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Androstenedione and follicle stimulating hormone involvement in the viability and development of goat preantral follicles *in vitro*

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Abstract

This study investigated the effects of androstenedione and follicle-stimulating hormone (FSH) on the viability and growth of caprine preantral follicles. Ovarian tissues were cultured for 1 or 7 days in Minimum Essential Medium (MEM^+) containing androstenedione (0, 1, 10, 50, or 100 ng/ml), FSH (50 ng/ml), or a combination of these two hormones. Cultured and non-cultured control tissues were processed for histological and fluorescence analysis. In comparison with non-cultured control, a significant reduction was noted in the percentage of normal follicles in all treatments after 1 and 7 days of culture (except treatment with 1 ng/ml of androstenedione for 1 day). As the culture period progressed from 1 to 7 days, treatments with 10 ng/ml of androstenedione + FSH or 50 ng/ml of androstenedione alone maintained the percentage of normal follicles. After 1 day, treatments with 10, 50, or 100 ng/ml of androstenedione + FSH, or with 50 ng/ml of androstenedione alone had more developing follicles than fresh control tissue. When comparing the culture periods, treatments with 1, 10 or 100 ng/ml of androstenedione alone, or FSH alone, or FSH with 1 ng/ml of androstenedione, showed an increase in the percentage of developing follicles. After 1 and 7 days, there were no differences in oocyte and follicular diameter among the treated samples and non-cultured control or MEM^+ cultured tissue. Fluorescence analysis demonstrated that only fragments cultured in 50 or 100 ng/ml of androstenedione + FSH had viable preantral follicles similar to those observed in MEM^+ alone. In conclusion, androstenedione at 50 or 100 ng/ml, either associated with FSH or at 50 ng/ml alone, plays an important role in the maintenance of caprine preantral follicle viability and activation after only a short *in vitro* culture period. In addition, after 7 days MEM^+ alone was efficient in the maintenance of viability and in follicular activation, showing the importance of basic medium composition.

Keywords: androstenedione, caprine, FSH, *in vitro* culture, ovarian follicles.

Introduction

Medium composition is an important factor for successful *in vitro* culture of preantral follicles. Several attempts have been made to develop a culture medium to maximize the number of viable preantral follicles reaching the maturation stage in different species (caprine: Silva *et al*., 2010; bovine: Gutierrez *et al*., 2000; ovine: Cecconi *et al*., 1999; bubaline: Gupta *et al*., 2008; swine: Wu *et al*., 2001). Therefore, it is important to obtain an *in vitro* culture system which allows the complete development of preantral follicles, making them capable of further fertilization with a subsequently achievement of healthy animals.

The α-Minimal Essential Medium (α-MEM), routinely used in caprine preantral follicle cultures, is the richest formulation of MEM, being composed of 21 essential amino acids, vitamins, inorganic salts, and pyruvate. Silva *et al*. (2004a) demonstrated a reduction in caprine preantral follicle survival after *in vitro* culture in MEM without hypoxanthine or energetic substrates, such as pyruvate and glutamine. It has also been demonstrated that addition of these substances to MEM supplemented with penicillin, streptomycin, bovine serum albumin (BSA), and ITS (insulin, transferrin, and selenium) significantly increases the rate of follicular survival. Moreover, hormones such as androstenedione and follicle stimulating hormone can also be added to the basic culture medium for preantral follicles. The effect of these substances (individually or associated) can be evaluated and controlled in an effort to minimize the effects of follicular atresia, which occurs naturally in vivo and leads to a significant loss in female reproductive performance.

Androstenedione is an androgen derived from progesterone, a precursor of estradiol, and is produced in theca cells of ovarian follicles in response to LH action (Couse *et al*., 2006; Taniguchi *et al*., 2007). Androstenedione acts through androgen receptors (AR), which are localized in granulosa cells of developing follicles of sheep (Campo *et al*., 1985), cattle (Hampton *et al*., 2004), chicken (Yoshimura *et al*., 1993), monkey (Hild-Petito *et al*., 1991), human (Horie *et al*., 1992; Otala *et al*., 2004), and rat preantral and antral follicles (Tetsuka *et al*., 1995). In addition, some studies have suggested that this hormone may also act through calcium channels present in the plasma membrane in sites where AR are not present (Machelon *et al*., 1998). The androgens spread through the basement membrane of the follicle and granulosa cells, acting through two

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pathways: (1) as hormones, via receptors, to increase FSH stimulus on aromatase, and (2) as an immediate substrate for estradiol synthesis by aromatase (Taniguchi *et al*., 2007). It has also been demonstrated that this class of hormones induces the appearance of receptors for certain growth factors, such as growth and differentiation factor-9 (GDF-9) and transforming growth factor-β (TGF-β) in granulosa cells and oocytes (Hickey *et al*., 2005). However, it is possible that there is a limit to the effects of androgens on follicular function, since antagonist activity may be observed in high concentrations (Zeleznik *et al*., 2004; Magoffin *et al*., 2005).

