RESEARCH ARTICLE

Effect of Epidermal Growth Factor Supplementation and Different Fertilization Media on *In Vitro* Maturation and Fertilization of Goat Oocytes

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Abstract

The present study evaluated the effect of epidermal growth factor (EGF) supplementation during *in vitro* maturation (IVM) and different fertilization media on nuclear maturation and fertilization rates of goat oocytes. Ovaries were collected from a local abattoir and oocytes were recovered by the slicing method. A total of 386 grade A and B oocytes were selected and matured in two media: medium I (TCM-199 + FSH 1 µg/mL + hCG 20 IU/mL + Estradiol 1 µg/mL + Gentamicin 50 µg/mL + 10% EGS -Estrus Goat Serum) and medium II (medium I + EGF -Epidermal Growth Factor, 20 ng/mL). Oocytes were incubated for 27 h at 38.5°C with 5% CO_2 in incubator. A proportion of the oocytes was fixed and stained with 1% aceto-orcein to assess nuclear maturation to metaphase II (M II). The remaining oocytes were fertilized in three different media (Fert-TALP, BO-IVF and SOF) using fresh buck semen for 18 h, and total penetration (2 pronuclei plus polyspermy) was evaluated. The proportion of oocytes reaching M II was significantly higher ($p < 0.05$) in the EGF-supplemented group than in the control (52.13 ± 3.19% vs. 16.16 ± 0.50%, respectively). Among EGF-matured oocytes, total penetration rate was significantly higher ($p < 0.05$) in BO-IVF medium (73.75 ± 5.25%) than in Fert-TALP (47.72 ± 7.14%) and SOF (20.98 ± 7.79%). In conclusion, supplementation of IVM medium with EGF combined with fertilization in BO-IVF medium improves nuclear maturation and fertilization rates of goat oocytes and may be beneficial for *in vitro* embryo production.

Key words: BO-IVF, EGF, Fert-TALP, Goat oocytes, *In vitro* fertilisation *In vitro* maturation, SOF

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Introduction

The *in-vitro* embryo production (IVEP) has become an important alternative to *in vivo* embryo harvesting by conventional superovulation, particularly in small ruminants, where repeated surgical collection of embryos is both impractical and unethical. IVEP provides an efficient, repeatable and cost-effective method to produce large numbers of embryos from slaughterhouse-derived ovaries or from oocytes obtained by follicular aspiration (Carolan *et al*., 1992). The success of IVEP is closely related to the developmental competence of oocytes, which in turn depends on the efficiency of the *in vitro* maturation (IVM) process (Yadav *et al*., 1998). Oocyte maturation is a complex sequence of nuclear and cytoplasmic events that prepare oocyte fertilization and subsequent embryo development. Epidermal growth factor (EGF) is a polypeptide involved in cell division, proliferation and signal transduction via the mitogen-activated protein kinase (MAPK) pathway. EGF is present in follicular fluid and binds to receptors on cumulus and granulosa cells, activating signalling pathways that promote cumulus expansion and oocyte maturation (Watson *et al*., 1992; Guler *et al*., 2000; Gall *et al*., 2005). Supplementation of IVM media with EGF has improved oocyte maturation rates in several species, including pigs, buffaloes and goats (Purohit *et al.,* 2005; Nagar and Purohit, 2005)

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The culture media used for *in-vitro* fertilization (IVF) must support several critical processes, including sperm motility, capacitation, gamete fusion, and the initiation of embryonic development. Various media formulations have been developed to support sperm capacitation and fertilization, such as Tyrode's albumin lactate pyruvate (TALP) medium for goats (Cox *et al*., 1994; Wang *et al.,* 2002), modified defined medium (Younis *et al.,* 1991) and Brackett and Oliphant (BO) medium for goats (Crozet *et al*., 1995), and synthetic oviduct fluid (SOF) medium for sheep (Wang *et al*., 2013). However, comparative information on the efficiency of these fertilization media for goat oocytes, especially after EGF-supplemented IVM, remains limited. Therefore, this study was aimed to evaluate the effect of EGF supplementation during IVM on nuclear maturation and fertilization rates of goat oocytes when co-incubated with sperm in different IVF media.

