International Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

Crossref

IJBSM September 2024, 15(9): 01-09 **Article** AR5539

Research Article *Natural Resource Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2024.5539

Effect of Epidermal Growth Factor on *In Vitro* Maturation, Fertilization and Early Embryonic Development of Cattle Oocytes

Dipannita Baishya[1], Arundhati Bora[2] and Champak Barman[2]

[1]Bovine Contagious Pleuro-Pneumonia, Khanapara, Guwahati, Assam (781 022), India
[2]Dept. of Veterinary Physiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam (781 022), India

Open Access

Corresponding drchampak80@gmail.com

0000-0001-9702-8680

ABSTRACT

The present experiment was conducted during January, 2018 to March, 2019 in the laboratory, Dept. of Veterinary Physiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam (781022), India to investigate the effect of epidermal growth factor on *in vitro* maturation, fertilization and early embryonic development of cattle oocytes. A total of 318 nos. cattle ovaries were collected from slaughter houses for one year and 1089 nos. culturable oocytes were collected. 381 out of 1089 nos. culturable oocytes were subjected to EGF supplemented serum and serum free basic maturation media. The mean percentage of *in-vitro* maturation of cattle oocytes based on polar body extrusion was found to be significantly higher in EGF supplemented serum free basic maturation media (70.00±14.49) than the serum basic maturation media without EGF (54.17±7.19) respectively. But the mean percentages of *in vitro* maturation of cattle oocytes based on the cumulus expansion and polar body extrusion were found to be significantly higher in EGF supplemented serum free basic maturation media (77.59±5.48 and 70.00±14.49) than the value recorded in serum free basic maturation media without EGF (64.20±3.77 and 45.95±8.19). The mean cleavage percentages recorded at 4 cell, 8 cell, 16 cell and morula stages of embryos in EGF supplemented serum free culture media were found to be significantly higher than the value recorded in serum culture media without EGF. From the present experiment, it can be inferred that addition of EGF in serum free basic maturation media had little beneficial effect than serum basic maturation media.

KEYWORDS: Cattle, cleavage, epidermal growth factor, oocyte

***Citation* (VANCOUVER):** Baishya et al., Effect of Epidermal Growth Factor on *In Vitro* Maturation, Fertilization and Early Embryonic Development of Cattle Oocytes. *International Journal of Bio-resource and Stress Management,* 2024; 15(9), 01-09. HTTPS://DOI.ORG/10.23910/1.2024.5539.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 17th June 2024 RECEIVED in revised form on 20th August 2024 ACCEPTED in final form on 03rd September 2024 PUBLISHED on 04th September 2024

1. INTRODUCTION

Improvement of *in vitro* embryo production (IVEP) is important for the production of high quality embryos for use in animal biotechnology and biochemical research and IVEP is greatly influenced by the events taking place during maturation, fertilization of oocytes followed by the subsequent development of presumptive embryos (Feugang et al., 2005; Chandra et al., 2012; Prasad et al., 2018; Kumar et al., 2020; Ahmed et al., 2023 and Yusuf, 2024). Production of high quality embryos depend on type of breed, quality of oocytes, follicular size and micro-environment, fertilization atmosphere and embryo culture milieu (Fiammetta, 2015; Borah and Biswas, 2020 and Kim et al., 2023). Growth factors (GF) have been shown to play a regulatory role in the functioning of the ovary (Echternkamp et al., 1994) and of the uterus (Boehm et al., 1990), resulting in a trophic effect on the endometrium and embryo. Growth factors are present *in vivo*, act on embryo receptors, are anti-apoptotic and increase development rates. *In vitro* produced embryos are exposed to *in vitro* sub-optimal conditions that greatly differ from the *in vivo* environment. The efficiency of an *in vitro* embryo production system is assessed based on cleavage of fertilized oocytes and their further development into blastocysts (Estrada et al., 1991; Lonergan et al., 2003; Yousef et al., 2018 and Currin et al., 2021). More recent observations imply that the effect of growth factor in the enhancement of maturation of oocytes is retarded by serum (Sakaguchi et al., 2000). The culture media supplemented with epidermal growth factor (EGF) and other growth factors have regulatory role in growth and development of *in vitro* cultured bovine oocytes and embryos. In particular, exogenous EGF enhances the developmental rate and mitogenesis of preimplantation bovine embryos (Lonergan et al., 1996; Singh et al., 2015; Arias et al., 2022; Yang et al., 2022) and also in *in vitro* maturation of sheep oocytes (Guler et al., 2000). The growth factor supplemented with serum-free system can also maintain *in vitro* bovine embryo production (Umdor et al., 2021). Among various factors in bovine embryo production, one of the most important factors regulating the number and quality of oocyte matured *in vitro*, is the culture system used for *in vitro* maturation (Neira et al., 2010; Dhali et al., 2011 and Ahumada et al., 2013). Inclusion of serum and combination of the hormones have been considered essential for obtaining high maturation and fertilization rates in buffaloes and cattle oocytes (Chuangsoongneon and Kamonpatana, 1991; Totey et al., 1992; Jainudeen et al., 1993 and Yang et al., 2019) and in development of preimplantation embryos in mouse *in vitro* (Gardner and Kaye, 1991). It is known that serum culture medium contains some unknown factors; therefore, to simplify and avoid contamination it is preferable to develop an *in vitro* culture (IVC) system of defined composition which is free of blood components or cell constituents. Supplementing specific paracrine and endocrine components during *in vitro* maturation (IVM) of bovine cumulus-oocyte complexes (COCs) improves the success of *in vitro* embryo production and maximize embryonic competency to be useful in oocytes maturation and growth. Although similar works have been conducted in different media, however literatures about the effect of epidermal growth factors on serum and serum free media for optimization of embryo production in bovine are very limited and needs further study. Moreover, embryos that will be produced have to be remained more viable without infection and has to be protected from cryopreservation damage. Henceforth, the effect of epidermal growth factor on serum and serum free media requires further study in relation to the early embryonic development of cattle oocytes.

