VETERINARSKI ARHIV 80 (1), 41-50, 2010

In vitro development of buffalo preantral follicles in co-culture with cumulus or granulosa cells

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RAMESH, H. S., P. S. P. GUPTA, S. NANDI, B. M. MANJUNATHA: *In vitro* development of buffalo preantral follicles in co-culture with cumulus or granulosa cells. Vet. arhiv 80, 41-50, 2010.

ABSTRACT

Low reproductive efficiency in buffalo limits the number of offspring produced during lifespan and thus results in under-exploitation of the superior female genetic material. *In vitro* production of transferable embryos is a method used to overcome such limitation. Developing a culture system for preantral follicles has important biotechnological implications, due to its potential for producing large number of oocytes for *in vitro* embryo production. Hence, the effect of the co-culture of buffalo preantral follicles with dispersed somatic cells, like cumulus cells and granulosa cells and their monolayers, was investigated in the present study. Large preantral follicles were isolated from trypsin (1%) digested ovarian cortical slices. Cumulus and granulosa cell monolayers were prepared by culturing cells until their confluency preantral follicles were co-cultured with dispersed cumulus cells (group 1), the monolayers of cumulus cells (group 2), dispersed granulosa cells (group 3) or the monolayers of granulosa cells (group 4). Large preantral follicles showed significantly higher size, growth rate and survivability when co-cultured with somatic cells dispersed in the medium, as compared to those grown in a monolayer. It was concluded that dispersed somatic cells from buffalo ovarian follicles were more effective in providing support for the growth and survivability of preantral follicles in culture, compared to their monolayers in buffalo.

Key words: preantral follicle, follicle culture, follicle development, somatic cells, monolayer, co-culture, buffalo

Introduction

Preantral follicles can be a source of oocytes for *in vitro* embryo production. Efforts are being made to develop an appropriate culture system with the necessary input and

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ISSN 0372-5480
Printed in Croatia

conditions for stimulating the growth of preantral follicles, oocyte maturation and its increasing capacity to be fertilized *in vitro*. Support from somatic cells under co-culture conditions could be of benefit in this direction. Co-culturing of bovine preantral follicles with bovine ovary mesenchymal cells, fetal bovine skin fibroblasts and bovine granulosa cells resulted in 50.7, 46.6 and 21.4% viable morphological normal follicles (ITOH and HOSHI, 2000). The growth rate and survival rate of porcine preantral follicle co-cultured with cumulus cells, mural granulosa cells and control was found to be 16.9, 9.7, 12.1 and 64.7, 37.5, 51.4 per cent, respectively (WU et al., 2002). *In vitro* culture of follicles on a monolayer, or co-culture with granulosa cells, resulted in an increase of 10 μm diameter in approximately 65% of growing follicles in the case of the domestic cat (JEWGENOW and GORITZ, 1995). Information on preantral follicle growth with somatic cells is lacking for buffalo. The development of protocols for the *in vitro* growth of preantral follicles is very important, in order to increase *in vitro* embryo production in the future. Somatic cells like cumulus cells, granulosa cells etc., were reported to support the growth of oocytes (NANDI et al., 2001) and embryos (NANDI et al., 2002) in buffalo. We hypothesized that preantral follicle and somatic cell co-culture would be beneficial for the growth and survivability of preantral follicles in buffalo. The present study was undertaken in order to examine the effect of the co-culture of buffalo large preantral follicles with dispersed somatic cells (cumulus and granulosa cells) or their monolayers, on the size, growth rate and survivability rate of preantral follicles in buffalo.

Materials and methods

All chemicals used in the present study were obtained from Sigma-Aldrich, USA, unless otherwise stated. Trypsin was obtained from Himedia Labs, Mumbai, India. Steer serum, collected by clotting the blood from a long-term castrated bullock, was heat inactivated at 56 ºC for 30 min, filtered (0.22 μm), sterilized and stored in 2 mL aliquots at -20 ºC until used.