Materials and Methods

The study was conducted after approval by the Institutional Animal Ethics Committee. Ovaries from slaughtered local goats were collected from a nearby slaughterhouse. Unless otherwise stated, all chemicals and reagents were procured from Sigma-Aldrich (USA) and HiMedia (India). Commercial BO-IVF fertilization medium and BO-W washing medium were obtained from IVF Biosciences, United Kingdom. All prepared media were sterilized by filtration through 0.22 µm syringe filters.

Collection of Ovaries and Recovery of Oocytes

Ovaries with visible follicles were collected irrespective of the age, physiological or nutritional status of the animals and transported to the laboratory in normal saline solution supplemented with gentamicin sulphate (50 µg/mL) at 37°C within 2-4 h post-slaughter. On arrival, ovaries were washed several times in warm saline, and oocytes were recovered by the slicing method as performed by Poornima *et al.* (2025). Only cumulus-oocyte complexes (COCs) of grade A and B were selected for the study as described by Kharche *et al*. (2008).

In-vitro Maturation (IVM) of Goat Oocytes

A total of 386 cumulus-oocyte complexes (COCs) were selected for *in vitro* maturation (IVM) and subsequent fertilization. The selected COCs were washed three times in BO-W washing medium and then randomly allocated to two IVM treatments. Control medium (medium I) consisted of TCM-199 supplemented with FSH (1 µg/mL), hCG (20 IU/mL), Estradiol (1 µg/mL), Gentamicin (50 µg/mL) and 10% Estrus Goat Serum (EGS). The EGF-supplemented medium (medium II) comprised medium I and EGF (20 ng/mL). Oocytes were cultured in 50 µL droplets (10-15 COCs per droplet) under mineral oil and incubated for 27 h at 38.5°C in a humidified atmosphere of 5% CO_2 in air.

Assessment of Nuclear Maturation Rate

After 27 h of maturation, the subset of oocytes from each group was examined for meiotic division. Matured oocytes were denuded by repeated gentle pipetting. Using a 21-gauge needle and syringe, two parallel lines of paraffin wax were carefully drawn on a coverslip. Under a stereo-zoom microscope, denuded oocytes (those without cumulus cells) were gently placed in the grease free microslide with a pipette with 5 µL washing media (BO Wash media). A cover slip was then carefully placed and slightly pressed to flatten the oocytes, securing them between the slide and the cover slip. The prepared slides were placed in a jar containing acetic alcohol (a fixative solution) and left undisturbed for 24 h. After the fixation period, the slides were stained with 1% aceto-orcein as documented by Pawshe *et al*. (1994) and Vijayalakshmi (2016). The nuclear status of mature oocytes was classified into four phases: Germinal vesicle (GV), Germinal breakdown (GVBD), Metaphase I (M I) and Metaphase II (M II) as described by Vijayalakshmi (2016). *In vitro* maturation was assessed based on nuclear maturation, and the number of oocytes reaching metaphase II (MII) was considered as matured oocytes.

Sperm Preparation

Fresh semen was collected from a trained Osmanabadi buck from the Dept of LFC, Veterinary College, Bidar using the artificial vagina (AV) technique. Prior to sperm processing for *in vitro* fertilization, the ejaculate was evaluated for key semen quality parameters including mass motility, sperm viability and plasma membrane integrity. For IVF, 30 µL of neat semen was used per swim-up procedure.

