International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 8, Issue 50, 2016, pp.-2154-2157.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

EFFECT OF STEM CELL FACTOR ON CAPRINE PREANTRAL FOLLICLE CULTURE

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Received: July 10, 2016; Revised: July 24, 2016; Accepted: July 25, 2016; Published: October 24, 2016

Abstract- The influence of stem cell factor on *in-vitro* culture of preantral follicles (PFs) isolated from goat ovaries was investigated. Preantral follicles were isolated from the cortex of goat ovaries and individually cultured for 10 days in different concentrations (25 ng/ml, 50 ng/ml, 100 ng/ml) of stem cell factor (SCF).The proportion of PFs exhibiting growth, increase in diameter and antrum formation was assessed to determine the *in vitro* development. The oocytes isolated from cultured PFs were kept *in-vitro* maturation medium for 27 h, to assess the nuclear maturation to the metaphase II (M II) stage. The proportion of PFs exhibiti ng growth, average increase in diameter, antrum formation, extrusion of oocytes and meiotic resumption of oocytes were highest when the culture medium was supplemented with 50 ng/ml of SCF individually. Based on the best concentration from the above results, the preantral follicles were cultured with thyroxin (T_4), follicle stimulating hormone (FSH), stem cell factor (SCF) and leptin in various combinations. It is concluded that *in vitro* culture of goat PFs in combination of stem cell factor and leptin i.e SCF + leptin (31.25 ± 1.15) did not improve the frequency of oocyte maturation to metaphase II stage when compared with SCF alone.

Keywords- Goat, Preantral follicles, Stem cell factor

Citation: Divya V., et al., (2016) Effect of Stem Cell Factor on Caprine Preantral Follicle Culture. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 8, Issue 50, pp.-2154-2157.

Academic Editor / Reviewer: V.C. Ingle, D.J. Talukdar

Introduction

The reproductive technologies like *in-vitro* embryo production, transgenic animal production and nuclear transfer, depends on the availability of mature oocytes from antral follicles [40]. However, success rates are low as they use the oocytes from antral follicles, which provide a limited number of these structures on the ovary. Although large populations of preantral follicles with meiotically incompetent oocytes are present in ovaries, most of them are lost at various stages of development owing to atresia. Hence, there is a considerable interest for the development of culture systems to promote the growth of preantral follicles and to acquire meiotically competent oocytes would certainly be useful to reduce the generation interval. Thus, there is considerable interest to produce fully-grown, developmentally competent oocytes from preantral follicles, Studies on *in-vitro* culture of preantral follicles (PFs) from the ovaries of goat [1-14] was actively pursued to enhance the utilization of oocytes in animals, as well as the embryo production in pig [15, 16], buffalo [17], sheep [18] and goats [8]. Among all species studied till date, the birth of live offspring from cultured preantral follicles was achieved only in the mouse [19, 20]. *In vitro* development of preantral follicles is influenced by supplementing various hormones, peptides and growth factors. Among growth factors, kit ligand (KL), also known as stem cell factor, steel factor or mast cell growth factor, plays an important role acting at different stages of follicular development enhancing follicular growth, antrum formation and meiotic maturation [21]. In the granulosa cells, the KL mRNA expression has been detected in many species [22-26] and it can be expressed as either a membrane-bound (KL-1) or a soluble protein (KL-2) [27]. Both KL isoforms influence target cells by binding to a tyrosine kinase receptor, c-kit. KL-1 promote the primordial to primary transition [28], KL-2 is required for oocyte growth, survival and formation of germ cells [29]. Both forms are present in goats [25]. In oocytes both c-kit mRNA and protein are expressed at all stages of follicular development, as well as in the interstitial and theca cells of antral follicles [25, 30, 31]. Effects of stem cell factor include establishment of primordial germ cells (PGC), activation of primordial follicle[28], oocyte survival and growth[32], granulosa cell proliferation [33], theca cell recruitment and maintenance of meiotic competence [29, 34]. In addition, some studies have shown that KL increase the expression of FSH receptors by inhibiting the BMP-15 receptors by the oocytes [33, 35]. FSH regulate the KL expression in a biphasic manner by decreasing the ratio of KL-1/KL-2 mRNA at a low concentration of FSH and vice versa [35]. Keeping the above points in view, the present investigation was undertaken to investigate the effects of different concentrations of SCF individually and in combination with T_4, FSH and leptin on *in-vitro* development of PFs as well as on the ability of oocytes to undergo meiotic resumption.