Recently, it has been demonstrated using ovine (Andrade *et al*., 2005) and caprine (Matos *et al*., 2007) tissue that the addition of FSH to the culture medium of preantral follicles is important to maintain viability and to promote activation and further follicular growth *in vitro*. Receptors for FSH are expressed in granulosa cells (Ulloa-Aguirre *et al.*, 1995; O'Shaughnessy *et al.*, 1996) from the appearance of primary follicles onward (Wandji *et al.*, 1992; Oktay *et al.*, 1997). Although FSH receptors are not present in primordial follicles, it is likely that this gonadotropin has an indirect effect on early follicular development through the release of paracrine factors produced by large follicles or by ovarian stromal cells (Van Den Hurk and Zhao, 2005).

Although advances have been made in studies related to folliculogenesis in the preantral phase, there are no reports on how androstenedione and FSH may act synergically to promote the maintenance of integrity or further follicular activation and development. Therefore, the aim of this study is to verify whether the presence or absence of different concentrations of androstenedione alone or in association with FSH can promote beneficial effects on viability, activation and growth of caprine preantral follicles cultured *in vitro* for 1 or 7 days.

Materials and Methods

Source of ovaries

Ovaries (n = 8) from four mixed-breed goats (four repetitions) were collected at a local slaughterhouse. Immediately postmortem, the ovaries were washed once in 70% alcohol for 10 s and then twice in Minimum Essential Medium (MEM) supplemented by HEPES and antibiotics (100 µg/ml penicillin and 100 µg/ml streptomycin) and transported in MEM at 4°C within 1 h to the laboratory.

Experimental protocol

In the laboratory, the ovaries from each animal were stripped of surrounding fat tissue and ligaments and then cut in half. The medulla, large antral follicles, and corpora lutea were removed. Subsequently, the ovarian cortex of each pair of ovaries was divided into 25 fragments of approximately 3 mm x 3 mm x 1 mm. One fragment was randomly selected and immediately fixed for histological and ultrastructural studies (to serve as day 0 control). The remaining pieces of ovarian cortex were individually cultured in 24-well culture plates with 1 ml of culture media for either 1 or 7 days at 39°C with 5% CO_2 in air. The basic control medium was called MEM^+ and consisted of α-MEM (pH 7.2 – 7.4) supplemented with ITS (insulin 6.25 µg/ml, transferrin 6.25 ng/ml, and selenium 6.25 ng/ml), 0.23 mM pyruvate, 2 mM glutamine, 2 mM hypoxanthine, and 1.25 mg/ml bovine serum albumin (BSA). Fragments were cultured in MEM^+ alone or in MEM^+ containing androstenedione (1, 10, 50, 100 ng/ml), FSH (50 ng/ml - porcine FSH, Stimufol®, FSH:LH=20:1, donated by Dr. J.F. Beckers, Liège, Belgium) or a combination of these two hormones (Table 1). Unless otherwise indicated, the reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Every other day the culture media was replaced with fresh media. The effects of the different culture treatments were compared using ovarian fragments from the same animal. The concentrations of androstenedione were based on physiological parameters for follicular fluid in cows whereas the concentration of FSH was based on preliminary attempts to culture caprine preantral follicles in our laboratory (Matos *et al*., 2007).

Table 1. Different media tested for the *in vitro* culture of caprine preantral follicles.

Culture medium	Abbreviation
MEM^+	MEM^+
MEM^+ + FSH	FSH
MEM^+ + Androstenedione (1 ng/ml)	A1
MEM^+ + Androstenedione (1 ng/ml) + FSH	A1F
MEM^+ + Androstenedione (10 ng/ml)	A10
MEM^+ + Androstenedione (10 ng/ml) + FSH	A10F
MEM^+ + Androstenedione (50 ng/ml)	A50
MEM^+ + Androstenedione (50 ng/ml) + FSH	A50F
MEM^+ + Androstenedione (100 ng/ml)	A100
MEM^+ + Androstenedione (100 ng/ml) + FSH	A100F

Morphological analysis of preantral follicles

After either 1 or 7 days of culture in each media, the ovarian fragments from each treatment regime, including the fresh control, were fixed individually in Carnoy for 12 h. After fixation, tissues were dehydrated in increasing concentrations of ethanol, clarified with xylene, and embedded in paraffin wax. Serial sections (7 µm) were cut, and every fifth section was mounted on a glass slide and stained with Periodic Acid Schiff – hematoxylin (PAS staining system, Sigma, Inc., St. Louis, MO, USA). Coded anonymized slides were examined using a microscope (Nikon, Japan) under 400X magnification.