Swim up procedure was performed as per Palomo *et al.* (1999) with some modification. Briefly, semen was layered under 1 mL of Sp-TALP medium supplemented with bovine serum albumin (BSA, 6 mg/mL), sodium pyruvate (1 mM), gentamicin (50 µg/mL) and HEPES (10 mM), and centrifuged at 224g for 5 min. The supernatant was discarded, and the sperm pellet was washed twice with Sp-TALP. The final pellet was resuspended in Sp-TALP containing heparin (10 µg/mL) at the bottom and incubated for 60 min at 38.5°C in a humidified CO_2 incubator. The upper sperm-rich layer was collected and used for insemination

In Vitro Fertilization (IVF)

After 27 h of IVM, only COCs exhibiting cumulus expansion (n=217) were selected for IVF. Oocytes were randomly divided into three IVF media: Fert-TALP, BO-IVF and SOF, each supplemented with heparin 10 µg/mL. Oocytes were washed in the respective pre-equilibrated fertilization media, partially denuded by gentle pipetting, and placed in 50 µL fertilization droplets (10-15 oocytes per droplet) under mineral oil in 35-mm Petri dishes. Capacitated spermatozoa were added to each droplet to achieve a final concentration of 1-2× 10^6 sperm/mL, and gametes were co-incubated for

18 h at 38.5°C in 5% CO_2 in air. The number of oocytes with total penetration *i.e.,* two pronuclei or polyspermy were considered as fertilized oocytes.

Assessment of Fertilization

After 18 h of co-incubation, presumptive zygotes from each group were denuded completely and fixed and stained with 1% aceto-orcein as described above. Oocytes were examined for the presence of two pronuclei and evidence of polyspermy (more than two pronuclei).

Statistical Analysis

Data on nuclear maturation and fertilization rates were expressed as mean ± standard error (SE). Differences between treatment groups for nuclear maturation was analysed by t-test and fertilization rate was performed using General Linear model (GLM) followed by Duncan's multiple range test (DMRT) to compare means. Statistical analyses were performed using SPSS 20.0, and differences were considered significant at $p < 0.05$.

Results and Discussion

Nuclear Maturation Rate of Goat Oocytes with or without EGF

The proportion of oocytes reaching MII after 27 h of IVM was significantly higher ($p < 0.05$) in the EGF supplemented group than in the control (52.13 ± 3.19% vs. 16.16 ± 0.50%; Table 1, Fig. 1). This finding was comparable to the maturation rates reported in goats by Nagar and Purohit (2005) and Yadav *et al.* (2013), who observed 64.51% and 60.00% oocytes at MII, respectively. However, the MII rate in the present study was lower than the 81.90% and 80.44 ± 1.60% reported in Boer goats and rabbits by Wang *et al.* (2007) and El-Ratel and Fouda (2016), respectively. Growth factors are widely recognized for their role in regulating IVM in domestic species. Goat cumulus cells express EGF receptors (Gall *et al.*, 2004), and EGF has been shown to activate the MAPK pathway in goat COCs during IVM (Gall *et al.*, 2005). Binding of EGF to its receptor activates intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and phosphorylation of downstream targets (Carpenter and Cohen, 1990). This signalling cascade supports progression to MII, acquisition of fertilization competence and subsequent embryonic development.

Fertilization Rate

Among oocytes matured with EGF, total penetration rate was significantly higher ($p < 0.05$) in BO-IVF medium (73.75 ± 5.25%) than in Fert-TALP (47.72 ± 7.14%) and SOF (20.98 ± 7.79%; Table 2, Fig. 2). In contrast, fertilization rates of control oocytes (without EGF) were low and did not differ significantly among Fert-TALP (13.19 ± 1.10%), BO-IVF (11.11 ± 3.04%) and SOF (8.75 ± 5.90%) media. These findings were in agreement with Sadeesh *et al.* (2014) and Ambalkar (2021), who reported higher fertilization rates when oocytes were fertilized in BO-based media. However, Sithole *et al.* (2023) observed lower total fertilization rates (13.3 ± 7.6 to 23.3 ± 15.3%) in cattle using BO medium, suggesting that species differences and protocol variations may influence the response to BO-IVF. The high fertilization rate observed in the EGF + BO-IVF group may be partly attributed to the presence of heparin and the PHE (penicillamine, hypotaurine and epinephrine) mixture in the commercial BO medium, which are known to accelerate oocyte penetration and improve cleavage rates compared with media lacking PHE (Miller *et al.,* 1994; Ambalkar, 2021). Conversely, TALP medium is usually prepared fresh in the laboratory and may show batch-to-batch variation due to differences in reagent quality, water purity, pH, osmolarity and handling. Such variability can contribute to lower and inconsistent IVEP outcomes unless rigorous quality control procedures are applied (Sangale *et al.*, 2024).

