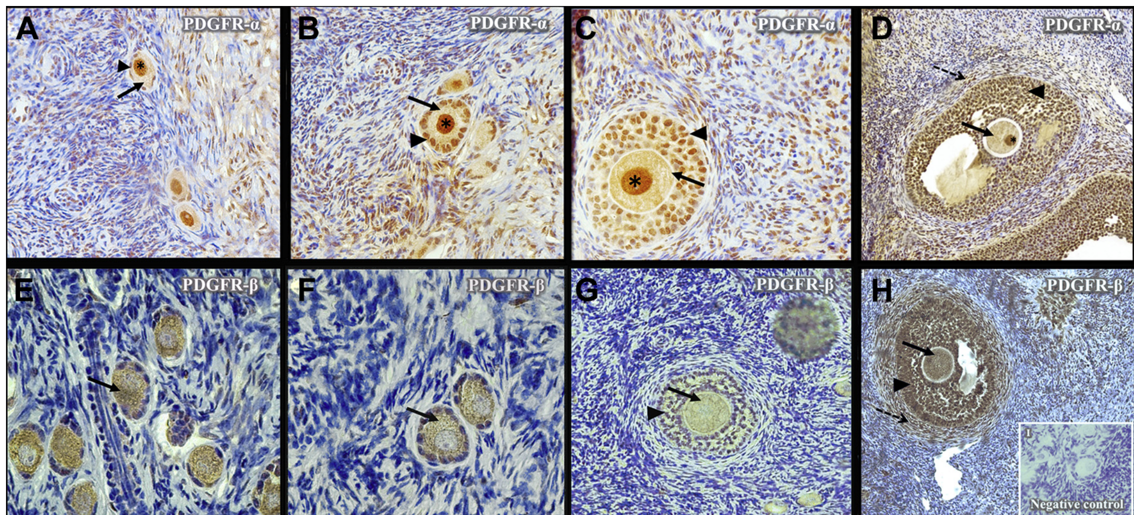

Fig. 6. Immunolocalization of PDGF receptor proteins in caprine ovary sections. Immunohistochemistry was performed with specific antibodies for PDGFR-α (A–D) and PDGFR-β (E–H) on 4 different follicular categories: primordial (A and E), primary (B and F), secondary (C and G), and antral (D and H). (I) Negative control (no staining). Immunoreactive cells of various types are indicated with the following symbols: oocyte cytoplasm (black arrows), oocyte nuclei (black asterisk), granulosa cells (black arrowheads), and theca cells (black-dashed arrows). Original magnification: A–C, E–G, and I, ×400; D and H, ×100. PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor. (For interpretation of the references to color in this figure, the reader is referred to the Web version of this article.)