2. MATERIALS AND METHODS

2.1. Collection of oocytes

Bovine ovaries of unknown reproductive status were collected from local slaughter house in and around Khanapara, Guwahati, Assam, India during January, 2018 to March, 2019 and carried to the laboratory in normal saline solution (0.85% NaCl) fortified with gentamicin (50 µg ml^{-1}) in a thermo flask at 37–38°C within 1–2 h of slaughter. In the laboratory, extraneous tissues were removed and ovaries were thoroughly washed with 70% ethanol followed by three rinses in phosphate buffer saline solution.

2.2. Grading of oocytes

Oocytes were aspirated from all the visible non-atretic surface follicles of the ovary by using a 10 ml sterile syringe fitted with 18 G needle containing oocytes collection medium after final washing and after that, oocytes were searched in oocytes collection media under stereo zoom microscope and cumulus oocytes complexes (COCs) were recovered. Oocytes possessing a full cumulus mass, unfragmented cytoplasm and intact zona were selected for culture and after that, the COCs were evaluated and graded (Hafez and Hafez, 2000). Good and excellent quality oocytes having more than 3–5 cumulus cell layers were cultured in 50 µl droplets (20–25 oocytes $droplet^{-1}$) of maturation media in 35 mm sterile petridish.

2.3. In vitro maturation (IVM) of bovine oocytes

The excellent (>5 layers) and good (>3 layers) quality of COCs were selected for *in vitro* maturation (IVM). Two different types of maturation and culture media *viz* serum basic maturation media (SBMM) containing modified TCM-199+serum (10% Fetal Bovine Serum)+Sodium pyruvate+L-glutamine+gentamicin+pFSH+hMGinj+E_2 (estradiol), serum free basic maturation media (SFBMM)

containing modified TCM-199+Polyvinylpyrrolidone (PVP)+Bovine serum albumin (BSA)+Sodium pyruvate+ L-glutamine+pFSH+gentamicin+hMGinj+E_2 (estradiol), serum basic culture media (SBCM) containing mCR2aa stock+10% FBS+Gentamicin, serum free basic culture media) (SFBCM) containing mCR2aa stock+BSA-V+PVP+Gentamicin were used for *in vitro* maturation and *in vitro* culture of the oocytes. Insulin-like growth factor-I (IGF-I) (100 ng) were added in maturation media as well as embryo culture media. Frozen bull semen straws of proven fertility were used and prepared for *in vitro* capacitation by density gradient method using B.O. media.

2.4. Epidermal growth factors (Sigma 4127) 0 .1 mg vial

EGF from mouse submaxillary glands (Code E-4127) was used. This product is lyophilized from a solution in 1 ml of 5 mM ammonium acetate at P^H 6.5. It should be reconstituted by adding the contents of the vial to a solution containing 0.1–1% BSA or 1–10% serum in buffered saline or tissue culture media.

To study the effect of epidermal growth factors on in vitro maturation and in vitro culture, a concentration of 30 ng ml^{-1} was used.

2.5. Statistical analysis

All the collected data were analyzed as per statistical procedures (Snedecor and Cochran, 1994) and expressed in Mean±SE. 'Z' test of SPSS (Anonymous, 2008) was performed for mean statistical significant difference.

3. RESULTS AND DISCUSSION

3.1. In vitro maturation of cattle oocytes based on cumulus cells expansion and extrusion of polar body in EGF supplemented serum and serum free basic maturation media

Table 1 shows *in vitro* maturation of bovine oocytes based on cumulus cells expansion and extrusion of the polar body in epidermal growth factor (EGF) supplemented (30 ng ml^{-1}) serum and serum free basic maturation media. A total of 318 nos. bovine ovaries were collected from slaughter houses for one year and 1089 nos. culturable oocytes were collected. 381 out of 1089 culturable oocytes were subjected to EGF supplemented serum basic and serum free basic maturation media. 207 and 174 nos. out of 381 nos. of culturable oocytes were subjected to *in vitro* maturation in both basic maturation media respectively. 168 out of 207 and 135 out of 174 oocytes were fully matured (+++) in EGF supplemented serum basic and serum free basic maturation media respectively. So the mean percentage of *in vitro* maturation (IVM %) based on cumulus cells expansion were recorded as 81.16±4.71 and 77.59±5.48 respectively in EGF supplemented serum basic and serum free basic maturation media. 60 out of 168 and 55 out of 135 cumulus oocyte complexes were denuded to see polar body in perivitelline space of matured denuded oocytes in EGF supplemented serum basic and serum free basic maturation media respectively. 42 out of 60 and 39 out of 55 matured bovine oocytes showed extrusion of polar body in EGF supplemented serum basic (70.00±10.25%) and serum free basic maturation media (70.00±14.49%). Z test revealed that there was no significant difference between EGF supplemented serum basic and serum free basic maturation media in respect of *in vitro* maturation (IVM%) of cattle oocytes on the basis of cumulus cells expansion and extrusion of polar body.