The relatively low fertilization rate in SOF in the present study may be related to the formulation of the medium, temperature fluctuations, storage and handling issues, or water quality (Yadav, 2004; Jharna *et al.*, 2025). In buffalo, inadequate glucose in the culture medium has been associated with developmental arrest at the morula stage and reduced cleavage and blastocyst rates (Abu El-Naga *et al.,* 2024), emphasizing the importance of optimizing energy substrates for each species and developmental stage.

Table 2: Fertilization rates (%) of *in vitro* matured goat oocytes with or without EGF in different fertilization media (Mean ± SE)

Maturation media	Replicates	Fertilization rate(%) in different media		
		Fert TALP	BO IVF	SOF
Control	5	$13.19 \pm 1.10^{A,a}$ (5/39)	$11.11 \pm 3.04^{A,a}$ (4/38)	$8.75 \pm 5.90^{A,a}$ (3/36)
EGF	5	$47.72 \pm 7.14^{AB,b}$ (16/34)	$73.75 \pm 5.25^{B,b}$ (25/34)	$20.98 \pm 7.79^{A,a}$ (8/36)

Values in parenthesis indicate total pooled across the replicates. Means bearing different superscripts (a,b) within the column and (A,B) within the row differ significantly ($p<0.05$).

Table 1: Nuclear maturation rate (Mean ±SE) with or without EGF in goat oocyte

Maturation media	No. of oocytes stained (n)	Nuclear maturation (Mean ±SE)							
		GV	Mean %	GVBD	Mean %	MI	Mean %	MII	Mean %
Control	80 (n=5)	24	31.28 ± 3.58^{a}	26	33.52 ± 1.91^{a}	18	21.26 ± 5.62^{a}	12	16.16 ± 0.50^{a}
EGF	89 (n=5)	12	13.13 ± 1.69^{a}	14	15.47 ± 2.77^{a}	17	19.27 ± 1.54^{a}	46	52.13 ± 3.19^{b}

Note: n indicates the number of replicates.

Means bearing different superscripts within the column differ significantly (p<0.05

The fertilization rate of oocytes matured with EGF in this study exceeded the 21.9% rate reported in goats by Nagar and Purohit (2005). Similar findings were noted by Grazul-Bilska *et al*. (2003) and Wani *et al*. (2012) in sheep. Kobayashi *et al*. (1994) observed that supplementing the maturation medium with epidermal growth factor enhanced blastocyst development from fertilized bovine ova. Lee and Fukui (1995) found that EGF exerted a stimulatory influence on post-fertilization development of bovine embryos, particularly at the morula/early blastocyst stages, rather than during early IVM. Additionally, bovine blastocysts cultured and vitrified in EGF-supplemented media displayed greater developmental potential (Mtango *et al*., 2003).