Preantral follicles were individually classified according to the shape and number of granulosa cell layers around the oocyte: as either primordial, with one layer of flattened granulosa cells; or as developing follicles, which were further classified as intermediate, with one layer of both flattened and cuboidal granulosa cells; primary, with a single layer of cuboidal granulosa cells; or secondary, with two or more layers of cuboidal granulosa cells (Silva *et al*., 2004a). Primordial and developing follicles were individually determined to be morphologically normal when they showed a healthy oocyte (without a pyknotic nucleus) with granulosa cells organized in layers. Otherwise, degenerated follicles were defined as those with a retracted oocyte, oocyte with a pyknotic nucleus, or disorganized granulosa cells detached from the basement membrane. Overall, 120 follicles were evaluated for each treatment regime (each medium and each culture period). The percentage of healthy primordial and developing follicles was calculated for day 0 (control) and after 1 or 7 days of culture in each medium. In addition, oocyte and follicular diameters were measured with the aid of an ocular micrometer, using only normal follicles from day 0 and *in vitro* cultured follicles thereafter. Two perpendicular diameters were recorded for each follicle and oocyte and the averages for each of these two values were reported for follicle and oocyte diameters, respectively. Care was taken to count each follicle only once, as was also done in our earlier studies (Matos *et al*., 2007). Only follicles with a visible oocyte nucleus were evaluated in order to avoid counting the same follicle twice.

Assessment of preantral follicle viability by fluorescence

Goat preantral follicles were isolated from the ovarian fragments using a modification of the mechanical method described by Lucci *et al*. (1999). Briefly, with a tissue chopper (The Mickle Laboratory Engineering Co., Gomshal, Surrey, UK) adjusted to a sectioning interval of 75 µm, samples were cut into small pieces, which were placed in MEM and suspended 40 times using a large Pasteur pipette (diameter of about 1600 µm) and then 40 times with a smaller Pasteur pipette (diameter of approximately 600 µm) in order to dissociate preantral follicles from stroma. The obtained material was passed through 200 µm nylon mesh filters, resulting in a suspension containing preantral follicles smaller than 100 µm in diameter. This procedure was carried out within 10 min at room temperature.

Preantral follicles were analyzed using a two-color fluorescence cell viability assay based on the simultaneous determination of live and dead cells by calcein-AM and ethidium homodimer-1, respectively. While the first probe detected intracellular esterase activity in viable cells, the latter labeled nucleic acids of non-viable cells showing plasma membrane disruption. Before being labeled, follicles were washed once by centrifugation at 100xg for 5 min and resuspended in MEM. The test was performed by adding 4 µM calcein-AM and 2 µM ethidium homodimer-1 (Molecular Probes, Invitrogen, Karlsruhe, Germany) to the suspension of isolated follicles, followed by incubation at 37°C for 15 min, then mounting on a glass microscope slide in 5 µl antifading medium (DABCO, Sigma, Deisenhofen, Germany) to prevent photobleaching, and finally examining using an a DMLB fluorescence microscope (Leica, Germany) (400X). The emitted fluorescent signals of calcein-AM and ethidium homodimer were collected at 488 and 568 ηm, respectively. Oocytes and granulosa cells were considered live if the cytoplasm was stained positively with calcein-AM (green) and chromatin was not labeled with ethidium homodimer (red).

Statistical analyses

Data were statistically analyzed with Kolmogorov-Smirnov and Bartlett's tests, which were applied to confirm normal distribution and homogeneity of variance, respectively. Analysis of variance was made using the GLM procedure of SAS (1999), and Dunnett's test was applied for comparison of hormone-treated samples with control groups. Student Newman Keuls' (SNK) test was used to compare percentages of surviving primordial or developing follicles between treatments and days of culture. Fluorescent probe data were analyzed by Chi-Square test. Differences among groups were considered significant when $P < 0.05$, and results were expressed as mean $\pm$ standard deviation (SD).