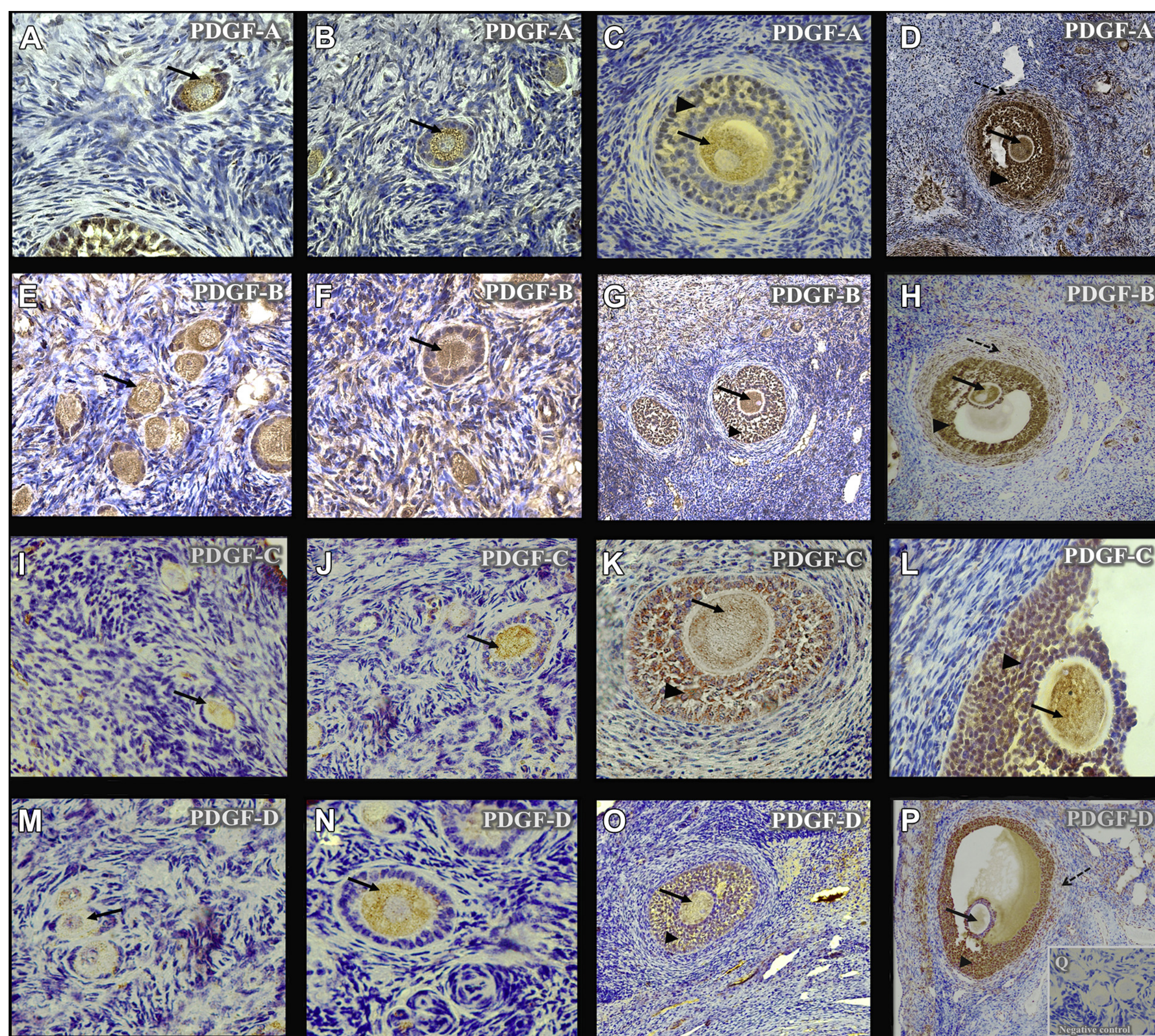

Fig. 7. Immunolocalization of PDGF proteins in caprine ovary sections. Immunohistochemistry was performed with specific antibodies for PDGF-A (A–D), PDGF-B (E–H), PDGF-C (I–L), and PDGF-D (M–P) on different follicular categories: primordial (A, E, I, and M), primary (B, F, J, and N), secondary (C, G, K, and O), and antral (D, H, L, and P). (Q) Negative control (no staining). Immunoreactive cells of various types are indicated as follows: oocyte cytoplasm (black arrows), granulosa cells (black arrowheads), and theca cells (black-dashed arrows). Original magnification: A–C, E, F, I–N, and Q, ×400; D, G, H, O, and P, ×100. PDGF, platelet-derived growth factor. (For interpretation of the references to color in this figure, the reader is referred to the Web version of this article.)

from small antral follicles ($P < 0.05$; Fig. 4C and D). In large antral follicles, no differences were observed ($P > 0.05$; Fig. 5C and D).

3.2. Immunolocalization of PDGF family proteins in caprine ovarian follicles

The immunohistochemical observations for the detection of PDGFs and PDGF receptors are summarized in Table 2. PDGFR-α immunoreactivity was detected in oocyte nucleus from all follicular categories (Fig. 6A–D). In contrast, a weak to moderate immunostaining was observed in the oocyte cytoplasm of all follicular categories (Fig. 7A–C, E–G, I–K, and M–O) for all PDGF family members except for PDGF-A and PDGFR-β, which showed a strong immunostaining in the oocyte cytoplasm of antral follicles (Figs. 7D and 6H, respectively). Granulosa cells from primordial and primary follicles only showed immunostaining for PDGFR-α (Fig. 6A and B), whereas the remaining proteins were expressed in the granulosa cells from secondary and antral stage follicles (Figs. 6G, H and 7C, D, G, H, K, L, O, P). Immunostaining for all members of the PDGF family, except PDGF-C, was observed in theca cells from antral follicles (Figs. 6D, H and 7D, H, L, P). No immunostaining was observed in the negative controls (Figs. 6I and 7Q).

4. Discussion

In this study, we reported for the first time an analysis of the mRNA and protein expression of all PDGF family

members in caprine ovaries. PDGF-A mRNA expression was not detected in primordial follicles, but PDGF-A protein was observed in the cytoplasm of oocytes from primordial follicles. We believe mRNA was undetectable by qPCR because of high translational activity. In mammalian cells, mRNAs are produced at a much lower rate than proteins; on average, a mammalian cell produces 2 copies of a given mRNA per hour, whereas it produces dozens of copies of the corresponding protein per mRNA per hour. Therefore, this result may indicate an important role for PDGF-A mRNA in caprine primordial follicles, because genes with specific functions in certain cells are susceptible to rapid transcriptional and/or translational regulation [18]. Indeed, Nilsson et al [19] showed that PDGF-A gene expression in rat ovaries is important for the primordial to primary follicle transition. Furthermore, in the present work, PDGF-A protein was detected in oocytes starting at the primordial stage. Another study showed that PDGF-AB stimulated the transition from primordial to primary follicles in *in vitro*-cultured rat ovaries [20], supporting the hypothesis that in primordial follicles, PDGF-A mRNA can be rapidly translated to ensure its protein activity in early follicular development.

