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Expression of steroidogenic enzymes and synthesis of steroid hormones during development of ovarian follicles in prepubertal goats☆

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Abstract

Expression of mRNAs encoding cytochrome P450 side-chain cleavage (P450scc), cytochrome P450 17 α-hydroxylase (P450c17), and cytochrome P450 aromatase (P450arom) were characterized by the RT-PCR technique and concentrations of progesterone (P4), testosterone (T0) and estradiol (E2) were measured by radioimmunoassay during follicular development of prepubertal goats. Synthesis of mRNAs encoding P450scc and P450c17 began in preantral follicles, but mRNA encoding P450arom was not detectable until early antral formation. While mRNA for P450scc was expressed in both theca and granulosa cells, mRNA for P450c17 was expressed only in theca cells while P450arom mRNA only in granulosa cells. In nonatretic follicles from prepubertal ovaries, the relative quantity of mRNA expression of all the three enzymes increased with follicle size; however, while the concentration of P4 and E2 increased, that of T0 decreased with follicle size. While expression of mRNA encoding P450scc was unaffected, that of P450c17 mRNA decreased to the lowest level and mRNA for P450arom became undetectable following atresia; accordingly, while the concentration of P4 increased in the atretic medium follicles, that of T0 and E2 decreased to the lowest level after atresia. While the adult follicular stage follicles showed a similar cytochrome expression as the nonatretic follicles of prepubertal goats, the former contained higher levels of E2 and P4 than the latter. The presence of corpus luteum in an ovary decreased expression of P450scc, significantly in large follicles while it increased concentration of P4. These findings indicated that (1) similar to other species, changes in follicular steroid production in goats were explained in large measure by changes in steroidogenic enzyme expression; (2) while mRNA expression was similar, activities of some of the steroidogenic enzymes may differ between sexually mature and immature goats.

Keywords: Steroid hormones; Follicular development; Prepubertal goat

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1. Introduction

The use of juvenile animals in embryo transfer programs would improve the genetic gain by reducing the generation interval [1,2]. Although oocytes obtained from prepubertal animals have produced viable offspring after in vitro maturation, fertilization and embryo culture [3–6], the developmental competence of these embryos was lower than that of embryos produced from adult animals (cattle: [3,7–9], sheep: [4,10,11], pig: [12]). A lack of blastocyst formation has been reported in prepubertal goat oocytes after in vitro maturation and fertilization [13]. These observations suggest incomplete maturation or cytoplasmic deficiencies of the oocytes obtained from prepubertal animals. However, the causes for the cytoplasmic deficiencies of prepubertal oocytes are poorly understood.

Mammalian oogenesis occurs concomitantly with folliculogenesis in a coordinated manner in the ovaries. It has been demonstrated that oocyte developmental competence is likely acquired both during follicle development, before meiotic resumption, and during meiotic progression, concurrent with nuclear maturation [14]. Ovarian steroids play an essential role in mammalian folliculogenesis. It has been shown that one prominent characteristic of dominant follicles is their tremendous capacity to produce steroids, especially 17β-estradiol [15–17]. The level of estradiol (E2) and the ratio of E2 to progesterone (P4) in healthy follicles were significantly higher than that in atretic follicles [16,18,19]. Furthermore, follicular fluid from sows has been found to contain more P4 and androstenedione than the follicular fluid from prepubertal pigs [20]. This suggests that acquisition of steroidogenic capacity may be involved in the selection of dominant follicles with good quality oocytes. However, studies on the expression of steroidogenic enzymes in prepubertal follicles are few [21].

Steroidogenesis in follicles requires the conversion of cholesterol to pregnenolone by cytochrome P450 side-chain cleavage (P450scc). In bovine follicles, pregnenolone (the precursor of progesterone) is mainly metabolized via the Δ5-pathway to dehydroepiandrosterone by cytochrome P450 17α-hydroxylase/17-20-lyase (P450c17) [22,23]. Dehydroepiandrosterone is converted to androstenedione (the precursor of testosterone), which is used as the substrate for 17β-estradiol synthesis, a process that is catalyzed by cytochrome P450 aromatase (P450arom) [23]. The expression of these key enzymes involved in ovarian steroidogenesis has been extensively studied in cattle [24,25], sheep [21,25] and pigs [25–27] and is found to be developmentally regulated and species specific. However, no similar study has been reported in goats.