Isolation of large preantral follicles. Buffalo ovaries were brought to the laboratory in warm (32 to 33 °C) normal saline, supplemented with gentamicin (50 μg/mL) for the isolation of preantral follicles, and in ice for the isolation of somatic cells, within one hour of slaughter. Ovaries were collected from mature buffalo in both the breeding and non-breeding seasons. Both ovaries, with or without corpus luteum, were used. The ovaries were washed thoroughly in 0.9% normal saline supplemented with gentamicin (50μg/mL). A combined enzymatic cum microdissection method was used for the isolation of large preantral follicles (GUPTA et al., 2007). Thin (approximately 1 mm thick) and small sections (approximately 5 mm^2) were dissected from the ovarian cortex and washed with preantral follicle isolation medium, consisting of Dulbecco's phosphate buffer saline, steer serum (10%) and gentamicin (50 μg/mL). The ovarian cortical sections were then

digested with trypsin (1%) by incubating at 37 °C for 10 minutes. Large preantral follicles 150 to 500 µm in size were isolated from ovarian pieces by micro-dissection, using a 26 G disposable needle and scalpel blade under a stereozoom microscope. The diameter of the preantral follicles was measured with using the precalibrated micrometer of the stereozoom microscope. Follicles of normal appearance and with no visible signs of degeneration (GUPTA et al., 2002a) were selected for the study.

Isolation of cumulus and granulosa cells. The follicles were evaluated for their atretic and non-atretic status. The degree of translucency, visualization of the follicle wall and integrity of the membrana granulosa, as seen under a stereoscopic microscope, were used to distinguish between non-atretic and atretic isolated follicles. Only non-atretic follicles were considered, as these follicles were found to contain oocytes with higher developmental competence. Oocytes with cumulus cells were aspirated from the medium sized surface follicles of buffalo ovaries. The cumulus-enclosed oocytes were denuded by repeated pipetting to facilitate the release of cumulus cells. The released cells were collected and washed 3 times in culture medium. For granulosa cells, the preantral follicles were dissected under a stereo zoom microscope and washed three times in preservation medium consisting of TCM-199 (90%) supplemented with follicular fluid (10%), mercaptoethanol (10%) and gentamycin (50 µg/mL). The oocytes were removed using a 26 G needle and the granulosa cells were aspirated from oocytectomized follicles and washed twice in the culture medium. As the isolation of granulosa cells by standard aspiration and centrifugation procedure from antral follicles might result in contamination of the granulosa cells with cumulus and thecal cells, we isolated the granulosa cells from the preantral follicles.

Preparation of the cumulus and granulosa cell monolayers. Cumulus cells (0.7×10^6) or granulosa cells (0.7×10^6) were placed in 100 µL droplet medium containing TCM199 + steer serum (10%) + gentamicin (50 µg/mL) and were cultured in CO_2 incubator until confluence. After 5 days of culture, the medium was replaced by a preantral follicle culture medium.

Culture of preantral follicles. Preantral follicles (2-3 preantral follicles in a group, equally distributed) were cultured in 100 µL droplets of standard culture medium, consisting of Minimum essential medium (MEM) supplemented with steer serum (10%), follicle stimulating hormone (FSH- 0.05 IU/mL), sodium pyruvate (0.23 mM), glutamine (2 mM), hypoxanthine (2 mM), insulin-transferin-selenium (ITS- 6.25 µg insulin, 6.25 µg transferin, 6.25 ng sodium selenite), mercaptoethanol (10 µM/mL) and gentamicin (50 µg/mL). The preantral follicles were cultured in groups, as group culture was found to be more beneficial to preantral follicle growth than individual culture (RAMESH et al., 2007). The preantral follicles were cultured under mineral oil in 35 mm Petri dishes placed in a CO_2 incubator (38 ºC, 5% CO_2 in air, 90-95% relative humidity). Supportive

cell cultures were established concomitantly with the follicles. On alternate days, the media were replenished along with the cells. The culture media were prepared fresh and kept for incubation in a CO_2 incubator for 30 min before being used for culture. The growth rate and viability of the follicles were assessed on days 0, 30, 60, and 80 of the culture period. The growth rate measured was the cumulative growth rate. The viability of the preantral follicles was assessed by the trypan blue staining technique (GUPTA et al. 2002b). In this technique, follicles were treated with 0.05% (w/v) trypan blue (Himedia Lab., Mumbai, India) and checked for viability after 2 minutes. Viable follicles excluded the stain, whereas degenerated follicles took up the stain and appeared blue in colour .