MaterialsandMethods

All culture media, chemicals, hormones, fetal calf serum (FCS) and growth factors were purchased from the Sigma (St. Louis, MO, USA) and plastics from Nunclon (Roskilde, Denmark).

Collection and processing of ovaries

Goat ovaries were collected from a local slaughterhouse at Ziaguda, Hyderabad immediately after slaughter. The ovaries were transported to the laboratory in sterile, warm (37ºC) PBS within 5-6 h. On reaching the laboratory, working area was cleaned with 70% alcohol and the ovaries were handled aseptically. The ovaries were trimmed off the adherent tissues and ligaments, washed twice in PBS supplemented with 50 µg/ml gentamycin sulphate. Then they were immersed in 70% alcohol for 3-5 s and rinsed twice in PBS with antibiotics. The subsequent procedures involved in *in-vitro* culture of preantral follicles were carried out in a laminar air flow [36-38, 18].

Isolation of Preantral Follicles by Micro-dissection Method

Briefly, the ovaries were cut into two halves and the medulla was scooped out. After medulla removal, the cortical portion of ovaries were placed in 35 mm plastic culture dishes containing handling medium which serves as basic culture medium{HEPES buffered tissue culture medium 199 [TCM 199] supplemented with 0.23mM of Sodium Pyruvate (TCL 015, HiMedia, India), 2mM L-Glutamine (TC 243, HiMedia, India), 80mM of ascorbic acid, 20mM cysteine and 50 µg/ml gentamycin sulphate (A 010, HiMedia, India)}. Intact preantral follicles in the size range of 250-400 µm were mechanically isolated by micro-dissection under a stereo zoom microscope (Olympus, SZX12, Japan) using two needles of 26 gauge fitted to 1 ml syringe barrels and a sterile surgical blade. A small amount of stromal tissue should be attached to the basement membrane of the follicles to avoid damage to the basement membrane while isolation of PFs. Good quality follicles with centrally placed, spherical oocyte with no signs of atresia were used in culture.

Culture of PFs in micro-droplets

The selected follicles were washed thrice in culture medium, placed individually in 40 µl droplets of culture medium and overlaid with lightweight mineral oil. These culture dishes were incubated at 39ºC in 5% CO_2 under humidified atmosphere in air for up to 10 days. Half of the medium was replaced by an equal volume of fresh medium every 48 h. The culture period of 10 days was found to be optimum from earlier studies in our lab. Based on the best combinations of various hormones, observed earlier [18, 38, 39] the following treatments were selected to evaluate their effect on goat PFs and subsequent meiotic maturation of oocytes. Each follicle was morphologically evaluated every 24 h during culture period using an inverted microscope (Olympus, IX51, Japan), for increase in diameter, antrum formation and extrusion of oocyte from the follicle if any takes place. At the end of the culture, the *in-vitro* cultured follicles were carefully opened (if oocytes were not extruded from follicles) using two 26 gauge needles attached to 1 ml syringe barrel, to release the oocytes.

Experimental design

1. To standardize the concentration of stem cell factor 2. To study the influence of inclusion of stem cell factor along with other hormones on development of PFs in culture.

Table-1 *Various Combinations of Hormones and Growth factors*

Treatments	Various Combination of Growth factors and Hormones
T1	SCF: 50 ng/ml + leptin: 10 ng/ml
T2	T_4: 1 µg/ml + FSH: 2 ng/ml + SCF: 50ng/ml + leptin: 10 ng/ml
T3	Control

***In-vitro* maturation of oocytes**

The oocytes collected from *in-vitro* cultured preantral follicles were washed thrice in the basic culture medium and once in the *in vitro* maturation medium (Basic culture medium supplemented with 10 µg/ml FSH, 10 µg/ml LH, 1 µg/ml estradiol 17β, 50 µg/ml gentamycin sulphate and 10 µg/ml BSA) [18]. The selected good quality oocytes were kept individually in 20 µl droplets of *in vitro* maturation medium. The droplets were overlaid with light weight mineral oil. These culture dishes were incubated at 39°C in 5% CO_2 under humidified atmosphere for 27 h. The maturation period of 27 h was found to be optimum from earlier studies in our lab.