The present study was undertaken to characterize, by the RT-PCR technique, the changes in expression of P450scc, P450c17, and P450arom mRNAs in prepubertal goat follicles collected at different stages of development or atresia. Attempts were also made to determine the localization of mRNAs for these enzymes in the theca cells, mural and cumulus granulosa cells, and oocytes of goat follicles. Concentrations of the three related steroids, P4, testosterone (T0) and E2, were measured in the follicular fluid to further confirm the expression of these enzymes.

2. Materials and methods

Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

2.1. Classification of ovaries and follicles

Ovaries from both prepubertal (2–3 months after birth) and adult goats were obtained from a local abattoir, transported within 3 h to the laboratory in sterilized saline containing 100 IU/ml penicillin and 0.05 mg/ml streptomycin, and maintained at 30–35 °C. Ovaries from adult goats with an obvious corpus luteum were designated as luteal stage ovaries, while the ovary pairs containing corpus luteum in neither ovary were used as follicular stage ovaries. All the ovaries used contained follicles 4 mm or larger in diameter.

Antral follicles were isolated from ovaries in Dulbecco's phosphate buffered saline (D-PBS) with fine forceps under a dissecting microscope. Follicles were classified into nonatretic and atretic groups according to Yang and Rajamahendran [28]. Briefly, the nonatretic follicles had vascularized (pink or red) theca interna and clear amber follicular fluid with no debris, but the atretic follicles had gray theca interna with no blood vessels and flocculent follicular fluid with many dark gobbets. Both the nonatretic and atretic follicles were classified according to diameter into 0.2–0.8, 1–1.8, 2–2.8, and 4–4.8 mm groups. To be more convenient for description, follicles 0.2–0.8, 1–1.8, 2–2.8 and 4–4.8 mm in diameter were designated as early antral, small, medium and large follicles, respectively, in this study. To obtain preantral follicles, the ovarian cortex was broken up with a tissue grinder (T18 basic, IKA, Germany) at 6000 rpm for 5 min, follicles were manually separated from stroma tissue and those 40–80 μm in diameter were selected under a stereomicroscope. Our histological observation indicated that an antrum appeared in goat follicles larger

than 100 μm in diameter and that the 40–80 μm follicles were surrounded with 1–3 layers of cuboidal granulosa cells.

2.2. Preparation of cells

Three cell samples were prepared per follicle diameter, and each sample was used for RT-PCR of all the three enzymes studied. Each sample of intact early antral, small, medium and large follicles consisted of 30–40, 20–25, 10–15 and 5–7 follicles, respectively. Cumulus cells, mural granulosa cells or thecal cells in each sample were collected from 20 to 30 medium follicles. Each oocyte sample consisted of $1–1.2 \times 10^3$ oocytes, and each preantral follicle sample was made of $1–1.2 \times 10^4$ follicles. Mural granulosa cells were collected by rupturing the follicles and scraping the theca interna. The theca interna were further washed after scraping. The released cumulus–oocyte complexes (COCs) were pipetted in D-PBS containing 0.1% hyaluronidase to separate cumulus cells and the oocyte. The mural granulosa cells, theca cells, cumulus cells, oocytes and intact preantral and antral follicles with oocytes inside were frozen immediately after collection and stored at −80 °C until RNA isolation.

2.3. Semi-quantitative reverse transcription polymerase chain reaction

Relative abundance of mRNA for P450scc, P450c17 and P450arom were determined in follicular tissues using semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). The reference gene used was the acidic ribosomal phosphoprotein (PO) gene. Total RNA was extracted from follicular tissues using Trizol reagent (Invitrogen TM, life technologies, USA) according to the manufacturer's instructions. The RNA was resuspended in diethylpyrocarbonate-treated MilliQ water (DEPC-dH_2O) and digested with RNase-free DNase I (Takara Biotechniques, Dalian, China). The purified RNA was dissolved in DEPC-dH_2O and spectroscopically quantified at 260 nm. Purity and integrity of the RNA was assessed by determination of the A260/A280 ratio and electrophoresis in 1% agarose.

The RNA (2 μg from antral follicles, theca cells or granulosa cells or all from around 100 oocytes or 10^3 preantral follicles) was used to generate single-strand cDNA in a 25 μl reaction mixture using 0.2 nM non-adeoxyribonucleotide pd (N) 9 (Takara Biotechniques, Dalian, China) as primers according to the protocol for the M-MLV Reverse Transcriptase Kit (Promega, Madison, WI). The RNA from 2 to 3.8 mm intact follicles was used to generate single-strand cDNA as a positive control under the same conditions. The optimal amount of total RNA and reaction mixture for reverse transcription was determined by testing different RNA concentrations. To eliminate false positives, a reverse transcriptase-negative control lacking the reverse transcriptase was run for each template.