The growth and viability rates of the preantral follicles were calculated as:

(a) Growth rate of follicle (μm/day) = (Final diameter (μm) of follicle as observed by day 30 or 60 or 80 - Initial diameter (μm) of follicle) / Days of culture,

(b) Viability rate (%) = (Number of follicles that were not stained (viable) / Follicle cultured) × 100.

Two to three preantral follicles were each co-cultured with either dispersed cumulus cells (0.7×10^6 per drop of preantral follicle culture medium) or dispersed granulosa cells (0.7×10^6 per drop of preantral follicle culture medium; or monolayer of cumulus cells or granulosa cells. The preantral follicles were also cultured without somatic cell support (Control). Preantral follicles were cultured in all groups on the starting day of the experiment.

After 80 days of culture, the preantral follicles or (by now) early antral follicles were broken down using a 26 G needle and the oocytes recovered. The viability of the oocytes was measured as described earlier (GUPTA et al., 2002b).

Statistical analysis. The differences in the size and viability of follicles on different days of *in vitro* culture were analyzed by ANOVA and the respective means were compared using the Bonneforni Multiple comparison test (GraphPad Prism, Version 4.00 for Windows, Graph Pad Software Inc., San Diego, California, USA 2006). Differences between the mean values were considered significant if the P values were less than 0.05.

Results

There was no significant difference ($P>0.05$) in the size of the preantral follicles between different groups on day 0 of the experiment. A significant increase in the growth of the preantral follicles was observed when cultured with somatic cells, compared to the control (Table 1, Table 2).

Table1. Effect of co-culture with dispersed somatic cells and their monolayers on size (mean ± SEM) profile of preantral follicles (PF) during *in vitro* culture

Days of culture	Size (diameter in μm) of preantral follicles				Control
	Cumulus cells		Granulosa cells		
	Dispersed cells (PF:94)*	Monolayer (PF: 99)*	Dispersed cells (PF: 93)*	Monolayer (PF: 93)*	PF: 118
0	$283.00^{1,a,p}$ ± 7.81	$311.80^{1,a,p}$ ± 14.52	$294.30^{1,a,p}$ ± 8.42	$290.30^{1,a,p}$ ± 16.91	$283.70^{1,a,p}$ ± 8.07
30	$428.10^{1,b,p}$ ± 11.86	$450.80^{1,b,x,p}$ ± 17.62	$458.00^{1,b,p}$ ± 12.23	$361.70^{2,b,y,q}$ ± 16.89	$354.20^{2,b,q}$ ± 9.04
60	$730.50^{1,c,x,p}$ ± 13.76	$539.50^{2,c,x,q}$ ± 18.67	$562.70^{1,c,y,q}$ ± 17.53	$398.60^{2,b,y,r}$ ± 16.19	$382.8^{2,b,r}$ ± 9.63
80	$865.60^{1,d,x,p}$ ± 14.06	$561.50^{2,c,q}$ ± 17.50	$618.30^{d,y,r}$ ± 18.11	**	$387.72^{2,b,s}$ ± 9.46

Number of preantral follicles employed in the group, **All follicles were degenerated. [a-q]Values with different superscript letters in a column differ significantly (P<0.05). [1,2]Values pertaining to specific category of cells with different superscript numbers in a row differ significantly (P<0.05). [x,y] Values pertaining to dispersed (Cumulus Vs. Granulosa) or monolayer (Cumulus Vs. Granulosa) with different superscript numbers in a row also differ significantly (P<0.05). [p,q,r,s] Comparison between control and addition of somatic cells. Values with different superscript in a row differ significantly (P<0.05).