Evaluation of IVM oocytes

After a period of 27 h of *in-vitro* maturation, the cumulus cells around the oocytes were denuded by repeated pipetting through a fine bore glass pipette. The denuded oocytes from *in vitro* maturation media were placed separately in 200 µl propidium iodide stain solution for 15 minutes in darkness at room temperature as studied earlier in our lab. The stained oocytes were examined through an inverted microscope to observe the nuclear maturation of oocytes.

Statistical Analysis

Comparison of the proportions of PFs exhibiting growth, average increase in diameter, antrum formation, extrusion of oocytes and maturation to metaphase II stage of the oocytes from the *in-vitro* cultured PFs among different treatment groups was undertaken separately were analysed by Analysis of variance, followed by Duncan's Multiple range Test for significance of differences among treatments (SPSS 17). The proportions of PFs exhibiting growth, average increase in diameter, antrum formation, extrusion of oocytes and maturation to metaphase II stage of the oocytes were dependent variables and independent variables were the different treatment groups.

Results

A total of 490 preantral follicles were isolated and used in two experiments. In experiment 1, the individual effects of stem cell factor at different concentrations in the medium on *in-vitro* development of PFs were tested. Preantral follicles cultured in basic culture medium supplemented variously with 25, 50 and 100 ng/ml of stem cell factor. Greater proportion of PFs exhibited antrum formation and average increase in diameter in the SCF 50 ng/ml and growth rate was not significantly different between treatments [Table-2]. While there was no significant difference was observed for extrusion of oocytes between different concentrations of stem cell factor. Oocytes from cultured PFs in SCF 50 ng/ml had exhibited higher percentage (50.78 ± 1.84) of M II stage and was significantly different when compared to the oocytes from other treatments of cultured PFs.

In experiment 2, PFs were cultured in best concentration of SCF of the experiment 1 with other hormones like T4, FSH and leptin. Although average increase in diameter, growth and antrum formation do not exhibit significant difference between the treatments but the proportion of oocytes extruded from PFs and meiotic maturation was highest in (T1) SCF + leptin and was significantly different [Table-3].

Table-2 *Effect of different concentrations of Stem Cell Factor (SCF) on in vitro development of goat preantral follicles*

Concentration of SCF (ng/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 oocytes extruded from PFs (Mean ± SE)	M-II (%)
25(9 / 49)	95.55 ± 2.93^{ab}	33.83 ± 3.43^{a}	90.0 ± 3.33^{b}	0.0 ± 0.0^{a}	13.32 ± 4.46^{b}
50(9 / 49)	100.0 ± 0.0^{b}	56.16 ± 3.12^{b}	100.0 ± 0.0^{c}	4.4 ± 2.93^{a}	50.78 ± 1.84^{c}
100(8 / 44)	97.5 ± 2.5^{b}	33.62 ± 2.78^{a}	87.5 ± 3.65^{b}	2.5 ± 2.5^{a}	2.5 ± 2.5a
Control(7 / 45)	88.57 ± 4.04^{a}	4.55 ± 3.30^{a}	45.63 ± 4.29^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}

Different superscripts within a column are indicating significantly difference between different treatment groups (P ≤ 0.05)

Table-3 *Effect of various Hormones in different combinations on in vitro development of goat preantral follicles*

Treatments	Combinations of diff. hormones (replicates/ no. of oocytes)	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 oocytes extruded from PFs (Mean ±SE)	Proportion of oocytes exhibiting M-II (%)
T1	SCF + leptin (9 / 101)	90.27 ±2.42^{b}	32.13±4.94^{b}	81.14 ±2.54^{b}	3.05± 0.98^{b}	31.25±1.15^{c}
T2	T_4 + FSH +SCF + leptin (9 / 101)	95.12 ±1.60^{b}	34.10 ±3.34^{b}	84.64±2.19^{b}	0.0 ±0.0^{a}	21.19±1.39^{b}
Control	TCM 199(9 / 101)	71.28± 5.30^{a}	13.61 ±1.93^{a}	53.14 ±3.04^{a}	0.0± 0.0^{a}	0.0± 0.0^{a}

Different superscripts within a column are indicating significantly difference between different treatment groups (P ≤ 0.05).