Table 1: *In vitro* maturation of cattle oocytes based on cumulus cells expansion and extrusion of polar body in EGF supplemented serum and serum free basic maturation media

Basic maturation media	COCs subjected to IVM (n)	Degree of cumulus expansion			IVM % (Mean±SE)	Total matured oocytes denuded (n)	Denuded oocytes showing polar body (n)	IVM % (Mean±SE)
		+	++	+++				
Serum+EGF	207	12	27	168	81.16±4.71	60	42	70.00±10.25
Serum free+EGF	174	12	27	135	77.59±5.48	55	39	70.00±14.49

From the present experiment, it can be revealed that EGF at the concentration of 30 ng ml^{-1} enhances cumulus expansion in bovine cumulus oocyte complexes (COCs) and also improves the percentage of oocytes undergoing nuclear maturation in the same way as demonstrated by Downs (1989) for rodent oocytes. The present results were in agreement with the previous reports (Harper and Brackett, 1993; Lonergun et al., 1996; Lonergun et al., 2003; Chandra et al., 2012; Prasad et al., 2018; Yousef et al., 2018 and Yang et al., 2022) where they found that epidermal growth factor at the concentrations between 20 ng to 30 ng ml^{-1} enhanced *in vitro* maturation, cleavage and pre embryonic development and observed that supplementation of EGF in maturation media positively affected cumulus expansion and maturation to Metaphase II . The results suggested that EGF might be one of the major follicular factors responsible for stimulating oocyte cytoplasmic as well as nuclear maturation. Lorenzo et al., 1994 and Nagar and Purohit, 2005 found that cumulus expansion of oocytes increased significantly with EGF supplementation

in a dose-dependent manner up to 50 ng ml^{-1} which can alter the pattern of proteins neosynthesized during *in vitro* maturation (IVM). However, the value recorded in the present experiment in respect of mean IVM percentages were found to be statistically non-significant between the both basic maturation media based on cumulus cell expansion and extrusion of polar body.

3.2. Comparison of in vitro maturation of cattle oocytes in serum and EGF supplemented serum free basic maturation media

Table 2 shows comparison of *in vitro* maturation of cattle oocytes in serum and EGF supplemented serum free basic maturation media. In the experiment, 175 and 174 nos. culturable oocytes were subjected to *in vitro* maturation in serum and EGF supplemented serum free basic maturation media respectively. 132 out of 175 and 135 out of 174 culturable oocytes were fully matured (+++) in both media. The mean percentage of *in vitro* maturation (IVM %) based on cumulus expansion were recorded as 75.43±3.25 and 77.59±5.48 respectively in serum basic maturation and EGF supplemented serum free basic maturation media. Simultaneously, 48 out of 132, and 55 out of 135 nos. of matured cocs were denuded to see polar body in both media. 26 out of 48 and 39 out of 55 matured oocytes showed extrusion of polar body in serum basic maturation (54.17±7.19) and EGF supplemented serum free (70.00±14.49) basic maturation media.

Table 2: Comparison of *in vitro* maturation of cattle oocytes in serum and EGF supplemented serum free basic maturation media

Basic maturation media	Nos. of COCs subjected to IVM (n)	Degree of cumulus expansion			IVM % (Mean±SE)	Total matured oocytes denuded (n)	Denuded oocytes showing polar body (n)	IVM % (Mean±SE)
		+	++	+++				
Serum	175	18	29	132	75.43±3.25	48	26	54.17±7.19*
Serum free+EGF	174	12	27	135	77.59±5.48	55	39	70.00±14.49*

*($p \leq 0.5$)

From the present experiment, it was observed that the mean *in vitro* maturation percentage based on extrusion of polar body was significantly higher in EGF supplemented serum free basic maturation media (70.00±14.49) than serum basic maturation media (54.17±7.19) without EGF. The result of present findings showed that though the mean *in vitro* maturation percentages (IVM %) were apparently higher in EGF supplemented serum free basic maturation media than the value recorded in serum basic maturation media based on cumulus cells expansion but statistically they were found to non-significant between the both media in respect of cumulus expansion. The present study demonstrated that supplementation of 30 ng EGF in serum free basic maturation media during IVM stimulated cumulus cell expansion and improved the percentage of bovine oocytes undergoing nuclear maturation. The findings recorded in the present experiment were in agreement with the earlier reports (Harper and Brackett, 1993; Chandra et al., 2012; Sadeesh et al., 2014; Singh et al., 2015; Arias et al., 2022; Rajesh et al., 2020; Currin et al., 2021; Yang et al., 2022 and Ahmed et al., 2023) where they observed that epidermal growth factor (EGF) has effects in the development of bovine oocytes and embryos in supplemented maturation and culture media and concluded that EGF might be one of the major follicular factors responsible for stimulating oocyte cytoplasmic as well as nuclear maturation. In contrast, Nagar and Purohit (2005) found that cumulus expansion of oocytes increased significantly with EGF supplementation in a dose-dependent manner up to 50 ng ml^{-1} and observed that supplementation of EGF in maturation media positively affected cumulus expansion and maturation to Metaphase II.