Table 2. Effect of co-culture with dispersed somatic cells and their monolayers on growth rate (mean ± SEM) profile of preantral follicles (PF) during *in vitro* culture

Days of culture	Increment in size/day (diameter in μm) of preantral follicles				
	Cumulus cells	Granulosa Cells	Cumulus cells	Granulosa cells	
	Dispersed cells	Dispersed cells	Monolayer	Monolayer	Control
30	$4.87^{1,a,x}$ ± 0.35	$4.63^{1,a,x}$ ± 0.42	$5.46^{1,a,x}$ ± 0.27	$2.38^{2,a,y}$ ± 0.28	$1.79^{2,a,z}$ ± 0.22
60	$7.48^{1,b,x}$ ± 0.21	$3.79^{2,a,b,y}$ ± 0.22	$4.47^{1,b,y}$ ± 0.24	$1.81^{2,b,z}$ ± 0.19	$1.10^{2,a,z}$ ± 0.11
80	$7.30^{1,b,x}$ ± 0.16	$3.12^{2,b,y}$ ± 0.16	$4.0^{5b,y}$ ± 0.18	*	$1.28^{2,b,z}$ ± 0.07

*All follicles were degenerated. No. of PFs are the same as in Table 1. [a,b] Values with different superscript letters in a column differ significantly (P<0.05). [1,2] Values pertaining to specific category of cells with different superscript numbers in a row also differ significantly (P<0.05). [x,y,z] Comparison between control and addition of somatic cells. Values with different superscript in a row differ significantly (P<0.05).

Table 3. Effect of co-culture with dispersed somatic cells and their monolayers on the viability percentage (% of live follicles) (mean ± SEM) observed during different days of *in vitro* culture

Days of Culture	% of live follicles				
	Cumulus cells		Granulosa cells		
	Dispersed cells	Monolayer	Dispersed cells	Monolayer	Control
30	$100.00^{1,a,x}$ ± 0.00	$100.00^{1,a,x}$ ± 0.00	$86.47^{1,a,x}$ ± 7.35	$67.60^{1,a,y}$ ± 25.10	$75.64^{1,a,y}$ ± 6.21
60	$86.60^{1,b,x}$ ± 12.23	$94.43^{1,a,x}$ ± 2.94	$86.60^{1,a,x}$ ± 7.28	$10.37^{2,b,y}$ ± 2.01	$43.72^{2,b,z}$ ± 9.22
80	$86.60^{1,b,x}$ ± 12.23	$94.43^{1,a,x}$ ± 2.94	$51.37^{1,b,y}$ ± 7.48	$0.00^{2,b,z}$ ± 0.00	$42.21^{2,b,y}$ ± 8.90

*All follicles were degenerated. No. of PFs are the same as in Table 1. ^{a,b} Values with different superscript letters in a column differ significantly ($P<0.05$). ^{1,2} Values pertaining to specific category of cells with different superscript numbers in a row also differ significantly ($P<0.05$). ^{x,y,z} Comparison between control and addition of somatic cells. Values with different superscript in a row differ significantly ($P<0.05$).

The growth rate of the preantral follicles decreased significantly in the control compared to those observed in the co-culture with somatic cells (Table 2). When preantral follicles were co-cultured with dispersed cumulus cells, a significant increase in the growth rate of the preantral follicles was observed after 60 days of culture, compared to 30 days of culture, but thereafter there was no increase in the growth rate. The growth rate of preantral follicles cultured in cumulus cell monolayer increased significantly after 30 days of culture, however, the growth rate decreased on further culture. On the other hand, the growth rate of preantral follicles progressively decreased when co-cultured with granulosa cells (both dispersed or monolayer). When the preantral follicles were co-cultured with cumulus cells, either dispersed or monolayer, the viability of the preantral follicles did not decrease significantly throughout the experiment (Table 3). However, when the preantral follicles were co-cultured with granulosa cells (monolayer), the viability of the preantral follicles decreased progressively. When the preantral follicles were co-cultured with granulosa cells (dispersed), there was no significant decline in the percentage of live follicles by day 30 or day 60; however, further culture significantly decreased the viability rate of the preantral follicles.