Discussion

Stem cell factor evidenced an important role in follicular development, acting at different follicular stages. The present results showed that follicular diameter was higher at a concentration of 50 ng/ml compared to other treatments after 10 days of culture. The results obtained from the present study were in accordance with regard to follicular diameter as Celestino et al. [26] and Lima et al. [40] studied a positive effect of stem cell factor (50 ng/ml) on the caprine preantral follicular diameter. Proportion of PFs exhibiting growth was highest when the follicles were supplemented with SCF at a concentration of 50 ng/ml. Wang and Roy [41] and Xian Jin et al. [42] confirmed these results showing, disproportionate follicle growth by stimulating granulosa cells development and by elevating the expression of steroidogenesis related proteins. In contrast, Carlsson et al. [24] observed that there was no effect on the stages of follicular development with 7 days culture period in humans which was independent of the concentration used suggesting that KL's action differ according to the stage of follicular development. Present findings were in line with the study of Reynaud et al. [43] and Yoshida et al. [44] who verified that the blockade of KL/c-kit system inhibited the antrum formation and follicular fluid accumulation suggesting a positive effect of kit ligand on antrum formation. However, supplementation of SCF(50 ng/ml) and/or IGF-1(50 ng/ml) did not enhance the antrum formation in 7-8 week old mice [45] and this may be due to species variation and stage of follicular development. In the present work, KL concentration (50 and 100 ng/ml) increased the number of oocytes destined for IVM. The stem cell factor with a concentration of 50 ng/ml increased the percentage of meiotic resumption with 50.78 % of oocytes in metaphase II stage. With regard to kit, Ismail et al. [46] observed that meiosis inducing effects of LH could be elicited by a decrease in oocyte kit expression, decrease in granulosa cell KL expression and altered biological activity of KL. The present results showed that SCF at a concentration of 50 ng/ml increased the percentage of M II stage oocytes as observed in goats [40]. In agreement with the present results Yeet al. [47], observed that the treatment of mouse cumulus oocyte complexes cultured in KL enhanced the extrusion of first polar body with an increase in cyclin B1 synthesis, which is important for the progression of meiotic maturation after GVBD, however Byung Chul Jee et al. [45] studied that SCF supplementation did not enhance the acquisition of M II stage oocytes compared to the non supplemented group. The results are interesting for goats, since few studies in this species have achieved meiotically competent oocytes from the *in vitro* culture of preantral follicles [8, 9, 40, 48], especially with the occurrence of nuclear maturation. In experiment 2, proportion of PFs exhibiting growth, antrum formation and average increase in diameter was similar irrespective of combinations of hormones and growth factors used. Similarly, Arunakumari et al. [18], observed that T_4 and FSH in combination supported better *in-vitro* growth, extrusion of oocytes , antrum formation and subsequent maturation of oocytes from cultured adult sheep PFs. The extrusion of oocytes was observed only in SCF and leptin combination, however the combination of T4 + FSH + SCF + leptin did not allow the extrusion of oocytes in the present study and this might be due to species variation and size of follicles used for culture or due to the presence of FSH in culture medium [40]. As documented in the previous research, T_4 and FSH in combination had no effect on extrusion of oocytes in goat fetal PFs [49]. In addition supplementation of SCF + leptin resulted in highest proportion of MII stage oocytes obtained from cultured PFs. SCF with a concentration of 50 ng/ml showed greater percentage of MII stage oocytes when supplemented individually, but the percentage of matured oocytes was less when supplemented in combination with leptin (50.78% vs 31.25%), this decreased percentage may be due to down regulation of SCF receptors on oocyte by the leptin as leptin receptors were present on oocyte surface [50]. From the present results it is concluded that SCF at a concentration of 50 ng/ml supported better *in-vitro* development of PFs in goat than SCF in combination with leptin.

Abbreviations: SCF- Stem cell factor, PFs- Preantral follicles, M II- Metaphase II, s- seconds, h- hours, FSH- Follicle stimulating hormone, °C- Degree Celsius, T_4- Thyroxine

Acknowledgements

Department of Biotechnology (DBT), Government of India supported this work through a research grant to Dr. G. Arunakumari.

Conflict of Interest: None Declared

References

[1] Huanmin Z. and Yong Z. (2000) *Theriogenology,* 54, 641-650.

[2] 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) *Theriogenology,* 61, 1691-1704.

[3] Silva J.R.V., Van den Hurk R., Costa S.H.F., Andrade E.R., Nunes A.P.A., Ferreira F.V.A., LoboR.N.B. and Figueiredo J.R. (2004b) *Animal Reproduction Science,* 81, 273-286.

[4] Zhou H. and Zhang Y. (2005a) *Animal Reproduction Science,* 90, 265-272.

[5] Zhou H.M. and Zhang Y. (2005b) *Reproduction in Domestic Animals,* 40, 161-165.