3.3. Comparison of in vitro maturation of cattle oocytes in serum free and EGF supplemented serum free basic maturation media

Table 3 shows comparison of *in vitro* maturation of cattle oocytes in serum free and EGF supplemented serum free basic maturation media. In the experiment, 162 and 174 nos. culturable oocytes were subjected to *in vitro* maturation in serum free and EGF supplemented serum free basic maturation media respectively. 114 out of 162 and 135 out of 174 culturable oocytes were fully matured (+++) respectively in both media. So the mean percentage of *in vitro* maturation based on cumulus expansion were recorded as 64.20±3.77 and 77.59±5.48 respectively in serum free and EGF supplemented serum free basic maturation media. Simultaneously, 37 out of 114, and 55 out of 135 nos. of matured cocs were denuded to see polar body in both media respectively. 17 out of 37 and 39 out of 55 matured oocytes showed extrusion of polar body in serum free (45.95±8.19) and EGF supplemented serum free (70.00±14.49) basic maturation media respectively. From the present experiment, it was observed that the mean percentages of *in vitro* maturation of cattle oocytes

Table 3: Comparison of *in vitro* maturation of cattle oocytes in serum free and EGF supplemented serum free basic maturation media

Basic maturation media	Nos. of COCs subjected to IVM (n)	Degree of cumulus expansion			IVM % (Mean±SE)	Total matured oocytes denuded (n)	Denuded oocytes showing polar body (n)	IVM % (Mean±SE)
		+	++	+++				
Serum free	162	19	37	114	64.20±3.77*	37	17	45.95±8.19*
Serum free+EGF	174	12	27	135	77.59±5.48*	55	39	70.00±14.49*

*($p \leq 0.5$)

were found to be significantly higher in EGF supplemented serum free basic maturation media than the value recorded in serum free basic maturation media.

The present results were in agreement with previous reports of enhancement of maturation after culture with EGF at the concentrations between 20 to 30 ng ml^{-1} (Park and Lin, 1993; Chandra et al., 2012; Richani et al., 2014; Singh et al., 2015 and Prasad et al., 2018). The results indicated that EGF enhances cumulus expansion in bovine COCs, in the same way as demonstrated by Downs (1989) for rodent oocytes. Epidermal Growth Factor (EGF) influenced oocyte maturation and blastocyst production rates in a number of mammals as bovine cumulus cells express EGF receptors (Purohit et al., 2005; Sadeesh et al., 2014; Arias et al., 2022; Yousef et al., 2018; Yang et al., 2022) and EGF triggers signaling through the MAPK pathway during IVM in goat cumulus oocyte complexes (Kumar and Purohit, 2004; Borah and Biswas, 2020) and is involved in the regulation of follicular growth and oocyte maturation in goats. Goat oocytes matured *in vitro* in the presence of EGF had greater cumulus oocyte complex expansion, higher maturation and fertilization rates than the oocytes matured without EGF (Nagar and Purohit, 2005).

The results of various researchers indicated that cumulus expansion and nuclear maturation rate of bovine oocytes are significantly higher when they are cultured in a medium (10% FCS with TCM 199) supplemented with various doses (1, 10, 100 ng ml^{-1} EGF) than without EGF. Epidermal growth factor in serum is one of the undetermined components contributing to enhanced oocyte maturation and the major site of action of growth factors that regulates oocyte maturation is the cumulus cells (Harper and Brackett, 1993; Ahumada et al., 2013 and Ahmed et al., 2023).

3.4. Effect of EGF supplemented serum culture and serum free culture media on in vitro fertilization and early embryonic development of in vitro matured cattle oocytes

Table 4 shows the effect of EGF supplemented serum culture and serum free culture media on *in vitro* fertilization and early embryonic development of *in vitro* matured cattle oocytes. Following *in vitro* maturation and denudation, a total of 108 and 105 *in vitro* matured cattle oocytes were fertilized in EGF supplemented serum culture and serum free culture media and out of 108, 78 and out of 105, 80 nos. *in vitro* matured cattle oocytes were cleaved respectively in both media. In the present experiment, the mean fertilization rates (%) were recorded as 72.22±4.31 and 76.19±4.16 respectively in EGF supplemented serum culture and serum free culture media. Statistical analysis revealed no significant differences between EGF supplemented serum culture and serum free culture media in respect of IVF percentages. The mean cleavage percentages (%) at 4 cell, 8 cell, 16 cell, morula & blastocyst stages of cattle embryos in EGF supplemented serum culture and serum free culture media were recorded as 55.56±4.78 and 57.14±4.83, 43.52±4.77 and 45.71±4.86, 34.26±4.57 and 37.14±4.72, 21.30±3.94 and 24.76±3.85 & 5.56±2.20 and 4.76±2.08 respectively but the mean cleavage percentages recorded at 4 cell, 8 cell, 16 cell, morula and blastocyst stages of cattle embryos were found to be statistically non-significant between the both culture media containing EGF.