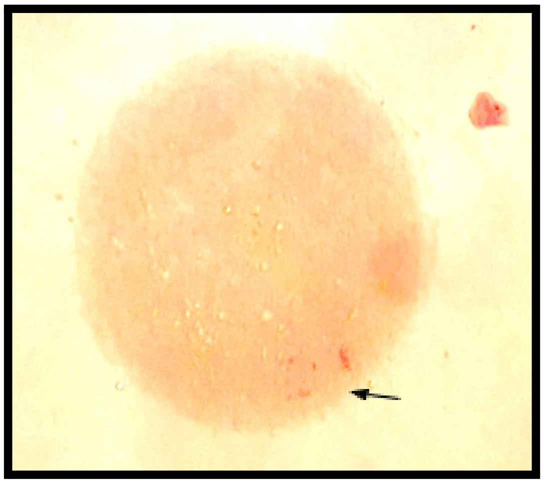

Fig. 1: Nuclear maturation stages of goat oocytes by 1% aceto-orcein stain: metaphase II (MII) (x 40 magnifications**)**

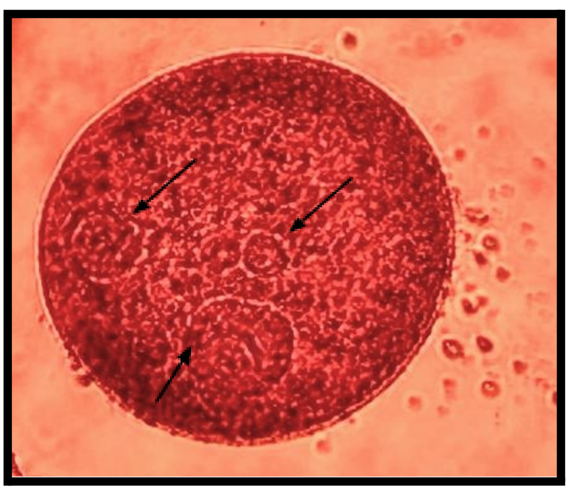

Fig. 2: Assessment of fertilization rate (Pronucleus) by 1% aceto-orcein stain (x40 magnifications)

Conclusion

It can be concluded that EGF supplementation (20 ng/mL) during IVM and the use of BO-IVF medium for fertilization significantly improve nuclear maturation and fertilization rates of goat oocytes *in vitro*.

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References

Abu El-Naga, E.M., Ali, M.E., Ali, R.H., Hozyen, H.F. & Hussein, H.A. (2024). Cleavage and *in vitro* cultivation rates monitoring in culture media supplemented with energy sources, non-essential amino acids, and antioxidants in the buffalo embryos. *BMC Veterinary Research*, *20*(1), 521.

Ambalkar, M.B. (2021). Effect of media and capacitating agent on in vitro fertilization of goat oocytes. *Ph.D. Thesis*, Department of Animal Reproduction, Gynaecology and Obstetrics, Maharashtra Animal and Fishery Sciences University, Nagpur, India.

Carolan, C., Monaghan, P., Mehmood, A., Lonergan, P., Gallagher, M., & Gorden, I. (1992). Slicing of bovine ovaries as a means of oocyte recovery. *Journal of Reproduction and Fertility*, *9,* 51.

Carpenter, G., & Cohen, S. (1990). Epidermal growth factor. *Journal of Biological Chemistry*, *265,* 7709-7712

Cox, J., Abila, J., Saravia, F., & Maria, A. (1994). Assessment of fertilizing ability of goat spermatozoa by in vitro fertilization of cattle and sheep intact oocytes. *Theriogenology*, *41,* 1621-1629.

Crozet, N.M., Ahmed-Ali, M., & Dubos, P. (1995). Developmental competence of goat oocytes from follicles of different size categories following maturation, fertilization, and culture *in vitro*. *Journal of Reproduction and Fertility, 103*(2), 293-298

El-Ratel, I.T., & Fouda, S.F. (2016). Potentiality of epidermal growth factor or/and cysteamine in maturation medium on *in vitro* rabbit embryo production and apoptosis. *Global Veterinaria*, *17*(6), 505-512.

Gall, L., Chene, N., Dahirel, M., Ruffini, D., & Boulesteix, C. (2004). Expression of epidermal growth factor receptor in the goat cumulus-oocyte complex. *Molecular Reproduction and Development*, *67,* 439-445.

