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RESEARCH ARTICLE

# In vitro culture of goat preantral follicles

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**ABSTRACT**

The main objective of study was to know the effect of Growth Hormone (GH), Insulin like Growth Factor-I (IGF-I), Epidermal Growth Factor (EGF), Thyroxine ($T_4$) and Follicle Stimulating Hormone (FSH) individually or in combination on *in vitro* development of goat preantral follicles (PFs). Bicarbonate buffered tissue culture medium 199 (TCM 199B) without any supplementation was used as control medium. Goat PFs (diameter ranging from 150-400µ) were cultured for a period of six days in different concentrations of above mentioned hormones and growth factors individually and also in different combinations. The proportion of PFs exhibiting growth, increase in diameter, antrum formation and extrusion of oocyte in culture were highest when the culture medium was supplemented with GH (2 mIU/ml), IGF-I (20 ng/ml), EGF (50 ng/ml), Thyroxine (1 µg/ml) and FSH (2 ng/ml) individually. The proportion of PFs exhibiting growth, increase in diameter, antrum formation and extrusion of oocytes was 98.07±1.92, 52.27±3.01, 88.46±4.57 and 25.0±0.00 respectively when cultured in $T_4$+FSH+GH+EGF. In conclusion, it can be said that, the addition of GH, IGF-I, EGF, $T_4$ and FSH in different concentrations significantly improved the *in vitro* development of goat PFs.

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## INTRODUCTION

Biotechnology techniques depend on the predictable production of fully developed oocytes, currently their availability is limited by the small number of antral follicles present in the ovaries. An alternative option is use of primordial and preantral follicles. Mammalian ovary is endowed with a large number of preantral follicles, which is a treasure of germ cells. Although large populations of preantral follicles with meiotically incomplete oocytes are present in ovaries, most of them are lost at various stages of development owing to atresia and only a minority of oocytes becomes available for ovulation. Therefore, to maximize the utilization of female gametes, repeatable *in vitro* techniques for maturing preantral follicles and subsequent fertilization of oocytes are desirable.

Several methods were developed to isolate and culture preantral follicles from ovaries of the sheep (Cecconi *et al.,* 1999 & 2004; Hemamalini *et al.,* 2003; Tamilmani *et al.,* 2005; Arunakumari *et al.,* 2007), goat (Silva *et al.,* 2004 a&b; Huanmin and Yong,2000; Zhou and Zhang, 2005 a,b & c and 2006), dog (Bolamba *et al.,* 1998 & 2002), human (Abir *et al.,* 2001; Zhang *et al.,* 2002; Carlsson *et al.,* 2006). In contrast, *in vitro* folliculogenesis in large mammals is still largely unsuccessful because of long culture time, greater follicle diameters and thicker follicular wall. Several laboratories have reported the use of growth factors (GFs) and hormones in *in vitro* culture of PFs from different farm animals. However, success in meiotic maturation of oocytes from cultured PFs of goat was achieved in only a few recent studies (Chelikani *et al.,* 1998; Silva *et al.,* 2004 a&b; Huanmin and Yong, 2000; Zhou and Zhang, 2005a, b & c and 2006). *In vitro* embryo production from cultured PFs was successfully reported in different animals like pig (Wu *et al.,* 2001 a&b), buffalo (Gupta *et al.,* 2008), sheep (Arunakumari *et al.,* 2010) and goat (Magalhaes *et al.,* 2011a). Keeping the above points in view, the present investigation was undertaken to study the influence of different hormones and growth factors like GH, EGF, FSH, IGF and Thyroxin on *in vitro* development of goat preantral follicles.

## MATERIALS AND METHODS

Unless otherwise stated, all culture media, hormones, growth factors, fetal calf serum (FCS) and chemicals were purchased from the Sigma (St. Louis, MO, USA) and plastics from Nunclon (Roskilde, Denmark). Barring the media supplemented with different growth factors and hormones all other solutions were filtered through a 0.22 µm sterilizing filter (Sartorius, Germany) prior to use. All media were incubated at 39°C under a humidified atmosphere of 5% $CO_2$ in air for 1 h prior to use.

### *Handling medium for follicles*

HEPES buffered tissue culture medium 199 (TCM 199H) was supplemented with 0.23mM of Sodium Pyruvate (TCL 015, HiMedia, India), 2mM L-Glutamine (TC 243, HiMedia, India) and 50 µg/ml Gentamicin sulfate (A 010, HiMedia, India). The medium was sterilized by filtration through a 0.22µm

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filter and incubated at 39°C under humidified atmosphere in 5% $CO_2$ in air for 1 h prior to use.