The sequences for P450scc, P450c17 and P450arom were based on the goat sequences obtained from the Entrez Nucleotides database (National Center for Biotechnology Information, Bethesda, MD), and primers were designed using software DNAMAN 5.2.2 to span introns to avoid false results from genomic DNA amplification. The PO primers were constructed from the fully conserved sequences among cattle (AB098956), human (AK129754), rabbit (X15096) and mouse (X15267) and covered the sequences used by Mazerbourg et al. [29], because DNA or RNA sequences for the goat were not available in GenBank. The primer sequences and resulting fragment sizes for all examined genes are shown in Table 1.

Polymerase chain reaction (PCR) was performed in 25 μl mixture containing 1U Takara TaqTM, 2 mM Mg^{2+}, 2.5 μl 10× PCR buffer (Takara Biotechniques, Dalian, China), 1 ng/μl cDNA, 1 mM dNTP, and 0.2 mM primer. The amplifications were done as follows: an initial denaturation step at 94 °C for 4 min, 35 cycles of 94 °C for 30 s, 54 °C for 1 min, and 72 °C for 1 min, and a final elongation step at 72 °C for 10 min. To determine the optimal quantity of reverse transcripts needed for semi-quantitative PCR and to verify that cDNA product was dependent on the amount of transcripts used, various quantities of transcript template and different numbers of cycles were tested before the PCR assay.

Aliquots of the PCR products (10 μl) were fractionated by electrophoresis using a 2% agarose gel containing ethidium bromide, and gel images were digitally captured under UV illumination. Densitometric analyses of the cDNA bands were performed using LabImage 2.7.2 (http://www.labimage.net). The relative abundance of mRNA for a target gene from each group was defined as the ratio of densitometric values relative to PO. No products were found when PCR were performed using RT negative reaction mixtures.

2.4. Radioimmunoassay

Follicular fluid samples were collected from ovarian follicles of different types. Each sample consisted of follicular fluid from 7 to 10 follicles of 2–2.8 mm or 1–2 follicles of 4–4.8 mm in diameter. Three samples were assayed per steroid per follicle diameter (each

Table 1
GenBank (Entrez Nucleotides database) accession numbers and sequences of primers used to amplify first strand cDNA for P450scc, P450c17, P450arom and PO

Gene	Sequences (5′–3′)	Position	PCR product size (bp)	Accession
P450scc	Forward: atccactttcgccacatcg Reverse: ggtctttcttccaggctcct	219–238 451–432	233	D50058
P450c17	Forward: actgaatgcctttgccctgt Reverse: ctgattatgttggtgaccgcc	426–445 581–561	146	AF251387
P450arom	Forward: catcctcaataccaggtccca Reverse: ggtttcctctccacataccca	163–183 314–294	151	AY148883
PO	Forward: gcgacctggaagtccaact Reverse: tcttgcccatcagcaccac	96–114 249–231	Not available	Not available

treatment). Follicular fluid was centrifuged at 3000 rpm for 5 min, and the supernatant was stored at −20 °C until hormone assay. Concentrations of progesterone (P4), testosterone (T0) and estradiol (E2) in follicular fluid in unextracted samples were determined by direct radioimmunoassay (RIA) using a commercial kit (Jiuding Biotechniques Ltd., Tianjin, China), which has been validated for goat samples [30]. Immediately before assay, 20 μl of follicular fluid from each sample was 10-fold diluted with sterilized saline and 100 μl of the diluted fluid was assayed. A sterilized saline assay showed no cross-reactivity and values below the limit of sensitivity (0.1 ng/ml for P4, 1 ng/ml for T0 and 10 pg/ml for E2). The intra- and inter-assay CVs for P4, T0 and E2 were 7.2% and 6.9%, 8.2% and 7.9%, and 8.0% and 7.7%, respectively.

2.5. Statistical analysis

Each treatment (each enzyme or steroid per follicle diameter) was repeated three times using three different follicle samples as indicated earlier in Sections 2.2 and 2.4. Statistical analyses were carried out by ANOVA. Differences between treatments were evaluated by the Duncan multiple comparison test. Data were expressed as means ± S.D. and $P < 0.05$ was considered significant.

3. Results

3.1. The onset of mRNA expression of cytochromes P450scc, P450c17 and P450arom in goat follicles

The expression of mRNAs encoding cytochromes P450scc, P450c17 and P450arom was analyzed in intact nonatretic preantral, 0.2–0.8, 1–1.8 and 2–3.8 mm antral follicles from prepubertal goats. While mRNAs encoding P450scc and P450c17 were well expressed in both antral and preantral follicles, mRNAs for P450arom were expressed only in antral follicles (Table 2, Fig. 1).