Results

Caprine preantral follicle survival after in vitro culture

A total of 2,520 preantral follicles were analyzed. Classic histological techniques showed that there were both morphologically normal (Fig. 1A) and degenerated follicles (Fig. 1B) before and after *in vitro* culture for 1 or 7 days, respectively. Degenerated follicles, had cytoplasmic retraction, nuclear pyknosis, and disorganization of granulosa cells. The percentages of morphologically normal preantral follicles in control

non-cultured tissues and after 1 or 7 days of culture are shown in Fig. 2. It was verified that when all treatments were compared to the fresh control, there was a significant reduction in the percentage of normal follicles after 1 and 7 days of culture, except in tissue treated with 1 ng/ml of androstenedione after 1 day. After 1 or 7 days, all treatments tested showed percentages of normal follicles similar to those observed after culture in MEM alone. As the culture period progressed from day 1 to 7, it was observed that only cultures treated with 10 ng/ml of androstenedione associated with FSH or with 50 ng/ml of androstenedione alone maintained a percentage of morphologically normal follicles on day 7 similar to that on day 1. In the tissues from other treatment regimes, the percentage of normal follicles was significantly reduced.

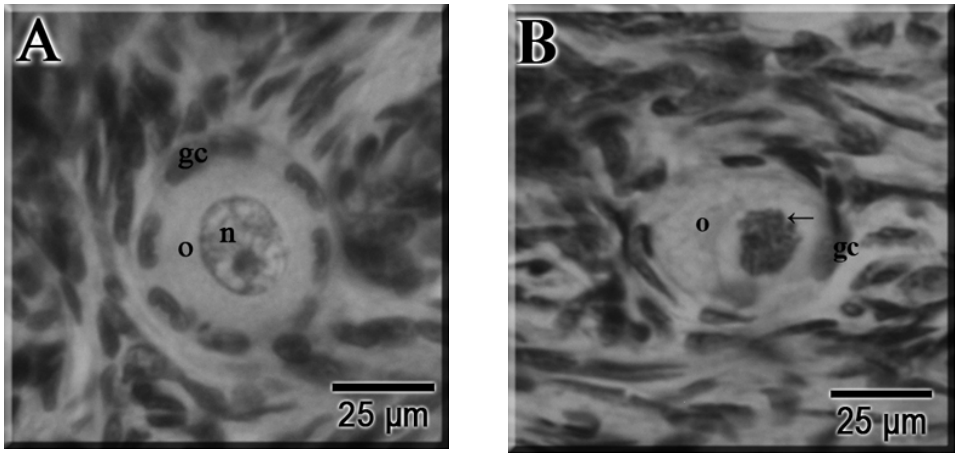
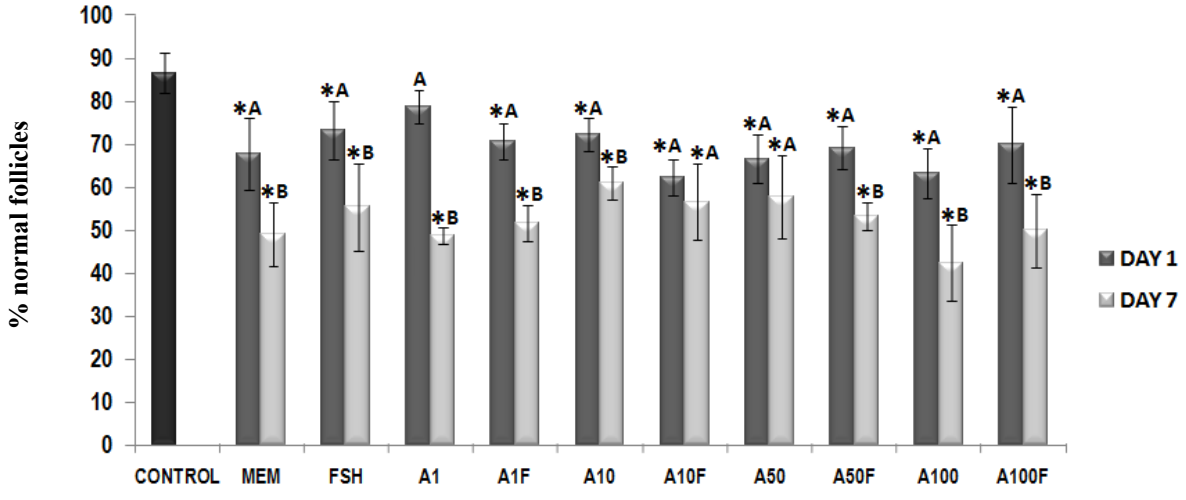


Figure 1. Histological sections (400X) of preantral follicles cultured for 7 days with FSH plus androstenedione (50 ng/ml; A) or in MEM^{+} alone (B). Figure 1A = normal follicle. Figure 1B = degenerated follicle. Gc = granulosa cell; o = oocyte; n = oocyte nucleus; arrow = nuclear pyknosis