***Control medium***

Bicarbonate buffered tissue culture medium 199 (TCM 199B) was supplemented with 50 µg/ml gentamycin sulphate. This medium was freshly prepared weekly and stored at 4°C.

***Preparation of culture media supplemented with different growth factors and hormones***

Preparation of phosphate buffered saline (PBS), the handling medium for PFs [HEPES-buffered tissue culture medium 199 (TCM199H), supplemented with 25 IU/ml heparin and 50 µg/ml gentamicin sulphate], 0.5% bovine serum albumin (BSA), stock solutions of EGF and FSH have been described by Tamilmani et al.(2005), Thyroxin stock solution by Arunakumari et al. (2007). Arunakumari et al. (2010) described the preparations of Growth hormone and IGF-I stock solutions so far.

***Collection of ovaries, isolation, selection and classification of preantral follicles***

Procedures for the collection of ovaries at slaughter house, transport to the laboratory, isolation, selection and classification of the PFs for the culture were described earlier (Hemamalini *et al.,* 2003; Tamilmani *et al.,* 2005; Rajarajan *et al.,* 2006; Arunakumari *et al.,* 2007).

Each ovary was cut into two halves along its longitudinal axis, the medulla part was scooped out and the remaining ovarian cortex was dissected into thin slices using a 26 gauge sterile surgical blade. Under a stereo zoom microscope (Nikon, Japan) these cortical slices were subjected to microdissection in handling medium for isolation of the PFs in a size range of 150–400 µm. Care was taken to leave a small amount of stromal tissue attached to the basement membrane of the follicles (Plate 1: A). PFs having a centrally placed spherical oocyte with an intact basement membrane (Plate 1: A) were chosen for culture.

***Culture procedure and assessment of PFs development***

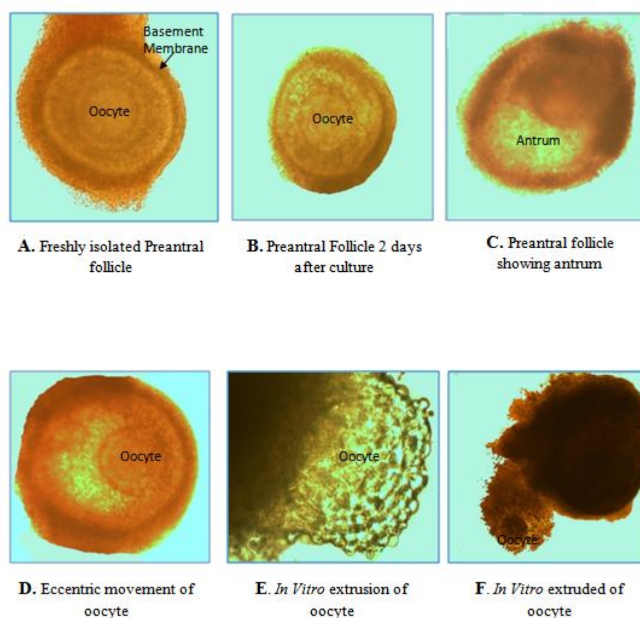
Culture procedures for PFs were followed, as standardized previously (Hemamalini *et al., 2003*; Tamilmani *et al.,* 2005; Arunakumari *et al.,* 2007

***Morphological evaluation of preantral Follicles***

Each follicle was morphologically evaluated every 24 h during culture period using an inverted microscope (Leica, DMIRB, Germany) for increasing diameter, antrum formation (Plate 1: B, C and D) and extrusion of oocyte from the follicle if any takes place (Plate 1: E and F). At the end of the culture, the *in vitro* cultured follicles were carefully opened (if they had not ovulated) using two 26 gauge needles attached to 1 ml syringe barrel, to release the cumulus oocyte complexes (COC).

***Experiments***

Two different experiments were conducted. In the first experiment influence of the individual growth factors and hormones on *in vitro* development of goat preantral follicles was investigated by supplementing the control medium with Thyroxin, FSH, GH, EGF or IGF-I (Table 1).