Table 2
mRNA expression of cytochromes P450scc, P450c17 and P450arom in nonatretic whole follicles of different sizes from prepubertal goats

Follicle diameter (mm)	P450scc	P450c17	P450acrom
Preantral	+	+	−
0.2–0.8	+	+	+
1–1.8	+	+	+
2–3.8	+	+	+

3.2. Localization of mRNAs encoding cytochromes P450scc, P450c17 and P450arom in goat follicles

The expression of mRNAs encoding cytochromes was analyzed in theca cells, mural and cumulus granulosa cells and oocytes from nonatretic 2–3.8 mm follicles of prepubertal goats. Among the three cytochromes examined, while P450scc mRNA was expressed in both theca and granulosa cells, P450c17 mRNA were expressed only in theca cells and P450arom mRNA only in granulosa cells (Table 3, Fig. 2). Cumulus granulosa cells showed the same pattern of cytochrome mRNA expression as the mural granulosa cells did. Oocytes expressed mRNAs for none of the three cytochromes.

Table 3
Localization for mRNA expression of cytochromes P450scc, P450c17 and P450arom in 2–3.8 mm nonatretic follicles from prepubertal goats

Tissues	P450scc	P450c17	P450arom
Theca cells	+	+	−
Mural granulosa cells	+	−	+
Cumulus cells	+	−	+
Oocytes	−	−	−

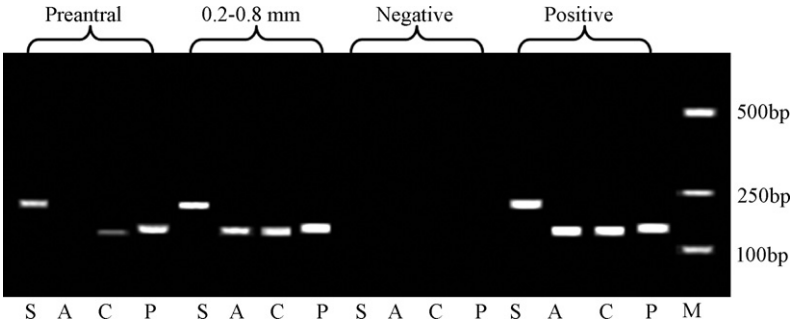

Fig. 1. Gel image of electrophoresis showing expression of mRNAs encoding P450scc (S), P450c17 (C) and P450arom (A) in preantral and 0.2–0.8 mm follicles from prepubertal goats. M, negative and positive stand for standard marker, negative and positive controls, respectively. The RNA from 2 to 3.8 mm intact follicles was used to generate single-strand cDNA as a positive control under the same conditions.

3.3. Cytochrome expression and concentration of steroid hormones in healthy and atretic follicles of different diameters from prepubertal goats

Cytochrome mRNA expression was examined in whole follicles of different diameters and atretic states whereas concentration of steroid hormones was measured in follicular fluid from different follicles. In nonatretic follicles, expression levels of mRNAs encoding all the three cytochromes increased significantly during follicular growth (Figs. 3 and 4). In atretic follicles, however, while the expression of P450scc mRNA increased with increased follicular diameters, expression of P450c17 was low and did not differ between follicular sizes. No expression of mRNA encoding P450arom was observed in atretic follicles.

In nonatretic follicles, concentrations of both P4 and E2 increased while the concentration of T0 decreased significantly from medium sized to large follicles (Fig. 5). When follicles became atretic, however, the level of both T0 and E2 became very low and did not differ between follicle sizes. The concentration of P4 increased to the highest level in atretic follicles of medium sizes, significantly higher than that in large follicles.