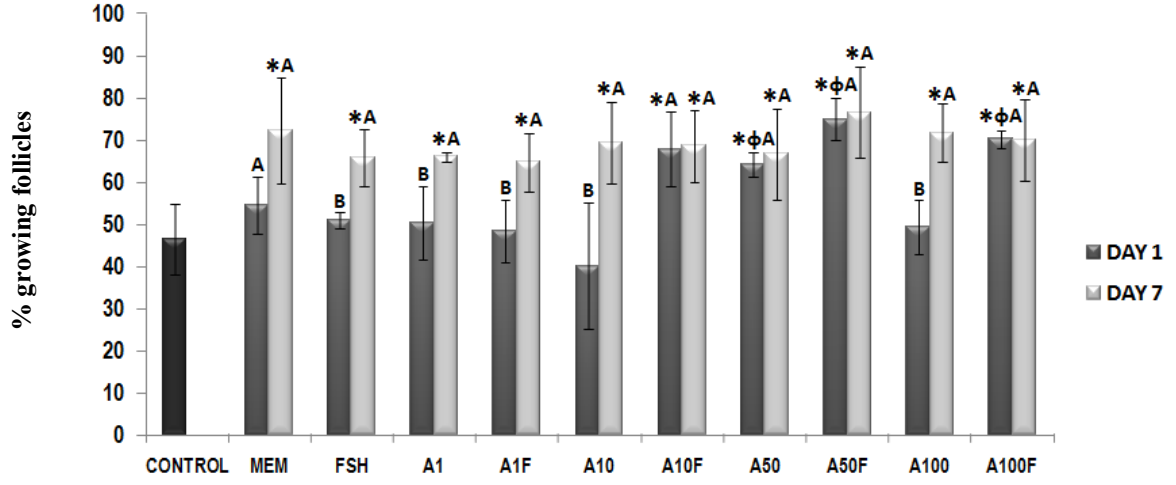
*Differs significantly from control follicles ($P < 0.05$).
AB Differs significantly between days of culture in the same treatments ($P < 0.05$).

Figure 2. Percentages of morphologically normal caprine preantral follicles in control non-cultured tissue and after 1 or 7 days of culture in different medium.

Activation of caprine preantral follicles after in vitro culture

After 1 day of culture, only treatments containing 10, 50, or 100 ng/ml of androstenedione associated with FSH or containing 50 ng/ml of androstenedione alone showed percentages of activated (developing) preantral follicles significantly higher than that of the non-cultured control (Fig. 3). After 7 days, a significant increase in the percentage of developing follicles was observed in all treated cultures, compared to non-cultured control tissue.

When follicles cultured in other treatments were compared to those cultured in MEM$^+$ after 1 day of culture only, treatments containing 50 or 100 ng/ml of androstenedione + FSH (A50F or A100F) or 50 ng/ml of androstenedione alone showed a significant higher percentage of developing follicles. After comparing the culture periods with each other, it was verified that a significant increase in the percentage of developing follicles after 7 days of culture, when compared to day 1, was observed in tissues treated with FSH alone, with 1, 10, or 100 ng/ml of androstenedione alone, or with 1 ng/ml of androstenedione + FSH.

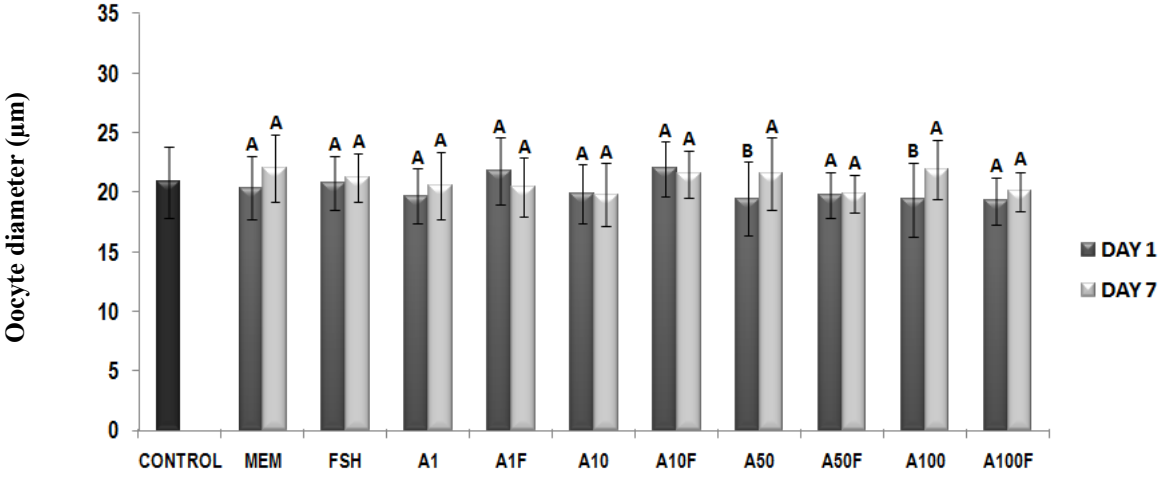


*Differs significantly from control follicles (P < 0.05).
Φ Differs significantly from MEM alone in each day of culture (P < 0.05).
AB Differs significantly between days of culture in the same treatments (P < 0.05).