The findings recorded in the present experiment were in

Table 4: Effect of EGF supplemented serum culture and serum free culture media on in vitro fertilization and early embryonic development of *in vitro* matured cattle oocytes

Culture Media	Total COCS subjected to IVF (n)	IVF (%) (Mean±SE)	Cleavage (%)				
			4 CELL% (Mean±SE)	8 CELL% (Mean±SE)	16 CELL% (Mean±SE)	Morula % (Mean±SE)	Blastocyst % (Mean±SE)
Serum+EGF	108	72.22±4.31	55.56±4.78	43.52±4.77	34.26±4.57	21.3 ± 3.94	5.56±2.2
Serum free+EGF	105	76.19±4.16	57.14±4.83	45.71±4.86	37.14±4.72	24.76±3.85	4.76±2.08

agreement with the earlier reports (Park and Lin,1993; Park et al.,1997; Sirisathien and Brackett, 2003; Richani et al., 2014; Prasad et al., 2018 and Yang et al., 2022) where they found that EGF improved oocyte fertilizing ability when cultured in defined medium. The present study demonstrated that supplementation of EGF (30 ng ml^{-1}) in serum free culture media during post fertilization period improved the proportion of embryos attaining the morula stage. Similar findings had been also reported by the earlier workers (Lonergun et al., 1996; Helmy and Abdel-Halim, 2014; Singh et al., 2015 and Ahmed et al., 2023) where they revealed that EGF supplemented *in vitro* matured bovine oocytes improved the percentage of oocytes undergoing nuclear maturation as well as cleavage.

Arias et al., 2022 investigated about *in vitro* culture medium supplementation with epidermal growth factor (EGF) in the development of oocytes and embryos in bovine and they observed that EGF treatment increased the cleavage rate and morula percentages of bovine embryos. They stated that epidermal growth factor supplementation increased embryo development rates and quality following *in vitro* fertilization (IVF) and *in vitro* culture (IVC).

3.5. Comparison of in vitro fertilization and early embryonic development of cattle oocytes in serum and EGF supplemented serum free basic culture media

Table 5 shows *in vitro* fertilization and early embryonic development of cattle oocytes in serum and EGF supplemented serum free culture media. To study *in vitro* fertilization performance, out of 91, 64 nos. *in vitro* matured oocytes and out of 105, 80 nos. *in vitro* matured oocytes were cleaved respectively and the mean fertilization rates (%) were found as 70.33±3.21 and 76.19±4.16 respectively in serum culture and EGF supplemented serum free culture media and statistical analysis revealed no significant differences between serum and serum free culture media in respect of IVF percentages.

Table 5: Comparison of *in vitro* fertilization and early embryonic development of cattle oocytes in serum and EGF supplemented serum free basic culture media

Culture Media	Total COCS subjected to IVF (n)	IVF (%) (Mean±SE)	Cleavage (%)				
			4 CELL% (Mean±SE)	8 CELL% (Mean±SE)	16 CELL% (Mean±SE)	Morula % (Mean±SE)	Blastocyst % (Mean±SE)
Serum	91	70.33±3.21	47.25±4.86*	31.87±4.99*	20.88±3.21*	7.69±4.32*	4.40±2.09
Serum free+EGF	105	76.19±4.16	57.14±4.83*	45.71±4.86*	37.14±4.72*	27.62±4.36*	4.76±2.08

*($p \leq 0.5$)

The mean cleavage percentages (%) at 4 cell, 8 cell, 16 cell, morula & blastocyst stages of embryos in serum culture and EGF supplemented serum free culture media were recorded as 47.25±4.86 and 57.14±4.83, 31.87±4.99 and 45.7±4.86, 20.88±3.21 and 37.14±4.72, 7.69±4.32 and 27.62±4.36 & 4.40±2.09 and 4.76±2.08 respectively but the mean cleavage percentages recorded at 4 cell, 8 cell, 16 cell and morula stages of embryos in EGF supplemented serum free culture media were found to be significantly higher than the value recorded in serum culture media and after that the differences during blastocyst stage of embryos were found to be statistically non-significant.

The present results were in agreement with previous reports (Sirisathien and Brackett, 2003; Richani et al., 2014; Sadeesh et al., 2014; Prasad et al., 2018 and Yang et al., 2022) where they have studied about cleavage and embryonic development of bovine oocytes after culture with epidermal growth factor. Epidermal growth factor supplementation increased embryo development rates and quality following *in vitro* fertilization and *in vitro* culture. The results suggested that EGF might be one of the major follicular factors responsible for stimulating oocyte cytoplasmic as well as nuclear maturation. The exposure of cumulus enclosed bovine oocytes during *in vitro* maturation to EGF improved the percentage of oocytes undergoing nuclear maturation as well as the cleavage (Park and Lin, 1993; Chandra et al., 2012; Singh et al., 2015 and Ahmed et al., 2023). The positive effect of EGF on embryonic development was shown to be due solely to specific mitogenic effects of EGF as evidenced through use of anti-EGF antibody (Buyalos and Cai, 1994; Helmy and Abdel-Halim, 2014).