3.4. Cytochrome expression and concentration of steroid hormones in healthy follicles of different diameters from adult goats

Cytochrome expression in whole follicles was examined and concentration of steroid hormones in follicular fluid was assayed in nonatretic follicles of different diameters in ovaries at either the follicular or the luteal stage. Changes in cytochrome expression and steroid concentration during follicle growth in adult ovaries at the follicular stage were similar to those in nonatretic follicles of prepubertal animals. Thus, the level of mRNA expression for all the three cytochromes tended to increase during follicular growth, although differences between follicular sizes were not always significant statistically (Fig. 6); while the concentration of T0 decreased significantly from medium to large follicles, concentrations of both P4 and E2 increased, although the increase in P4 was not statistically significant (Fig. 7). In follicles from ovaries at the luteal stage, however, while expression of P450arom increased significantly and that of P450c17 tended to increase, the level of P450scc expression remained low during follicular growth (Fig. 6). However, changes in steroid concentrations during follicular growth were similar

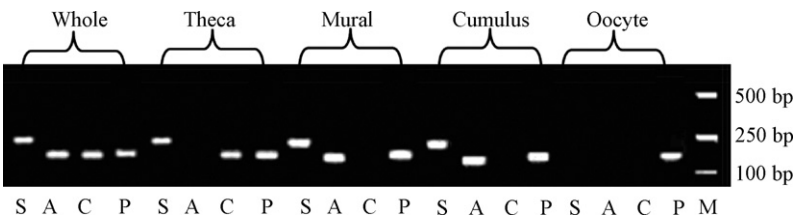

Fig. 2. Gel image of electrophoresis showing expression of mRNAs for P450scc (S), P450c17 (C) and P450arom (A) in the whole follicle, theca interna, mural and cumulus granulosa cells and oocytes from 2 to 4.8 mm follicles of prepubertal goats. P stands for mRNA from the PO gene while M represents the standard marker.

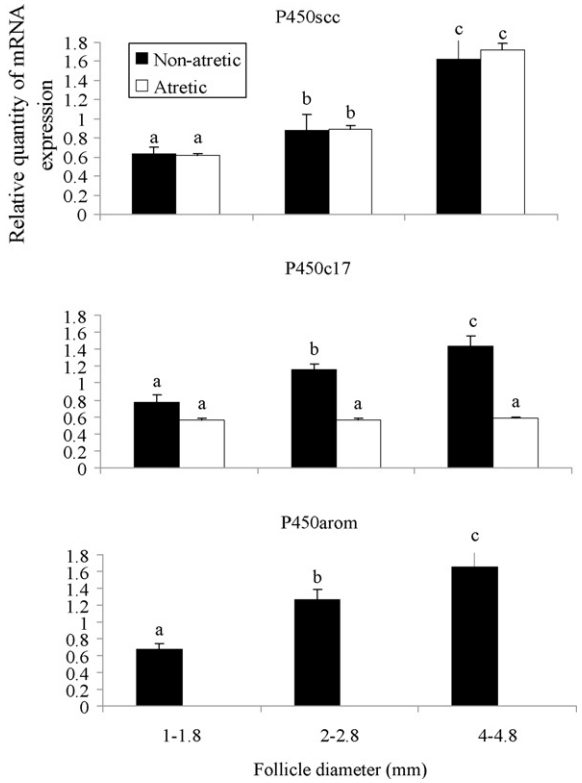

Fig. 3. Relative quantities of mRNAs encoding cytochromes P450scc, P450c17 and P450arom in nonatretic and atretic follicles of different diameters from prepubertal goats. The relative abundance of mRNA for a target gene from each group was defined as the ratio of densitometric values relative to PO. Values without a common letter above their bars differ ($P < 0.05$).

between adult ovaries at the luteal stage and those at the follicular stage (Fig. 7). This suggests that an increased accumulation of P4 occurred in the medium and large follicles of luteal stage ovaries even though there was a relatively low amount of the P450scc enzymes.

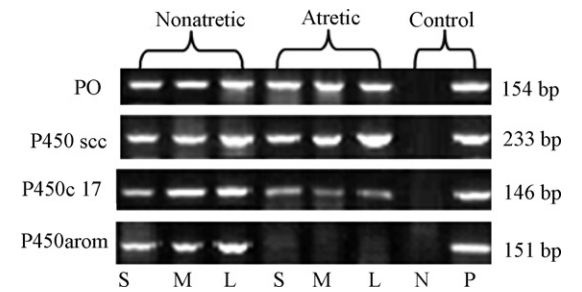

Fig. 4. Gel image of electrophoresis showing expression of mRNAs encoding P450scc, P450c17, P450arom and PO in nonatretic and atretic follicles of small (S, 1–1.8 mm), medium (M, 2–2.8 mm) and large (L, 4–4.8 mm) sizes from prepubertal goats. N and P stand for negative and positive controls, respectively. The RNA from 2 to 3.8 mm intact follicles was used to generate single-strand cDNA as a positive control under the same conditions.