The percentage of viability of the oocytes recovered from the preantral follicles after 80 days of culture were: a) cumulus dispersed: 56.6 ± 5.2; b) cumulus monolayer: 57.3 ± 7.2; c) granulosa dispersed: 32.6 ± 5.0; d) granulosa monolayer: 4.1 ± 1.1and control: 30.2 ± 3.3.

Discussion

In the present study, the value of development indicators (size, growth rate and viability) of buffalo large preantral follicles *in vitro* was better when co-cultured with dispersed cumulus cells in comparison with their monolayer. The observations made in this study support the findings of WU et al. (2002), in which they obtained a significantly higher growth rate in preantral follicles and a growth rate (%) and survival rate (%) in preantral follicular oocytes when co-cultured with cumulus cells taken from large antral follicles (>3 mm). Porcine cumulus and granulosa cells produce a cumulus expansion enabling factor (CEEF) *in vitro* (PROCHÁZKA et al., 1998). FSH induces cumulus cells to produce a diffusible heat stable meiosis activating substance that helps in oocyte maturation (BYSKOV et al., 1997). Co-culture with cumulus cells, granulosa cells and mesenchymal cells was beneficial for the development of large buffalo preantral follicles *in vitro* compared to the control, which suggests that the these somatic cells might have secreted certain factors to promote *in vitro* growth of the developing follicles. The observations made in this study support the findings of WU et al. (2002), in which they obtained significantly a higher growth rate in preantral follicles; and the growth rate and survival rate in oocytes enclosed in preantral follicles when co-cultured with cumulus cells taken from large antral follicles. The higher growth rates of preantral follicles obtained when cultured with cumulus cells might be due to secretions such as an oocyte growth promoting factor (WU et al., 2002), a cumulus expansion enabling factor (PROCHAZKA et al., 1998) or a diffusible heat stable meiosis activating substance (BYSKOV et al., 1997). Cumulus cells also secreted some unknown proteins of 158.5, 123.0, 31.6, 15.1, 12.6 and 2.0 kDa when cultured along with preantral follicles, which might be responsible for the increased growth rates of the follicles in the culture (Gupta, unpublished data). The inclusion of somatic cells in the preantral follicle culture media had a beneficial effect on the growth of preantral follicles, while cumulus cells supported maximum growth and the survival of the preantral follicles *in vitro*.

With granulosa cells also, co-culture with dispersed cells was beneficial, compared to their monolayer, for the development of buffalo large preantral follicles *in vitro*. Granulosa cell products like activin (LI et al., 1995), inhibin (CAMPBELL et al., 1995), thecal differentiation factor (MAGARELLI et al., 1996) and fibronectin (CARNEGIE, 1990) might have enhanced the growth and survivability of preantral follicles. A granulosa cell secreted protein called kit ligand (KL) regulated thecal cell function, cell proliferation and growth (PARROTT and SKINNER, 1997) and had a positive effect on oocytes, thecal cells and ovarian stromal cells (DOLCI et al., 1991; GODIN et al., 1991; MATSUI et al., 1991; PACKER et al., 1994; PARROTT and SKINNER, 1997). We observed increased growth rates in the preantral follicles cultured in the granulosa cell monolayer, however, the survivability rates decreased compared to the control. Secretion of some factors from granulosa cells

after monolayer formation might be the reason. However, a more detailed study regarding this aspect is required.