3.6. Comparison of in vitro fertilization and early embryonic development of cattle oocytes in serum free and EGF supplemented serum free culture media

Table 6 shows *in vitro* fertilization and early embryonic development of cattle oocytes in serum free culture media with or without epidermal growth factor**.** To study *in vitro* fertilization performance, out of 86, 48 nos. *in vitro* matured cattle oocytes and out of 105, 80 nos. *in vitro* matured cattle oocytes were cleaved respectively in serum free and EGF supplemented serum free culture media and the mean fertilization rates (%) were recorded as 55.81±4.33 and 76.19±4.16 respectively in both culture media and

Table 6: Comparison of *in vitro* fertilization and early embryonic development of cattle oocytes in serum free and EGF supplemented serum free culture media

Culture Media	Total COCS subjected to IVF (n)	Cleavage (%)					
		2 CELL% (Mean±SE)	4 CELL% (Mean±SE)	8 CELL% (Mean±SE)	16 CELL% (Mean±SE)	Morula % (Mean±SE)	Blastocyst % (Mean±SE)
Serum free	86	55.81±4.33*	31.40±4.99*	18.60±3.88*	10.47±2.23*	3.49±2.23*	1.16±2.23
Serum free+EGF	105	76.19±4.16*	57.14±4.83*	45.71±4.86*	37.14±4.72*	27.62±4.36*	4.76±2.08

*($p \leq 0.5$)

statistical analysis revealed significant differences between serum free culture media with or without EGF in respect of IVF percentages.

The mean cleavage percentages (%) at 4 cell, 8 cell, 16 cell, morula & blastocyst stages of cattle embryos in serum free and EGF supplemented serum free culture media were recorded as 31.40±4.99 and 57.14±4.83, 18.60±3.88 and 45.71±4.86, 10.47±2.23 and 37.14±4.72, 3.49±2.23 and 27.62±4.36 & 1.16±2.23 and 4.76±2.08 respectively.

From the present experiment, it can be revealed that the mean cleavage percentages (%) recorded at 4 cell, 8 cell, 16 cell and morula stages of cattle embryos in EGF supplemented serum free culture media were found to be significantly higher than the values recorded in serum free culture media (without EGF) and after that it was observed that the differences during blastocyst stage of cattle embryos were found to be statistically non-significant between the serum free culture media with or without EGF. Similar findings have been also reported by earlier workers (Lonergun et al., 1996; Richani et al., 2014; Sadeesh et al., 2014; Singh et al., 2015; Prasad et al., 2018; Arias et al., 2022; Ahmed et al., 2023) where they have observed that supplementation of EGF in serum free culture media increased the embryo development rate and quality. The results indicated that EGF enhances cumulus expansion in bovine COCs, in the same way as demonstrated by Downs (1989) for rodent oocytes. Epidermal Growth Factor (EGF) influenced oocyte maturation and blastocyst production rates in a number of mammals where cumulus cells express EGF receptors (Purohit et al., 2005; Yang et al., 2022). The positive effect of EGF on embryonic development might be due to specific mitogenic effects of EGF as evidenced through use of anti-EGF antibody (Buyalos and Cai, 1994; Helmy and Abdel-Halim, 2014).

4. CONCLUSION

Supplementing serum free maturation media with epidermal growth factor (EGF) significantly increased the nuclear maturation of cattle oocytes than serum added basic maturation media and EGF was shown to have a stimulatory effect on post fertilization period. The *in vitro* blastocyst production in EGF supplemented serum free media was comparable to serum basic maturation media.

5. REFERENCES

Ahmed, A.E., Sindi, R.A., Yousef, N.A., Hussein, H.A., Badr, M.R., Syaad, K.M.A., Al-Saeed, F.A., Hassaneen, A.S.A., Abdelrahman, M., Ali, M.E., 2023. Impact of epidermal growth factor and/or b-mercaptoethanol supplementations on the *in vitro* produced buffaloes' embryos. Frontiers in Veterinary Science 10, doi: 10.3389/fvets.2023.1138220.

Ahumada, C.J., Salvador, I., Cebrian-Serrano, A., Lopera, R., Silvestre, M.A., 2013. Effect of supplementation of different growth factors in embryo culture medium with a small number of bovine embryos on *in vitro* embryo development and quality. Animal 7(3), 455–462.

Anonymous, 2008. SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. 233 South Wacker Drive, 11th Floor Chicago, IL 60606-6412 (IBM SPSS Statistics).

Arias, M.E., Vargas, T., Gallardo, V., Aguila, L., Felmer, R., 2022. Simple and efficient chemically defined in vitro maturation and embryo culture system for bovine embryos. Animals 12(21), 3057 https://doi.org/10.3390/ani12213057.