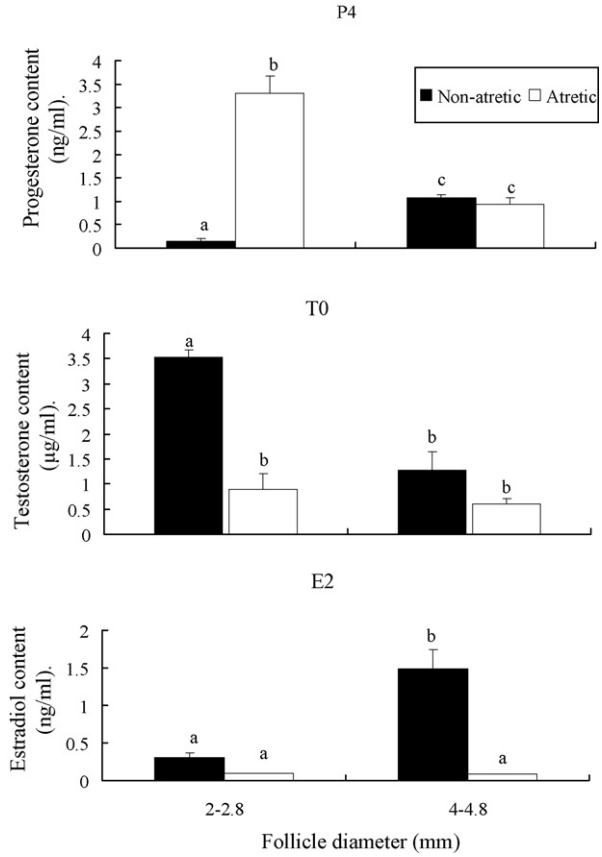

Fig. 5. Contents of progesterone (P4), testosterone (T0) and estradiol (E2) in follicular fluid from nonatretic and atretic follicles of different diameters in prepubertal goats. Values without a common letter above their bars differ ($P < 0.05$).

4. Discussion

This study demonstrated that synthesis of mRNAs encoding P450scc and P450c17 began early in preantral follicles of prepubertal goats, but mRNA encoding P450arom was not expressed until early antral formation. In cattle [31] and sheep [21], expression of P450scc and P450c17 enzymes were also initiated in preantral follicles. In cattle, expression of mRNAs for P450arom was first detected in follicles 4–6 mm in diameter [24,32]. In sheep, growth of ovarian follicles from 1 to 5 mm in diameter was characterized by an increase in P450 aromatase levels in follicles larger than 3.5 mm [33]. Although expression of P450scc and P450arom mRNAs within granulosa cells was associated with follicular recruitment in cattle [32], the follicular stage at which follicular recruitment begins is unknown in the goat. However, since goat oocytes did not acquire their competence to resume meiosis until 0.8–1.2 mm follicles [34], the early expression of P450arom in 0.2–0.8 mm antral

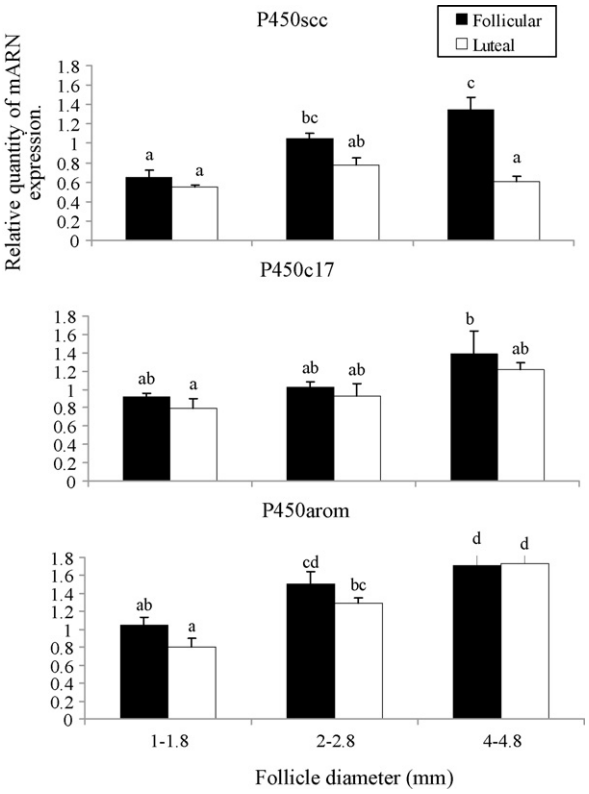

Fig. 6. Relative quantities of mRNAs encoding cytochromes P450scc, P450c17 and P450arom in whole follicles of different diameters from adult goat ovaries at either follicular or luteal stage. The relative abundance of mRNA for a target gene from each group was defined as the ratio of densitometric values relative to PO. Values without a common letter above their bars differ ($P < 0.05$).