Dispersed cells provided better results compared to monolayers, which might be due to less adherence of the preantral follicles to the culture plate when cultured along with dispersed cells than that observed when cultured in a monolayer. In a previous study (GUPTA et al., 2002a), preantral follicles could be cultured for a maximum period of 30 days with low survival rates, with the addition of growth factors (vasoactive intestinal peptide, fibroblast growth factor and epidermal growth factor). However, under co-culture conditions in the present study, even after 80 days of culture, >85% live follicles were present in both the cumulus cell groups and >50% in the dispersed granulosa cell group. This is the first report on the prolonged culture of preantral follicle for 80 days. Thus, in buffalo, larger preantral follicles can survive for a longer period and with a more significant growth rate than small preantral follicles. The isolation and characterization of the factors secreted by the somatic cells in co-culture with preantral follicles, responsible for the growth of preantral follicles, merit further investigation. Similarly, the effect of the extra somatic cells on the growth of preantral follicles cultured individually could be the subject of future research work.

Conclusion

The present study showed that co-culture with dispersed somatic cells i.e., cumulus cells and granulosa cells had a beneficial effect on the development and survivability of large preantral follicles, compared to their monolayers.

Acknowledgements

The authors would like to thank Dr. K.T. Sampath, Director, National Institute of Animal Nutrition and Physiology, for providing the necessary facilities for carrying out this work.

References

BYSKOV, A. G., C. Y. ANDERSEN, A. HOSSAINI, X. GUOLIANG (1997): Cumulus cells of oocyte-cumulus complexes secrete a meiosis-activating substance when stimulated with FSH. Mol. Reprod. Dev. 46, 296-305.

CAMPBELL, B. K., R J. SCARAMUZZI, R. WEBB (1995): Control of antral follicle development and selection in sheep and cattle. J. Reprod. Fertil. Suppl., 49, 335-350.

CARNEGIE, J. A. (1990): Secretion of fibronectin by granulosa cells occurs primarily during early follicular development. J. Reprod. Fertil. 89, 579-589.

DOLCI, S., D. E. WILLIAMS, M. K. ERNST, J. L. RESNICK, C. L BRANNAN, L. F. LOCK, S. D. LYMAN, H. S. BOSWELL, P. J. DONOVAN (1991): Requirements for mast cell growth factor for primordial germ cell survival in culture. Nature 352, 809-811.

GODIN, I., R. DEED., J. COOKE, K. ZSEBO, M. DEXTER, C. C. WYLIE (1991): Effects of the steel gene product on mouse primordial germ cells in culture. Nature 353, 750-752.

GUPTA, P. S. P., S. NANDI, B. M. RAVINDRANATHA, P. V. SARMA (2002a): *In vitro* culture of buffalo (*Bubalus bubalis*) preantral follicles. Theriogenology 57, 1839-1854.

GUPTA, P. S. P., S. NANDI, B. M. RAVINDRANATHA, P. V SARMA (2002b): Trypan blue staining to differentiate live and dead buffalo oocytes and its effect on embryo development *in vitro*. Buffalo J. 18, 321-329.

GUPTA, P. S. P., H. S., RAMESH, S. NANDI, J. P. RAVINDRA (2007): Recovery of large preantral follicles from buffalo ovary: Effect of season and corpus luteum. Animal Reprod. Sci. 101, 145-152.

ITOH, T., H. HOSHI (2000): Efficient isolation and long-term viability of bovine small preantral follicles *in vitro.* Soc. *in vitro* Biol. J. 36, 235-240.

JEWGENOW, K., F. GORITZ (1995): The recovery of preantral follicles from ovaries of domestic cats and their characterization before and after culture. Anim. Reprod. Sci. 39, 285-297.

LI, R., D. M. PHILIPS, J. P. MATHER (1995): Activin promotes ovarian follicle development *in vitro*. Endocrinol. 136, 849-856.

MAGARELLI, P. C., R. J. ZACHOW, D. A. MAGOFFIN (1996): Development and hormonal regulation of rat theca cell differentiation factor secretion in ovarian follicles. Biol. Reprod. 55, 416-420.

MATSUI, Y., D. TOKSOZ, S. NISHIKAWA, D. WILLIAMS, K. ZSEBO, B. L. HOGAN (1991): Effects of steel factor and leukemia inhibitory factor on murine primordial germ cells in culture. Nature 353, 750-752.