Figure 3. Percentages of growing preantral follicles in control non-cultured tissue and after 1 or 7 days of culture in medium containing androstenedione and/or FSH.

In vitro growth of caprine preantral follicles

After 1 and 7 days of culture, oocyte (Fig. 4) and follicular diameters (not shown) did not differ when all treatments tested were compared with the fresh control (27.2 ± 9.8 µm) or with MEM$^+$ alone (26.8 ± 7.2 and 26.7 ± 10.1 µm). When comparing the culture periods, it was noted that cultures performed in 100 ng/ml of androstenedione alone promoted a significant increase in both oocyte and follicular diameter after 7 days. This comparison also showed that treatment with 50 ng/ml of androstenedione alone promoted an increase in oocyte diameter only (P < 0.05).

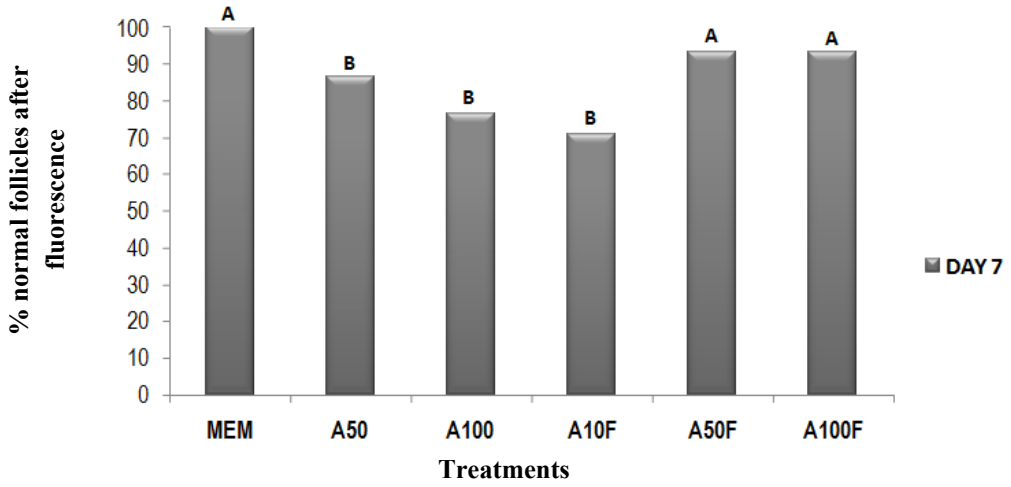


AB Differs significantly between days of culture in the same treatments (P < 0.05).

Figure 4. Mean oocyte diameter (µm) in the control non-cultured tissue and after 1 or 7 days of culture in medium containing androstenedione and/or FSH.

Assessment of preantral follicle viability by fluorescence

Based on the results obtained in histological analysis, we performed an assessment of follicular viability through fluorescence microscopy (Fig. 5 and 6) of fragments from fresh control tissue and in those cultured for 7 days in MEM$^+$ alone, or in MEM$^+$ supplemented by FSH (50 ng/ml) and 10, 50, or 100 ng/ml of androstenedione, or in MEM$^+$ supplemented by 50 and 100 ng/ml of androstenedione alone. The results showed that only treatments with 50 or 100 ng/ml of androstenedione + FSH produced a percentage (both 93.3%) of viable preantral follicles similar to MEM$^+$ alone (100%); percentages in the other concentrations tested were significantly lower than those in MEM$^+$.



AB Differs significantly from MEM alone ($P < 0.05$).

Figure 5. Percentages of normal follicles after fluorescent analysis.