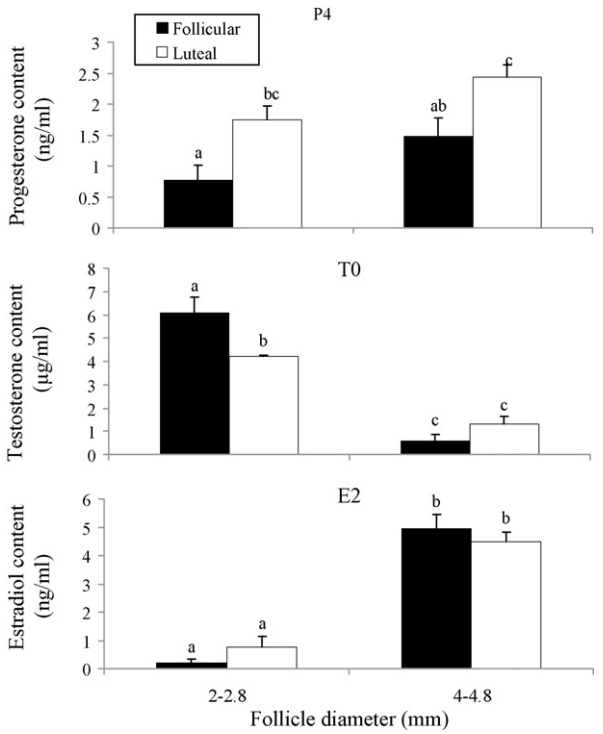

Fig. 7. Contents of progesterone (P4), testosterone (T0) and estradiol (E2) in follicular fluid from follicles of different diameters from adult goat ovaries at either follicular or luteal stage. Values without a common letter above their bars differ ($P < 0.05$).

follicles, together with the higher level of E2 in adult goat follicles compared to prepubertal goat follicles, may suggest that the product of P450 aromatase (E2) is important for goat oocytes to gain their developmental competence.

In this study, mRNA encoding P450scc was expressed in both theca and granulosa cells, whereas P450c17 mRNA was expressed only in theca cells while the P450arom mRNA only in granulosa cells of medium-sized follicles from prepubertal goats. In both cows [24] and ewes [21], while P450scc mRNA was localized to the theca interna cells starting in preantral follicles, expression of P450scc mRNA in granulosa cells was detectable only in follicles about 4 mm (cow) or 2.5 mm (ewe) in diameter. In prepubertal rats, while P450scc was markedly expressed in theca cells, no labeling was observed in granulosa cells until after PMSG treatment [35]. However, no significant differences between follicles from neonatal lamb and those from adult ewe ovaries were observed for the stage of onset of expression of P450scc, P450c17 or P450arom [21]. The mRNA for P450c17 was localized exclusively to the theca interna cells in cattle [24,31,36], sheep [21], pig [37] and human beings [38]. This indicates that conversion of progestagens to androgens occurs solely in the theca interna. Exclusive localization of P450arom mRNA to granulosa cells has also been demonstrated in cattle [24,39], human [38], sheep [21] and rat [40]. In addition, this study showed that goat cumulus cells expressed both P450arom and P450scc mRNAs as the mural granulosa cells did. Xu et al. [24] showed that bovine cumulus cells expressed P450arom mRNA. This is different from results obtained in rats [40] and mice [41], where cumulus cells did not express P450arom mRNA. While mouse cumulus cells promoted maturation of cumulus-denuded oocytes, mouse mural granulosa cells did not [42]. However, both cumulus and mural granulosa cells of goats promoted maturation of cumulus-free oocytes (our unpublished data). Thus, there may be species differences in the functional differentiation between cumulus and mural granulosa cells. In addition, this study demonstrated that goat oocytes did not express mRNAs for any of the three steroidogenic enzymes examined. No expression of the P450arom, P450c17 or P450scc enzymes was observed in ovine oocytes [21].