**A.** Freshly isolated Preantral follicle **B.** Preantral Follicle 2 days after culture **C.** Preantral follicle showing antrum

**D.** Eccentric movement of oocyte **E.** *In Vitro* extrusion of oocyte **F.** *In Vitro* extruded of oocyte

Different concentrations of the individual growth factors and hormones investigated were selected based on preliminary results (data not shown). After determining the best concentration for the each of the above five growth factors and hormones (Table 1), the effect of using them in different combinations was examined in the second experiment (Table 2).

***Statistical analysis***

After confirming that the (i) variation between replicates in each experiment and (ii) treatment by replicate interactions were not significant (ANOVA, SPSS 17), the data from different replicates in each experiment were pooled. Analysis of variance, followed by Duncan's multiple range test, was undertaken in different experiments separately to compare the mean increase in follicle diameter, proportion of follicles exhibiting growth, antrum formation and *in vitro* extrusion of oocyte of oocytes from cultured follicles.

## RESULTS AND DISCUSSION

This was the first study to investigate the effect of different concentrations of various hormones and growth factors on *in vitro* development of goat preantral follicles (PFs). From a total of 150 ovaries, 1950 preantral follicles were isolated and 1444 were used in the two experiments Tables (1-6). The present results indicated that among different concentrations of GH, 2mIU/ml showed highest proportion of PFs exhibiting growth, increase in diameter, antrum formation and extrusion of oocytes (Table 1). Extrusion of the oocyte was observed in the PFs when cultured in 1 mIU/ml and 2 mIU/ml of GH supplemented medium as reported in adult sheep PFs (Arunakumari *et al.,* 2010). These results are in agreement with the present results. Whereas, in caprine PFs the antrum formation and oocyte maturation was observed irrespective of GH concentrations (Magalhaes *et al.,* 2011b). Among IGF-I 20 ng/ml showed better results in all the parameters as compared to 10, 15 ng/ml and control medium (Table 2). Both IGF-I and IGF-II have recently been reported to increase bovine PF and oocyte diameter in serum-free culture, and to promote follicular differentiation (Itoh *et al.,* 2002). Similarly,

**Table 1** Effect of different concentrations of Growth Hormone (GH) on *in vitro* development of goat preantral follicles

| Concentration of GH (m IU/ml) (Replicates / No. Follicles) | Proportion of PFs Exhibiting Growth (Mean ± SE) | Average Increase in Diameter (µ) of PFs (Mean ± SE) | Proportion of PFs Exhibiting Antrum Formation (Mean ± SE) | Proportion of PFs Extruded Oocytes (Mean ± SE) |
|---|---|---|---|---|
| 1.0 (8/48) | 68.21±8.79[a] | 25.89±2.56[a] | 64.20±6.90[ab] | 14.60±2.11[a] |
| 2.0 (8/48) | 84.33±8.22[b] | 33.78±2.72[b] | 83.90±9.16[c] | 22.69±2.38[b] |
| 4.0 (8/48) | 74.37±8.24[a] | 24.10±2.71[a] | 70.41±7.54[ab] | 16.33±3.21[a] |
| Control (8/48) | 58.32±7.78[a] | 21.33±2.38[a] | 43.33±13.33[a] | 0.00 |

Figures with different superscripts within a column are significantly different. One way ANOVA followed by Duncan's Multiple Range Test ($P \leq 0.05$).

**Table 2** Effect of different concentrations of Insulin like Growth Factor-I (IGF-I) on *in vitro* development of goat PFs

| Concentrations of IGF-I (ng/ ml) (Replicates/ No. of Follicles) | Proportion of PFs Exhibiting Growth (Mean ± SE) | Average Increase in Diameter (µ) of PFs (Mean ± SE) | Proportion of PFs Exhibiting Antrum Formation (Mean ± SE) | Proportion of PFs Extruded Oocytes (Mean ± SE) |
|---|---|---|---|---|
| 10(8/48) | 56.66±8.47[a] | 25.48±4.24[ab] | 42.85±8.37[a] | 12.33±4.1[a] |
| 15 (8/48) | 60.91±7.04[a] | 18.45±3.29[b] | 38.74±12.15[a] | 11.42±5.2[a] |
| 20 (8/48) | 80.47±10.70[b] | 39.69±3.92[c] | 57.47±13.73[b] | 20.12±2.61[b] |
| Control(8/48) | 34.04±8.19[a] | 15.41±2.40[a] | 22.76±9.83[a] | 0.00 |

Figures with different superscripts within a column are significantly different. One way ANOVA followed by Duncan's Multiple Range Test ($P \leq 0.05$).