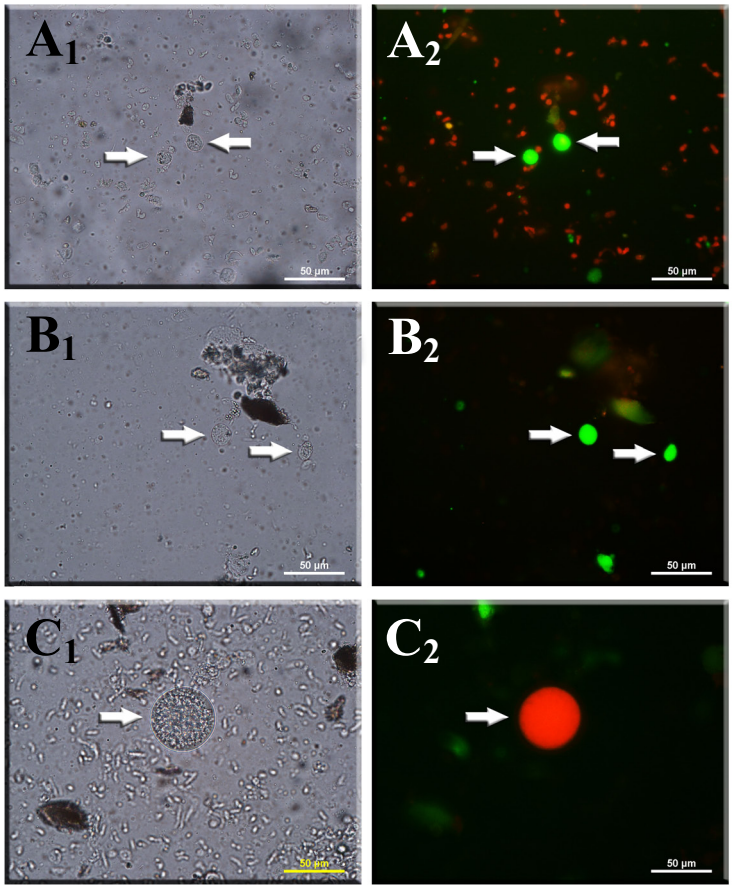


Figure 6. Fluorescent analysis in cultured follicles for 7 days with MEM alone (A_2) and with FSH plus 50 ng/ml (B_2) or 100 ng/ml (C_2) of androstenedione.
A_1, B_1, C_1 = preantral follicles without fluorescent color.

Discussion

The importance of androstenedione and/or FSH in the *in vitro* culture of caprine preantral follicles was demonstrated for the first time in this study. It is important to emphasize that the concentrations of androstenedione were chosen based on studies which measured the level of this hormone in bovine follicular fluid.

Upon comparison with fresh control tissue, a significant reduction in the percentage of normal preantral follicles was observed in most of the treatment regimes tested in this study (except 1 ng/ml of androstenedione). Similar reductions were also observed in previous studies with caprine preantral follicles using different factors such as EGF (Epidermal Growth Factor; Celestino *et al*., 2009) and VEGF (Vascular Endotelial Growth Factor; Bruno *et al*., 2008a) and may be due to changes in temperature, pH, and osmolarity originating from *in vitro* culture. In the present study, in all treatments tested after 1 or 7 days, the percentage of normal follicles was similar to that found in the culture with MEM^+ alone. It is known that medium composition is an important factor for success during *in vitro* culture of preantral follicles. The α-MEM used in our study is one of the richest formulation of MEM and is composed of 21 essential amino acids, B-vitamin complexes, vitamins C and D, inorganic salts, and pyruvate. This medium was also supplemented with glutamine, hypoxanthine, BSA, and ITS. Silva *et al*. (2004a) have demonstrated that addition of hypoxanthine and energetic substrates, such as pyruvate and glutamine, as well as BSA and ITS to the basic culture medium (MEM) significantly increases follicular survival that is the percentage of morphologically normal follicles. Furthermore, in our study, as the culture period progressed, only fragments cultured in 10 ng/ml of androstenedione + FSH or in 50 ng/ml androstenedione alone maintained a percentage of morphologically normal follicles comparable with day 1 after 7 days of culture. These results suggest that, in addition to the basic culture medium that is rich in supplements, the presence of androstenedione in specific concentrations may act in the maintenance of follicular survival after 7 days of *in vitro* culture. Indeed, in post-partum rats treated in vivo with androstenedione, there was a reduction in the levels of follicular apoptosis (Goyeneche *et al*., 2002).

To confirm the results obtained with classical histology, fluorescence microscopy was performed. Only fragments cultured in 50 and 100 ng/ml of androstenedione + FSH were similar to those cultured in MEM^+ in follicular viability. The technique of fluorescence microscopy has been successfully used in the analysis of bovine (Schotanus *et al*., 1997) and caprine (Rossetto *et al*., 2009) preantral follicles. This method can be used to analyze parameters of viability in follicles, and thus offers a new approach for investigating metabolic and developmental aspects of folliculogenesis *in vitro*.