In this study, the relative quantity of mRNA expression of all the three enzymes examined increased with follicular size in the nonatretic follicles of prepubertal goats. In the atretic follicles, however, while the level of mRNA encoding P450scc increased with follicle diameter, the amount of P450c17 mRNA remained at the lowest level and no mRNA for P450arom was detectable. Follicle growth in sheep [33] and cattle [32] was also associated with a progressive increase in levels of P450scc, P450arom and P450c17. In horses, granulosa cells from dominant follicles had increased levels of P450scc and P450arom during growth, and these two enzymes were lower in granulosa cells from subordinate than in those from dominant follicles [43]. Since oocyte developmental competence increased with follicular sizes [44], an increased expression of these enzymes may be associated with the selection of dominant follicles and oocytes that are more competent. Follicular atresia in cattle [24] and sheep [33] was also characterized by a loss of P450arom in granulosa cells and a decrease in levels of P450c17 in the theca interna. However, expression of P450scc, P450c17 and/or P450arom has been observed in some of the atretic follicles of cattle [24,31] and human beings [38]. In early atresia, bovine thecal cells expressed mRNAs for P450c17 and the steroidogenic acute regulatory protein (StAR), and their expression decreased gradually as atresia progressed [36].

The present results indicated that contents of P4 and E2 increased while that of T0 decreased significantly from medium to large nonatretic follicles of both prepubertal and adult goats, although the increase in P4 in adult follicles did not reach the level of statistical significance. However, the expression of P450c17 increased significantly with follicular growth in nonatretic follicles of prepubertal goats, and it tended to increase in nonatretic follicles of adult animals. In porcine, among individual follicles, P450c17 mRNA concentration was not significantly correlated with follicular fluid T0 concentration on any day of follicular maturation [45]. This suggested that while P4 could be promptly converted into T0 in both the large and medium follicles, T0 could not be efficiently converted into E2 in medium follicles. Although the relatively low level of P450arom mRNAs indicated that the inefficient conversion of T0 into E2 in medium follicles could be due to a low activity of P450arom in these follicles, factors other than mRNA levels could also influence the enzyme activity since mRNA levels for P450arom were not very much different between medium and large follicles. Previous studies showed that concentrations of both P4 and E2 in follicular fluid increased with follicular growth [30,45]. In sheep [46] and cattle [47], concentrations of T0 and androstenedione decreased as follicular diameter increased. Furthermore, the theca interna of bovine antral follicles less than 5 mm in diameter synthesized androstenedione in response to LH before the granulosa cells developed an appreciable ability to metabolize androgen to oestrogen [17]. However, levels of T0 and E2 became very low following atresia of both the medium and large follicles while that of P4 became very high in the atretic medium-sized follicles in this study. This indicated an inability of goat follicles to convert P4 into T0 and E2 after atresia due to a low expression of the P450c17 and P450arom enzymes. In addition, while the level of P450scc expression was significantly lower, the concentration of P4 was significantly higher in medium than in large follicles after atresia. Whether the expression of 3β-hydroxysteroid dehydrogenase (3β-HSD), which is responsible for the conversion of pregnanolone into P4, differs between large and medium goat follicles after atresia needs investigation.

An increased accumulation of P4 was observed in this study in medium and large follicles of the luteal stage ovaries, although the expression of P450scc enzymes was low in these follicles, compared to that in follicles from the follicular stage ovaries. In pigs, P450scc mRNA concentration in newly formed luteal tissue was eightfold greater than that in follicles recovered on the same day of the estrous cycle, but mRNAs for P450c17 or P450arom were not detectable in luteal cells [45]. According to McNatty et al. [48], although steroid synthesis may not be rigidly compartmentalized during follicular development, appreciable amounts of the steroids secreted by the granulosa and theca may enter different compartments before leaving the ovary. Therefore, it is postulated that the large quantity of P4 produced by the corpus luteum may enter the follicle, which not only causes an accumulation of P4, but also inhibits its further synthesis in the affected follicle. In addition, this study showed that changes in cytochrome expression in follicles from adult ovaries at the follicular stage were similar to those in nonatretic follicles of prepubertal animals. Logan et al. [21] found no significant differences between follicles from neonatal lamb and those from adult ewe ovaries for the stage of onset of expression of P450scc, P450c17 or P450arom. Furthermore, since almost all the previous studies cited above dealt exclusively with adult animals, the similar changes observed between those studies and the present study in the three cytochrome enzymes and steroids studied during follicular growth or atresia would suggest a similarity between adult and prepubertal animals in these aspects. However, while displaying similar levels of mRNA expression for the three cytochrome

enzymes studied, adult follicles contained much higher levels of E2 and P4 than the prepubertal follicles did. Follicular fluid from sows has been found to contain more P4 and androstenedione than that from prepubertal pigs [20]. This suggests that translation or posttranslational processing or activation of the P450arom and P450scc enzymes might differ between sexually mature and immature animals, and these events are more influenced by the very complicated environment in mature than in immature animals.

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