Gall, L., Boulesteix, C., Ruffini, D., & Germain, G. (2005). EGF-induced EGF receptor and MAP kinase phosphorylation in goat cumulus cells during *in vitro* maturation. *Molecular Reproduction and Development*, *71,* 489-494.

Grazul-Bilska, A.T., Choi, J.T., Bilski, J.J., Weigl, R.M., Kirsch, J.D., Kraft, K.C., Reynolds, L.P., & Redmer, D.A. (2003). Effects of epidermal growth factor on early embryonic development after *in vitro* fertilization of oocytes collected from ewes treated with follicle stimulating hormone. *Theriogenology*, *59,* 1449-1457.

Guler, A., Poulin, N., Mermillod, P., Terqui, M., & Cognie, Y. (2000). Effect of growth factors, EGF and IGF-I, and estradiol on *in vitro* maturation of sheep oocytes. *Theriogenology*, *54*(2), 209-218.

Jharna, J.F., Bari, M.W., Alfian, N.F., & Kishigami, S. (2025). The absence of bovine serum albumin (BSA) in preimplantation culture media impairs embryonic development and induces metabolic alterations in mouse offspring. *International Journal of Molecular Science*, 26(14): 6989.

Kharche, S.D., Goel, A.K., Jindal, S.K., Sinha, N.K., & Yadav, P. (2008). Effect of somatic cells co-culture on cleavage and development of *in-vitro* fertilized caprine embryos. *Indian Journal of Animal Sciences*, *78*(7), 686.

Kobayashi, K., Yamashita, S., & Hoshi, H. (1994). Influence of epidermal growth factor and transforming growth factor-α on *in vitro* maturation of cumulus cell-enclosed bovine oocytes in a defined medium. *Journal of Reproduction and Fertility*, *100*(2), 439-446.

Lee, E.S., & Fukui, Y. (1995) Effect of various growth factors in a defined culture medium on *in vitro* development of bovine embryos matured and fertilized *in vitro*. *Theriogenology*, *44,* 71-83.

Miller, G.F., Gliedt, D.W., Rakes, J.M., & Roriel, R.W. (1994). Addition of penicillamine, hypotaurine and epinephrine (PHE) or bovine oviductal epithelial cells (BOEC) alone or in combination to bovine in vitro fertilization medium increases the subsequent embryo cleavage rate. *Theriogenology*, *41,* 689-696.

Mtango, N.R., Varisanga, M.D., Dong, Y.J., Rajamahendran, R., & Suzuki, T. (2003) Growth factors and growth hormone enhance *in vitro* embryo production and post –thaw survival of vitrified bovine blastocyst. *Theriogenol*ogy, *59,* 1393-1402.

Nagar, D., & Purohit, G.N. (2005). Effect of epidermal growth factor on maturation and fertilization *in vitro* of goat follicular oocytes in a serum free or serum supplemented medium. *Veterinarski Arhiv*, *75*(6), 459.

Palomo, M.J., Mogas, T., & Paramio. M.T. (1999). Effect of semen preparation on IVF of pre-pubertal goat oocytes. *Theriogenology*, *51,* 927-940.

Pawshe, C.H., Rao, K.A., Jain, S.K., & Totey, S.M. (1994). Biochemical studies on goat oocytes: Timing of nuclear progression, effect of protein inhibitor and pattern of polypeptide synthesis during *in vitro* maturation. *Theriogenology*, *42*(2), 307-320.

Poornima, A., Bijurkar, R.G., Doddagoudar, V & Gond, M. (2025). oocyte recovery efficiency from slaughtered goat ovaries: A comparison of collection techniques. *The Indian Journal Of Veterinary Sciences and Biotechnology*, *21*(5), 145-149.