[6] Zhou H. and Zhang Y. (2005c) *Domestic Animal Endocrinology,* (28), 235-242.

[7] Zhou H.M. and Zhang Y. (2006) *Reproduction in Domestic Animals,* 41, 444-447.

[8] 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) *Reproduction in Domestic Animals,* 46, 134-140.

[9] 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) *Theriogenology,* 75, 182-188.

[10] Duarte A.B.G., Araújo V.R., Chaves R.N., Silva G.M., Magalhães D.M., Satrapa R.A., Donato M.A.M., Peixoto C.A., Campello C.C., Matos M.H.T., Barros C.M. and Figueiredo J.R. (2011)*Fertility and Development,* 24(3), 490-500.

[11] Saraiva M.V.A., Brito I.R., Chaves R.N., Rodrigues G.Q., Almeida A.P., Lima I.M.T., Rossetto R., Frota I.M.A., Leitão C.C.F., Costa J.J.N., Silva J.R.V. and Figueiredo J.R (2012) *Animal Reproduction Science,* 9(2), 71-79.

[12] Leitao C.F.F., Nascimento J.J., Saraiva V.A., Rocha A.V., Figueiredo J.R, Van den Hurk, J andViana Silva (2013) *Ciência Rural,Santa Maria,* 43, 107-113.

[13] Rocha R.M.P., Limaa L.F., Alvesa A.M.C.V., Celestinob J.J.H., Matosc M.H.T., Lima-Verde I.B., Bernucie M.P., Lopesa C.A.P., Báof S.N., Campelloa C.C., Rodriguesa A.P.R. and Figueiredoa J.R (2013) *Domestic Animal Endocrinology,* 44, 1–9.

[14] Cunha E.V., Costa J.J.N., Rossi R.O.D.S., Silva A.W.B., Passos J.R.S., Portela A.M.L.R., Pereira D.C.S.T., Donato M.A.M., Campello C.C., Saraiva M.V.A., Peixoto C.A., Silva J.R.V. and Santos R.P.(2013) *Brazilian Journal of Medical and Biological Research,* 46, 245-252.

[15] Wu J., Emery B.R and Carrell D.T. (2001a) *Biology of Reproduction,* 64, 375-381.

[16] Wu J., Carrell D.T. and Wilcox A.L. (2001b) *Biology of Reproduction,* 65, 1579-1585.

[17] Gupta P.S.P., Ramesh H.S., Manjunatha B.M., Nandi S and Ravindra J.P.(2008) *Zygote,* 16, 57-63.

[18] Arunakumari G., Shanmugasundaram N. and Rao V.H. (2010) *Theriogenology,* 74, 884-894.

[19] Eppig J.J. and Schroeder A.C. (1989) *Biology of Reproduction,* 41, 268–276.

[20] O'Brien M.J., Pendola J.K. and Eppig J.J.(2003) *Biology of Reproduction,* 68, 1682–1686.

[21] Celestino J.J.H., Matos M.H.T., Saraiva M.V.A. and Figueiredo J.R.(2009) *Animal Reproduction,* 6, 431–439.

[22] Ismail R.S., Okawara Y., Fryer, J.N. and Vanderhyden, B.C.(1996) *Molecular Reproduction Development,* 43, 458–469.

[23] Tisdall D.J., Fidler A.E., Smith P., Quirke L.D., Stent V.C., Heath D.A. and Mcnatty K.P. (1999) *Journal of Reproduction Fertility,* 116, 277–291.