**Table 3** Effect of different concentrations of Epidermal Growth Factor (EGF) on *in vitro* development of goat preantral follicles

| Concentrations of EGF (ng/ ml) (Replicates/ No. of Follicles) | Proportion of PFs Exhibiting Growth (Mean ± SE) | Average Increase in Diameter (µ) of PFs (Mean ± SE) | Proportion of PFs Exhibiting Antrum Formation (Mean ± SE) | Proportion of PFs Extruded Oocytes (Mean ± SE) |
|---|---|---|---|---|
| 10 (8/48) | 60.86±6.72[a] | 25.78±4.42[a] | 45.97±9.37[ab] | 16.41±2.27[a] |
| 25 (8/48) | 68.15±9.69[a] | 31.52±4.47[a] | 54.50±9.55[ab] | 22.42±4.3[a] |
| 50 (8/48) | 78.95±7.54[a] | 38.64±3.32[b] | 62.20±10.01[c] | 26.87±3.82[a] |
| Control (8/48) | 44.44±3.67[a] | 23.20±4.0[a] | 33.32±4.17[a] | 0.00 |

Figures with different superscripts within a column are significantly different. One way ANOVA followed by Duncan's Multiple Range Test ($P \leq 0.05$).

**Table 4** Effect of different concentrations of Thyroxine ($T_4$) on *in vitro* development of goat preantral follicles

| Concentrations of $T_4$ (µg/ml) (Replicates/ No. of Follicles) | Proportion of PFs Exhibiting Growth (Mean ± SE) | Average Increase in Diameter (µ) of PFs (Mean ± SE) | Proportion of PFs Exhibiting Antrum Formation (Mean ± SE) | Proportion of PFs Extruded Oocytes (Mean ± SE) |
|---|---|---|---|---|
| 0.5 (7/42) | 77.14±8.08[a] | 27.50±3.11[ab] | 72.37±5.71[b] | 16.66±0.00[a] |
| 1.0(7/42) | 97.14±2.85[b] | 30.88±2.61[b] | 91.42±4.04[c] | 16.66±0.00[a] |
| 2.0(7/42) | 80.00±0.00[a] | 25.35±3.69[ab] | 68.57±4.04[b] | 16.66±0.00[a] |
| Control (7/42) | 42.71±3.68[a] | 14.07±1.65[a] | 31.42±7.37[a] | 0.00 |

Figures with different superscripts within a column are significantly different. One way ANOVA followed by Duncan's Multiple Range Test ($P \leq 0.05$).

Gutierrez *et al.,* (2000) also found that IGF–I had positive effect on follicular diameter and antrum formation in bovines. In contrast, Derrar *et al*. (2000) found no effect of IGF–I on the growth of primary versus secondary follicles in bovine cortical pieces *in vitro*. The different sensitivity of IGF-I may depend on the different developmental stages of the follicles. The present results (Table 3) clearly demonstrated that EGF supported better *in vitro* growth, antrum formation and *in vitro* extrusion of oocytes at a concentration of 50 ng/ml. The earlier studies in sheep proposed that EGF might initiate the growth of sheep PFs, but requires other factors to sustain and/or complete the process (Hemamalini *et al.,* 2003 and Tamilmani *et al.,* 2005), which also supports the present study. EGF stimulated the follicular DNA synthesis at the concentration of 50 ng/ml resulted in increase in the diameter of sheep PFs (Hemamalini *et al.,* 2003) which is in agreement with the present findings. EGF to culture medium alone or in combination with another growth factor stimulates the survival growth of caprine preantral follicles (Zhou and Zhang, 2005a, b & c). Thyroxine at a concentration of 1 µg/ml favorably influenced the per cent increase in the growth of PFs, average increase in diameter and exhibition of antrum formation (Table 4). However, there was no difference observed in the rate of extrusion of oocytes from among different concentrations of $T_4$ like 0.5 and 2 µg/ml. Thyroxine ($T_4$) alone at a concentration of 1 µg/ml supported maturation to the M-II stage in small proportion of oocytes from cultured large PFs of sheep and in combination with FSH (2 µg/ml) supported better *in vitro* growth, antrum development and subsequent maturation of oocytes to the M-II stage in both large and small sheep PFs (Arunakumari *et al.,* 2007). FSH is one of the commonly supplemented hormones in culture medium to stimulate the growth of follicles *in vitro*. In the present study FSH at lower concentration (2 ng/ml) had shown better results when compare the higher concentrations (Table 5). Growth rate, increase in diameter, antrum formation and extrusion of oocytes was more with 2 ng/ml. In contrast to the observation of Cecconi *et al*. (1999) that 1 ng/ml FSH supported the best development in sheep adult PFs. This difference could be either due to the differences in size of the PFs used physiological status of the animal or species difference. FSH required for better follicular development, it increases the number of oocytes grown upto M.II stage (Arunakumari *et al.,* 2007). However, the optimal