Purohit, G.N., Brady, M.S. & Sharma, S.S. (2005). Influence of epidermal growth factor and insulin-like growth factor-1 on nuclear maturation and fertilization of buffalo cumulus oocyte complexes in serum free media and their subsequent development *in vitro*. *Animal Reproduction Science*, *87,* 229-239.

Sadeesh, E.M., Fozia Shah, F.S., Balhara, A.K., Thirumaran, S.M., Sarita Yadav, S.Y., & Yadav, P.S. (2014). Effect of growth factor and antioxidant on *in vitro* maturation of oocytes and cleavage rates of *in vitro* produced Indian buffalo (*Bubalus bubalis*) embryos. *Veterinarski Arhive*, *84*(5), 459-474.

Sangale, R.R., Pawshe, C.H., & Ambalkar, M.B. (2024). Comparative efficiency of different culture media on *in vitro* embryonic development of goat oocytes. *Indian Journal of Animal Research*, *1,* 1-6.

Sithole, S.M., Mphaphathi, M.L., Sebopela, M.D., & Nedambale, T.L. (2023). Comparison of *in vitro* maturation media on cattle oocytes after *in vitro* embryo production. *American Journal of Animal and Veterinary Science*, *18,* 27-39.

Vijayalakshmi (2016). Evaluation of different antioxidant supplements on in vitro maturation of buffalo oocytes. *M.V.Sc Thesis*, Department of Veterinary Physiology and Biochemistry, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India.

Wang, B., Baldassarre, H., Tao, T., Gauthier, M., Neveu, N., Zhou, J.F., Leduc, M., Duguay, F., Bilodeau, A.S., Lazaris, A., & Keefer, C. (2002). Transgenic goats produced by DNA pronuclear microinjection of *in vitro* derived zygotes. *Molecular Reproductionand Development: Incorporating Gamete Research*, *63*(4), 437-443.

Wang, Z.G., Xu, Z.R. & Yu, S.D. (2007). Effects of oocyte collection techniques and maturation media on *in vitro* maturation and subsequent embryo development in Boer goat. *Czech Journal of Animal Science*, *52*(1), 21-25.

Wang, Z., Lin, P., & Yu, S. (2013). Effects of ghrelin on developmental competence and gene expression of *in vitro* fertilized ovine embryos. *Theriogenology*, *79,* 695-701.

Wani, A.R., Khan, M.Z., Sofi, K.A., Lone, F.A., Malik, A.A., & Bhat, F.A. (2012). Effect of cysteamine and epidermal growth factor (EGF) supplementation in maturation medium on *in vitro* maturation, fertilization and culturing of embryos in sheep. *Small Ruminant Research*, *106*(2-3), 160-164.

Watson, A.J., Hogan, A., Wiemer, K., & Schultz, G.A. (1992). Expression of growth factor ligand and receptor genes in the pre-implantation embryos. *Molecular Reproduction and Development*, *31,* 87-95.

Yadav, P.S., Saini, A., Kumar, A., & Jain, G.C. (1998). Effect of oviductal cell co-culture on cleavage and development of goat IVF embryos. *Animal Reproduction Science*, *51,* 301-306.

Yadav, R. (2004). *In vitro* fertilization and cleavage rate of in vitro matured pubertal goat oocytes. *M.V.Sc Thesis*, Pandit Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyala Evam Go Anusandhan Sansthan, Mathura, U.P, India.

Yadav, P., Kharche, S.D., Goel, A.K., Jindal, S.K., & Goel, P. (2013). Assessment of nuclear maturation and subsequent *in-vitro* embryo development of caprine oocytes with different supplementations in maturation medium. *Indian Journal of Animal Sciences*, *83*(10), 1048-1052.

Younis, A.I., Zuelke, K.A., Harper, K.M., Oliveira, M.A.L., & Brackett, B.G. (1991). *In vitro* fertilization of goat oocytes. *Biology of Reproduction*, *44*(6), 1177-1182.