After 1 day of culture, only tissues treated with 10, 50 or 100 ng/ml of androstenedione + FSH and with 50 ng/ml of androstenedione alone showed percentages of follicular activation significantly higher than that of non-cultured (fresh) control tissue. These data suggest that androstenedione, beginning from a low concentration (10 ng/ml), may act synergically with FSH to promote follicular activation as quickly as within 1 day of culture. In addition, intermediate concentrations of androstenedione (50 ng/ml) may also promote follicular activation in a short period, either in the presence or absence of FSH. Androstenedione is an androgen derived from progesterone and is produced in theca cells of ovarian follicles in response to LH (Couse *et al*., 2006; Taniguchi *et al*., 2007). After its production, androstenedione is converted in testosterone, which is converted to estradiol in granulosa cells, under aromatase action. This latter has its action modulated by FSH (Yarak *et al*., 2005), suggesting that androstenedione and FSH may have a synergic role in follicular development. Moreover, it was recently demonstrated that addition of FSH to the culture medium of ovine (Andrade *et al*., 2005) and caprine (Matos *et al*., 2007; Magalhães *et al*., 2009) preantral follicles is important to the maintenance of viability and the activation of follicles, as well as for further follicular growth *in vitro* (Silva *et al*., 2004b). Joyce *et al*. (1999) reported that FSH stimulates the expression of some growth factors, such as Kit Ligand, Bone Morphogenetic Protein-15 and Growth Differentiation Factor-9 (Thomas *et al*., 2005), which are important for the regulation of early folliculogenesis. Furthermore, after 7 days of culture, all treatments tested showed percentages of follicular activation higher than those observed in fresh control, suggesting that culture conditions did not affect the potential of follicle development. In addition, after comparison with MEM^+ after 7 days, no significant differences in follicular activation were observed among treatments. In goats, it has been observed that MEM^+ alone promoted activation of primordial follicles even after 1 day of *in vitro* culture of ovarian tissue fragments (Bruno *et al*., 2008b; Lima-Verde *et al*., 2009), probably due to its rich composition. Moreover, *in vitro* conditions possibly improve follicle development, thus providing the release of stimulatory factors or cessation of the production of inhibitory factors by stromal, granulosa, or theca cells.

After 1 and 7 days of culture, both oocyte and follicular diameters did not differ in all treatments in comparison with fresh control tissue or tissue cultured in MEM^+ alone. The culture medium used in the present study was α-MEM without ribonucleosides. Hartshorne (1997) reported that addition of ribonucleosides to the *in vitro* culture medium is essential only when rapid cell proliferation is necessary. In our case, accelerated

oocyte growth or rapid proliferation of granulosa cells in small preantral follicles may be inappropriate, leading to a rupture of the basement membrane and to follicle deformation due to the non-preservation of their spherical structure (Lenie *et al*., 2004). Furthermore, some studies have shown that MEM^+ alone, with the same supplementation used in our study, can promote oocyte and follicular growth (Martins *et al*., 2005; Matos *et al*., 2006). Upon comparing the culture periods, we observed that fragments cultured in 100 ng/ml of androstenedione showed an increase in both oocyte and follicular diameters after 7 days, and fragments cultured in 50 ng/ml of androstenedione showed only an increase in oocyte diameter. Some studies performed with primates showed that androgens positively regulate follicular development, increasing FSH receptors in granulosa cells and promoting the growth of small follicles (Vendola *et al*., 1998; Weil *et al*., 1999). Moreover, Yang and Fortune (2006) showed that testosterone promotes the growth of bovine follicles activated *in vitro* and suggested that its stimulatory effect is mediated through androgen receptors in the stroma and/or follicular cells. The increase in oocyte and follicular diameters observed in the highest androstenedione concentration (100 ng/ml) may be indicative of follicular degeneration, since this treatment did not maintain the same percentage of morphologically normal follicles after 7 days of culture as did lower concentrations. Moreover, in high concentrations, androgens may have antagonist effects on follicular functions, inhibiting development and promoting follicular degeneration (Goyeneche *et al*., 2002; Zeleznik *et al*., 2004, Magoffin, 2005).

It can be concluded that androstenedione, at 50 or 100 ng/ml, associated with FSH, or at 50 ng/ml alone, could have an important role in the maintenance of caprine preantral follicle viability and activation after only a short *in vitro* culture period. Moreover, at 50 ng/ml, androstenedione promotes oocyte growth after 7 days of culture. In addition, after 7 days, MEM^+ alone was efficient in the maintenance of viability, as well as in follicular activation, showing the importance of basic medium composition and its supplements. However, more research is necessary, both to develop an efficient culture medium to promote caprine preantral follicle development and to provide complete elucidation of the role of androstenedione and its synergic action with FSH on early caprine folliculogenesis.

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