concentrations of FSH for development of PFs were highly variable with species and size of the PFs (Wu *et al.,* 2001a and Ralph *et al.,* 1995).

After combination of best concentrations of above hormones and growth factors the highest proportion of PFs exhibiting growth, increase in diameter, antrum formation and *in vitro* extrusion of oocytes was observed in $T_4$+FSH+GH+EGF supplemented medium (Table 6). These results are comparable with the findings observed by Arunakumari *et al.,* (2010). However, the proportion of PFs exhibiting growth, antrum formation and extrusion of the oocytes were considerably increased in the presence of $T_4$+FSH to the above combinations, this might be due to synergistic effect. Gutierrez *et al.* (2000) reported that FSH led to a larger follicular diameter and antrum formation in bovine preantral follicles in insulin-containing medium and also found that FSH, IGF-I and EGF stimulated follicle growth but no oocyte growth. Further present findings suggested that the stimulatory effect of GH+EGF was more pronounced in the presence of $T_4$+FSH. But, the synergistic action of $T_4$ +FSH on PFs is in accordance with Arunakumari *et al.,* (2007).

It is concluded that (*i*) GH, IGF-I, EGF, $T_4$ and FSH improve independently the *in vitro* development of caprine PFs, (*ii*) $T_4$+FSH+GH+EGF supplementation of culture media supported the best development on *in vitro* culture of goat PFs.

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## References

Abir R, Fisch B, Nitke S, Okon E, Raz A and Ben Rafael Z. 2001. Morphological study of fully and partially isolated early human follicles. Fertility and Sterility 75: 141-146.

Arunakumari G, Vagdevi R, Rao B S, Naik B R, Naidu K S, Suresh Kumar R V and Rao V H. 2007. Effect of hormones and growth factors on in vitro development of sheep preantral follicles. Small Ruminant Research 70: 93 - 100.

Arunakumari G, Shanmugasundaram N and Rao V H. 2010. Development of morulae from the oocytes of cultured sheep preantral follicles. Theriogenology 74: 884-894.

Bolamba D, Borden-Russ K D and Durrant B S. 1998. In vitro maturation of domestic dog oocytes cultured in advanced preantral and early antral follicles. Theriogenology 49: 933 - 942.

Bolamba D, Russ K D, Olson M A, Sandler J L and Durrant B S. 2002. In vitro maturation of bitch oocytes from advanced preantral follicles in synthetic oviduct fluid medium: serum is not essential. Theriogenology 58: 1689 - 1703.

Carlsson I B, Scott J E, Visser J A, Ritvos O, Themmen A P N and Hovatta O. 2006. Anti-Mullerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro. Human Reproduction 21(9): 2223-2227.

Cecconi S, Barboni B, Coccia M and Mattioli M. 1999. In vitro development of sheep preantral follicles. Biology of Reproduction 60: 594-601.

Cecconi S, Rossi G, Coticchio G, Macchiarelli G, Borini A and Canipari R. 2004. Influence of thyroid hormone on mouse preantral follicle development in vitro. Fertility and Sterility 81(1): 919-924.

Chelikani P K, Amarnath D, Reddy K K, Naidu K S Rao K V and Rao V H. 1998. Isolation of preantral follicles in sheep and goats. Theriogenology 49: 343.

Derrar N,Priceta C A, and Sirard M A. 2000. Effect of growth factors and co-culture with ovarian medulla on the activation of primordial follicles in explants of bovine ovarian cortex. Theriogenology 54: 587-598.

Gupta P S P, Ramesh H S, Manjunatha B M, Nandi S and Ravindra J P. 2008. Production of buffalo embryos using oocytes from in vitro grown preantral follicles. Zygote 16: 57-63.