Boehm, K.D., Daimon, M., Gorodeski, I.G., Sheen, L.A., Utian, W.H., Ilan, J., 1990. Expression of the insulin-like and platelet derived growth factor genes in human uterus tissues. Molecular Reproduction and Development 27(2), 93–101.

Borah, D., Biswas, R.K., 2020. Effect of additives in medium on *in vitro* maturation of goat oocytes. Indian Journal of Animal Research 54(1), 11–15.

Buyalos, R.P., Cai, X., 1994. Preimplantation embryo development enhanced by epidermal growth factor. Journal of Assisted Reproduction and Genetics 11, 33–37.

Chandra, V., Mishra, A., Sharma, G.T., 2012. Effect of growth factors (epidermal growth factor, platelet derived growth factor, and insulin-like growth

factor-1) on buffalo embryos produced *in vitro*. Indian Journal of Animal Sciences 82(12), 1510–1514.

Chuangsoongneon, U., Kamonpatana, M., 1991. Oocyte maturation, *in vitro* fertilization and culture system for developing preimplantation swamp buffalo embryos using frozen thawed semen. Buffalo Journal 7(2), 189–198.

Currin, L., Baldassarre, H., Bordignon, V., 2021. *In vitro* production of embryos from prepubertal holstein cattle and mediterranean water buffalo: problems, progress and potential. Animals 11(8), 2275. doi.org/10.3390/ani11082275.

Dhali, A., Anchamparuthy, V.M., Butler, S.P., Mullarky, I.K., Pearson, R.E., Gwazdauskas, F.C., 2011. Development and quality of bovine embryos produced *in vitro* using growth factor supplemented serum free system. Open Journal of Animal Sciences 1(3), 97–105.

Downs, S.M.,1989. Specificity of epidermal growth factor action on maturation of the murine oocyte and cumulus oophorus *in vitro*. Biology of Reproduction 41(2), 371–379.

Echternkamp, S.E., Howard, H.J., Roberts, A.J., Grizzle, J., Wise, T., 1994. Relationship among concentrations of steroids, insulin-like growth factor-I and insulin-like factor binding proteins in ovarian follicular fluid of beef cattle. Biology of Reproduction 51(5), 971–981.

Estrada, J.L., Jones, E.E., Johnson, B.H., Petters, R.M., 1991. Effect of insulin-like growth factor-I on protein synthesis in porcine embryonic disks cultured *in vitro*. Journal of Reproduction and Fertility 93(1), 53–61.

Feugang, J.M., Camargo, R.O., Erdogan, M., 2005. Culture systems for bovine embryos. Livestock Science 121(2–3), 141–149.

Fiammetta, B., 2015. Embryo production. in: reproduction in livestock. Encyclopaedia of Life Support Systems, 203–217.

Gardner, H.G., Kaye, P.L., 1991. Insulin increases cell numbers and morphological development in mouse preimplantation embryos *in vitro*. Reproduction Fertility and Development 3(1), 79–91.

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 59(2), 209–218.

Hafez, E.S.E., Hafez, B., 2000. Assisted reproductive technology. Part VI. In: Reproduction in farm animals (3rd Edn.), Blackwell Publishing, 363–443.

Harper, K.M., Brackett, B.G.,1993. Bovine blastocysts development after *in vitro* maturation in a defined medium with epidermal growth factor and low concentrations of gonadotropins. Biology of Reproduction 48(2), 409–416.

Helmy, N.A., Abdel-Halim, B.R., 2014. Effect of insulin-like growth factor-I and epidermal growth factor on *in vitro* embryo production in cattle. Assiut Veterinary Medical Journal 60(140), 139–144.

Jainudeen, M.R., Takahashi, Y., Nihayah, M., Kanagawa, H., 1993. *In vitro* maturation and fertilization of swamp buffalo (*Bubalus bubalis*) oocytes. Animal Reproduction Science 31(3–4), 205–212.

Kim, M., Park, J.E., Lee, Y., Lee, S.T., Lee, G.S., Hyun, S.H., Lee, E., Lee, J., 2023. Effect of growth factors and hormones during *in vitro* growth culture of cumulus oocyte complexes derived from small antral follicles in pigs. Animals 13(7), 1206. doi.org/10.3390/ani13071206.

Kumar, D., Purohit, G.N., 2004. Effect of epidermal and insulin-like growth factor-1 on cumulus expansion, nuclear maturation and fertilization of buffalo cumulus oocyte complexes in simple serum free media DMEM and Ham's F-10. Veterinary Archives 74(1), 13–25.

Kumar, R., Chandra, P., Konyak, P., Karunakaran, M., Santra, A., Das, S.K., 2020. *In vitro* development of preimplantation caprine embryo using cryopreserved black bengal buck semen. Indian Journal of Animal Research 54(10), 1210–1214.

Lonergan, P., Carolan, C., Langendonckt, A.V., Donnay, I., Khatir, H., Mermillod, P., 1996. Role of epidermal growth factor in bovine oocyte maturation and preimplantation embryo development *in vitro*. Biology of Reproduction 54(6), 1420–1429.