In the present study, the mRNAs of all the remaining PDGF family ligands (PDGF-B, PDGF-C, and PDGF-D) and the immunoreactivity of the corresponding proteins were detected in all categories of preantral follicles. The results showed high PDGF-B mRNA expression in primary follicles, whereas PDGF-B protein was more abundant starting at the secondary stage. These data support the effects of this growth factor when used in an in vitro culture of preantral follicles. In goats, the addition of PDGF-BB, in association with FSH, promoted the growth of secondary follicles [12]. Similarly, in rat ovaries, the addition of PDGF-AB or PDGF-BB to the culture medium stimulated the growth of secondary follicles [7].

In our study, high levels of PDGF-C and PDGF-D mRNA were observed in secondary follicles. A similar result was observed in rat ovaries by in situ hybridization; PDGF-C and PDGF-D mRNA was observed in the theca cells of secondary follicles [7]. However, in the rat study, PDGF-C protein expression was also observed in the theca cells of secondary follicles, whereas in our study, PDGF-C protein expression was not detected in theca cells at any follicular stage. Although previous studies have demonstrated the presence of PDGF-C and PDGF-D mRNA and protein in preantral follicles, there is no information on their role in early folliculogenesis.

In antral follicles, the mRNA levels of PDGF-A and PDGF-C were higher in the COCs and GT cells, respectively, from small antral follicles than in those from large antral follicles. Moreover, PDGF-A and PDGF-C mRNA expression was higher than that of the other isoforms in COCs and GT cells, respectively, from small antral follicles. These results are in agreement with the findings of Sleer and Taylor [7]. They showed that the levels of both genes in rat ovaries were not affected by equine chorionic gonadotropin stimulation, suggesting higher PDGF-A and PDGF-C activity in the early stages of antral folliculogenesis. However, Rappolle et al [21] detected PDGF-A mRNA expression in ovulated and unfertilized oocytes and blastocysts in mice. Similarly, in cows, PDGF-A mRNA was expressed during development in the oocyte to blastocyst stages, demonstrating that PDGF-A also plays an important role in the later stages of folliculogenesis and in preimplantation embryonic development [22]. Corroborating these findings, in the present study, strong PDGF-A protein immunoreactivity was observed in all compartments of the caprine antral follicles. In contrast, in pig ovaries, only weak expression of this protein was observed in oocyte and granulosa cells [11].

In the present study, the mRNA levels of PDGF-B and PDGF-D were higher in GT cells from large antral follicles than in GT cells from small antral follicles and in their respective COCs. These findings are in agreement with the results of the immunohistochemical analysis, which showed higher PDGF-B and PDGF-D immunostaining in granulosa cells than in the oocyte. These results are consistent with previous studies suggesting a possible function for these isoforms in the later stages of antral folliculogenesis. Sleer and Taylor [8] showed that the levels of PDGF-B and PDGF-D mRNA in rat ovaries increased after hCG administration. In humans, PDGF-D mRNA expression increased after stimulation with FSH and LH, indicating an important role for PDGF during luteogenesis [23]. Studies have also demonstrated an effect for PDGF on theca cell proliferation in rats [24] and pigs [25,26]. Moreover, it has been shown that PDGF-B supports the in vitro development of bovine embryos [27–29].

In the present study, PDGFR-α and PDGFR-β mRNA and protein were detected in all follicular categories. However, in the COCs and GT cells from small antral follicles, the mRNA levels of PDGFR-α were higher than the levels of PDGFR-β. Similar results were obtained in mice by Chen et al [30]. They observed higher levels of PDGFR-α mRNA than PDGFR-β mRNA. Immunohistochemical analysis showed the presence of PDGFR-α in all compartments, including oocyte nuclei, starting with primordial follicles and increasing after follicular development. In contrast, weak PDGFR-β immunoreactivity was observed in all preantral follicles; however, the expression increased starting with the antral stage. These variations in the expression patterns may reflect the many functions of these receptors and their involvement in the regulation of folliculogenesis at different stages. Herrera et al [31] detected PDGFR-β in ovarian stromal cells of newborn mice, around newly formed primary follicles, and in a few cells that intercalate oocyte clusters. These data suggest an early role for PDGFR-β in the differentiation of ovarian stroma. Moreover, the results may be explained by the fact that all the isoforms of PDGF, except PDGF-DD, can bind to PDGFR-α [6], suggesting that this receptor is more important than PDGFR-β. In fact, one study revealed that mutations in PDGFR-α mRNA may affect steroidogenesis, and result in infertility. The ovaries of these mutant mice were unusually small, and the number of later-stage antral follicles was lower [32].

In summary, these data demonstrate that PDGF isoforms are differentially expressed in the follicular compartments of caprine ovaries, according to the stage of follicular development. These results suggest roles for specific PDGF isoforms at different stages of caprine folliculogenesis. Thus, the results of this study may provide the

basis for future studies on the importance of PDGF in follicular development.

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