Gutierrez C G, Ralph J H, Telfer E E, Wilmut I and Webb R. 2000. Growth and antrum formation of bovine preantral follicles in long-term culture in vitro. Biology of Reproduction 62: 1322-1328.

Hemamalini N C, Rao B S, Tamilmani G, Amarnath D, Vagdevi R, Naidu K S, Reddy K K and Rao V H. 2003. Influence of transforming growth factor-alpha, insulin like growth factor-II, epidermal growth factor or follicle stimulating hormone on in vitro development of preantral follicles in sheep. Small Ruminant Research 50: 11 - 22.

Huanmin Z and Yong Z. 2000. In vitro development of caprine ovarian preantral follicles. Theriogenology 54: 641-650.

Itoh T, Kacchi K, Abe H, Sendai Y and Hoshi H. 2002. Growth, antrum formation, and estradiol production of bovine preantral follicles cultured in a serum-free medium. Biology of Reproduction 67: 1099-1105.

Magalhaes D M, Fernandes D D, Mororo M B S, Silva C M G, Rodrigues G Q, Bruno J B, Matos M H T, Campello C C and Figueiredo J R. 2011a. Effect of the medium replacement interval on the viability, growth and in vitro maturation of isolated caprine and ovine pre-antral follicles. Reproduction in Domestic Animals 46: 134-140.

Magalhaes D M, Duarte A B G, Araujo V R, Brito I R, Soares T G, Lima I M T, Lopes C A P, Campello C C, Rodrigues A P R and Figueiredo J R. 2011b. In vitro production of a caprine embryo from a preantral follicle cultured in media supplemented with growth hormone. Theriogenology 75: 182-188.

Rajarajan K, Rao B S, Vagdevi R, Tamilmani G, Arunakumari G, Sreenu M, Amarnath D, Naik B R and Rao V H. 2006. Effect of various growth factors on the in vitro development of goat preantral follicles. Small Ruminant Research 63: 204-212.

Ralph J H, Wilmut I and Telfer E E. 1995. In vitro growth of bovine preantral follicles and the influence of FSH on follicular and oocyte diameters. Journal of Reproduction and Fertility 15: 6 (Abstract).

Silva J R V, Van den Hurk R, Matos M H T, dos Santos R R, Pessoac C, Manoel O de Moraes M O and de Figueiredo J R. 2004a. Influences of FSH and EGF on primordial follicles during in vitro culture of caprine ovarian cortical tissue. Theriogenology 61: 1691-1704.

Silva J R V, Van den Hurk R, Costa S H F, Andrade E R, Nunes A P A, Ferreira F V A, Lobo R N B and Figueiredo J R. 2004b. Survival and growth of goat primordial follicles after in vitro culture of ovarian cortical slices in media containing coconut water. Animal Reproduction Science 81: 273-286.

Tamilmani G, Rao B S, Vagdevi R, Amarnath D, Naik B R, Mutharao M and Rao V H. 2005. Nuclear maturation of

oocytes in sheep preantral follicles cultured in vitro. Small Ruminant Research 60: 295-305.

Wu J, Emery B R and Carrell D T. 2001a. In vitro growth, maturation, fertilization, and embryonic development of oocytes from porcine preantral follicles. Biology of Reproduction 64: 375-381.

Wu J, Carrell D T and Wilcox A L. 2001b. Development of in vitro matured oocytes from porcine preantral follicles following intracytoplasmic sperm injection. Biology of Reproduction 65: 1579-1585.

Zhang P U, Hreinsson J G, Telfer E and Hovatta O. 2002. Few instead of many: human follicle collection from follicular aspirates at oocyte retrieval. Human Reproduction 17(12): 3190 - 3192.

Zhou H and Zhang Y. 2005a. Effect of growth factors on in vitro development of caprine preantral follicle oocytes. Animal Reproduction Science 90: 265-272.

Zhou H M and Zhang Y. 2005b. Impact of growth factors on in vitro development of caprine oocytes at pre-antral stage. Reproduction in Domestic Animals 40: 161-165.

Zhou H and Zhang Y. 2005c. Regulation of in vitro growth of preantral follicles by growth factors in goats. Domestic Animal Endocrinology (28): 235-242.

Zhou H M and Zhang Y. 2006. In vitro growth and maturation of caprine oocytes. Reproduction in Domestic Animals 41: 444-447.

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