[24] Carlsson I.B., Laitinen M.P.E., Scott J.E., Louhio H., Velentzis L., Tuuri T., Aaltonen J., Ritvos O., Winston R.M.L. and Hovatta O. (2006) *Reproduction,* 131, 641–649.
[25] Silva J.R.V., Van den Hurk R., Van Tol H.T.A., Roelen B.A.J. and Figueiredo J.R. (2006) *Zygote,* 14, 317–328.
[26] Celestino J.J.H., Bruno J.B., Lima-Verde I.B., Matos M.H.T., Saraiva M.R.V.A., Chaves R.N., Martins F.S., Almeida A.P., Cunha R.S., Lima L.F., Name K.P.O., Campello C., Silva J.R., Bao S.N.N. and Figueiredo J.R.(2010) *Molecular Reproduction & Development,* 77, 231–240.
[27] Huang E.J., Nocka K.H., Buck J. and Besmer P. (1992) *Mol. Biol. Cell.,* 3, 349–362.
[28] Parrott J.A. and Skinner M.K. (1997) *Endocrinology,* 138, 3819–3827.
[29] Thomas F.H., Ismail R.S., Jiang J. and Vanderhyden B.C. (2008) *Biology of Reproduction,* 78, 167-175.
[30] Motro B. and Bernstein A. (1993) *Developmental Dynamics,* 197, 69–79.
[31] Clark D.E., Tisdall D.J., Fidler A.E. and McNatty K.P. (1996) *Journal of Reproduction Fertility,* 106, 329–335.
[32] Jin X., Han C.S., Yu F.Q., Wei P., Hu Z.Y. and Liu Y.X. (2005) *Molecular Reproduction Development,* 70, 82–90.
[33] Otsuka F. and Shimasaki S. (2002) *Proc. National Academy Sci,* 99, 8060–8065.
[34] Hutt K., McLaughlin E.A. and Holland M.K. (2006a) *Moecular. Human Reproduction,* 12, 61–69.
[35] Thomas F.H., Ethier J.F., Shimasaki S. and Vanderhyden B.C. (2005) *Endocrinology,* 146, 941–949.
[36] Hemamalini N.C., Rao B.S., Tamilmani G., Amarnath D., Vagdevi R., Naidu K.S., Reddy K.K and Rao V.H. (2003)*Small Ruminant Research,* 50: 11 - 22.
[37] Tamilmani G., Rao B.S., Vagdevi R., Amarnath D., Naik B.R., Mutharao M and Rao V.H. (2005) *Small Ruminant Research,* 60, 295-305.
[38] Arunakumari G., Vagdevi R., Rao B.S., Naik B.R., Naidu K.S., Suresh Kumar R.V. and Rao V.H.(2007) *Small Ruminant Research,* 70, 93 - 100.
[39] Kamalamma P., Kona S.S.R., Praveen Chakravarthi V., Siva Kumar A.V.N., Punyakumari B. and Rao V.H (2015) *Theriogenology,* 85, 224-229.
[40] Lima I.M.T., Brito I.R., Rodrigues G.Q., Silva C.M.G., Magalhães-Padilha D.M., Lima L.F.,Celestino J.J.H., Campello C.C., Silva J.R.V., Figueiredo J.R. and Rodrigues A.P.R. (2011) *Molecular and Cellular Endocrinology,* 345, 38-47.
[41] Wang J. and Roy S.K. (2004) *Biology Of Reproduction,* 70, 577–585.
[42] Xuan Jin., Chun-Sheng Han., Xue-Sen Zhang., Fu-Qing Yu., Shu-Hua Guo., Zhao-Yuan Hu And Yi- Xun Liu (2005) *Frontiers in Bioscience,* 10, 1573-1580.
[43] Reynaud K., Cortvrindt R., Smitz J. and Marc-Antoine D. (2000) *Molecular Reproduction And Development,* 56, 483-494.
[44] Yoshida H., Takakura N., Nataoka H., Kunisada T., Okamura H. and Nishikawa S.I.(1997) *Developmental Biology,* 184, 122-137.
[45] Byung Chul Jee., Kim J.H., Park D.H., Hyewon Youm., Chang Suk Suh and Seok Hyun Kim (2012) *Clinical Experimental Reproductive Medicine,* 39(3), 107-113.
[46] Ismail R.S., Dube M. and Vanderhyden B.C. (1997) *Developmental Biol*ogy, 184, 333–342.
[47] Ye Y., Kawamura K., Sasaki M., Kawamura N., Groenen P., Gelpke M.D.S., Rauch R, Hsueh A.J.W. and Tanaka T.(2009) *Reproductive Biology and Endocrinology,* 7,26.
[48] Silva C.M.G., Matos M.H.T., Rodrigues G.Q., Faustino L.R., Pinto L.C., Chaves R.N., Araújo V.R., Campello C.C. and Figueiredo J.R.(2010) *Animal Reproduction Science,* 117, 83–89.
[49] Rooh Ul Amin., Chandrashekar Reddy K., Sadasiva Rao K., Raghavender K B P., Teja A., Ramesh T.and Arunakumari G.(2013) *Small ruminant research,* 115, 71-76.
[50] Ryan N.K., Woodhouse C.M., Van der Hoek K.H., Gilchrist R.B., Armstrong D.T. and Norman R.J. (2002) *Biology of Reproduction,* 66,1548–1554.