NANDI, S., B. M. RAVINDRANATHA, P. S. P. GUPTA, P. V. SARMA (2001): Effect of somatic cells monolayer on maturation of buffalo oocytes *in vitro*. Indian J. Anim. Sci. 71, 936-937.

NANDI, S., H. RAGHU, M. M. RAVINDRANATHA, P. S. P. GUPTA, P. V. SARMA (2002): Effect of somatic cell co-culture and synthetic oviductal fluid on development of buffalo embryos *in vitro*. Online J. Physiol. 1, 26-32.

PACKER, A. L., Y. C. HSU, P. BESMER, R. F. BACHVAROVA (1994): The ligand of the c-kit receptor promotes oocyte growth. Dev. Biol. 161, 194-205.

PARROT, J. A., M. K. SKINNER (1997): Direct actions of KL on theca cell growth and differentiation during follicle development. Endocrinol. 138, 3819-3827.

PROCHAZKA, R., E. NAGGOVA, G. BREM, K. SCHELLANDER, J. MOTLIK (1998): Secretion of cumulus expansion enabling factor (CEEF) in porcine follicles. Mol. Reprod. Dev. 49, 141-149.

RAMESH, H. S., J. P. RAVINDRA, P. S. P. GUPTA, V. G. KUMAR (2007): Effect of individual and group culture on *in vitro* development of buffalo preantral follicles. Indian J. Anim. Sci. 77, 953-955.

WU, M. F., W. T. HUANG, C. TSAY, H. F. HSU, B. T. LIU, C. M. CHIOU, S. C. YEN, S. P. CHENG, J. C. JU (2002): The stage- dependent inhibitory effect of porcine follicular cells on the development of preantral follicles. Anim. Reprod. Sci. 73, 73-88.

Received: 7 August 2008
Accepted: 22 December 2009

RAMESH, H. S., P. S. P. GUPTA, S. NANDI, B. M. MANJUNATHA: Razvoj *in vitro* preantralnih folikula u uzgoju s kumulusnim ili granuloznim stanicama. Vet. arhiv 80, 41-50, 2010.

SAŽETAK

Niska rasplodna sposobnost bivolica ograničava broj potomaka podrijetlom od jedne bivolice tijekom njezina životnoga vijeka, a time i iskorištavanje velikih mogućnosti ženskoga genetskoga materijala. Proizvodnja zametaka *in vitro* u svrhu prijenosa u plotkinju metoda je koja može premostiti to ograničenje. Razvoj tehnologije uzgoja preantralnih folikula od važnoga je biotehnološkog značenja zahvaljujući mogućnostima proizvodnje velikoga broja jajašaca radi proizvodnje zametaka *in vitro*. Stoga je istraživan učinak suuzgoja bivoljih antralnih folikula s raspršenim somatskim stanicama kao što su kumulusne i granulozne stanice i njihovim jednoslojnim kulturama. Veliki preantralni folikuli bili su izdvojeni iz komadića kore jajnika u postupku probave 1%-tnim tripsinom. Kumulusne i granulozne stanice bile su uzgajane sve dok se nisu spojile u jednom sloju. Preantralni folikuli bili su uzgajani zajedno s raspršenim kumulusnim stanicama (skupina 1), kumulusnim stanicama uzgojenima u jednom sloju (skupina 2), raspršenim granuloznim stanicama (skupina 3) i granuloznim stanicama uzgojenima u jednom sloju (skupina 4). Veliki preantralni folikuli bili su značajno veći, brže su rasli i duže preživljavali u suuzgoju sa somatskim stanicama raspršenima u hranjivoj tekućini nego u suuzgoju sa stanicama naraslima u jednom sloju. Zaključeno je da su raspršene somatske stanice podrijetlom od jajničnoga folikula bivolice bile učinkovitija podloga za rast i preživljavanje preantralnih folikula od uzgojenih u jednom sloju.

Ključne riječi: preantralni folikul, uzgoj folikula, razvoj folikula, somatske stanice, jednoslojna stanična kultura, bivolica