Lonergan, P., Rizos, D., Gutierrez-Adan, A., Fair, T., Boland, M.P., 2003. Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. Reproduction in Domestic Animals 38(4), 259–267.

Lorenzo, P.L., Illera, M.J., Illera, J.C., Illera, M., 1994. Enhancement of cumulus expansion and nuclear maturation during bovine oocyte maturation *in-vitro* by the addition of EGF and IGF-I. Reproduction and Fertility 101(3), 697–701.

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. Veterinary Archives 75(6), 459–467.

Neira, J.A., Tainturier, D., Pena, M.A., Martal, J., 2010. Effect of the association of IGF-I, IGF-II, bFGF, TGF-β1, GM-CSF, and LIF on the development of bovine embryos produced *in vitro*. Theriogenology 73(5), 595–604.

Park, K.W., Iga, K., Niwa, K., 1997. Exposure of bovine oocytes to EGF and IGF-1 during maturation allows

them to develop to blastocysts in a chemically defined medium. Theriogenology 48(7), 1127–1135.

Park, Y.S., Lin, Y.C., 1993. Effect of epidermal growth factor (EGF) and defined simple media on *in vitro* bovine oocyte maturation and early embryonic development. Theriogenology 39(2), 475–484.

Prasad, S., Prakash, C., Rohit, K., Karunakaran, M., Santra, A., Das, S.K., 2018. Development of cattle embryo through *in vitro* technique using epidermal growth factor as a media supplement. International Journal of Bio-resource and Stress Management 9(6), 691–694.

Purohit, G.N., Brady, M.S., Sharma, S.S., 2005. Influence of epidermal growth factor and insulin-like growth factor I on nuclear maturation and fertilization of buffalo cumulus oocyte complexes in serum free media and their subsequent development *in vitro*. Animal Reproduction Science 87(3–4), 229–239.

Rajesh, K., Swathi, B., Aruna, K.G., Shanmugam, M., 2020. Effect of kisspeptin on *in vitro* maturation of buffalo oocytes. Indian Journal of Animal Research 54(10), 1218–1222.

Richani, D., Melanie, L., McDowall, S., Frank, L.A., Gilchrist, R.B., Thompson, J.G., 2014. Effect of epidermal growth factor-like peptides on the metabolism of *in vitro* matured mouse oocytes and cumulus cells. Biology of Reproduction 49(3), 1–10.

Sadeesh, E.M., Shah, F., Balhara, A.K., Thirumaran, S.M.K., Yadav, S., 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 Arhiv 84(5), 459–474.

Sakaguchi, M., Dominko, T., Leibfried, R.M.L., Nagel, T., First, N.L., 2000. A combination of EGF and IGF-I accelerated the progression of meiosis in bovine follicular oocytes *in vitro* and fetal calf serum neutralizes the acceleration effect. Theriogenology 54(8), 1327–1342.

Singh, V., Misra, A.K., Kumar, S., Kumar, V., 2015. Effect of growth factors on *in vitro* maturation and *in vitro* culture of buffalo oocytes. Ruminant Science 4(2), 219–224.

Sirisathien, S., Brackett, B.G., 2003. Tunel analyses of bovine blastocysts after culture with EGF and IGF-I. Molecular Reproduction and Development 65(1), 51–56.

Snedecor, G.W., Cochran, W.G., 1994. Statistical methods (7th Edn.), The Iowa State University Press, Ames.

Totey, S.M., Singh, G., Taneja, M., Pawshe, C.H., Talwar, G.P., 1992. *In vitro* maturation, fertilization and development of follicular oocytes from buffalo (*Bubalus bubalis*). Journal of Reproduction and Fertility 95(2), 597–607.

Umdor, S.B., Karunakaran, M., Mandal, D.K., Santra, A., Das, S.K., 2021. Enhancement of developmental competence of immature oocytes supplemented with growth factors in culture media. Indian Journal of Animal Research 55(5), 493–497.

Yang, R.F., Xionga, X.R., Ziab, X.D., 2019. Effect of cysteine, insulin like growth factor-1 and epidermis growth factor during *in vitro* oocyte maturation and *in vitro* culture of yak-cattle crossbred embryos. Journal of Applied Animal Research 47(1), 463–466.

Yang, S., Yang, Y., Hao, H., Du, W., Pang, Y., Zhao, S., Zou, H., Zhu, H., Zhang, P., Zhao, X., 2022. Supplementation of EGF, IGF-1, and Connexin 37 in IVM medium significantly improved the maturation of bovine oocytes and vitrification of their ivf blastocysts. Genes 13(5), 805. doi.org/10.3390/genes13050805.

Yousef, N.A., Hussein, H.A., Badr, M.R., Ahmed, A.E., 2018. Effect of epidermal growth factors (EGF) on the maturation and developmental competence of buffalo's oocytes and embryo stages *in vitro*. SVU- International Journal of Veterinary Sciences 1(2), 85–94.

Yusuf, A.M., 2024. Promise of *in vitro* embryo production technology for improvement of cattle reproductive potential. EAS Journal of Veterinary Medical Science 6(2), 44–58. DOI: 10.36349/easjvms.2024.v06i02.003.