International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(1): 1444-1447

Received: 27-11-2017
Accepted: 28-12-2017

M Bhajoni
Department of ARGO, C.V.Sc, Khanapara, Assam, India

D Bhuyan
Professor, Department of ARGO, CVSc, Khanapara, Assam, India

RK Biswas
Professor, Department of Vety Physiology, CVSc, Assam, India

DJ Dutta
Department of Vety Physiology, CVSc, Assam, India

H Dev
Department of Vety Physiology, CVSc, Assam, India

G Hazarika
Department of Animal, Biotechnology, CVSc, Assam, India

Correspondence
M Bhajoni
Department of ARGO, C.V.Sc, Khanapara, Assam, India

Effect of Cysteamine, Epidermal Growth Factor (EGF) and Estrous Cow Serum (ECS) on *In-vitro* Maturation of Bovine Oocytes

M Bhajoni, D Bhuyan, RK Biswas, DJ Dutta, H Dev and G Hazarika

Abstract
A total of 526 good quality bovine oocytes were subjected to *in-vitro* maturation of oocytes at 38.5 ^{0}C in humidified atmosphere of 5% CO_2 tension for 24 hours and matured *in-vitro* in medium-I or control (TCM-199+10% FBS+L-glutamine+sodium pyruvate+Gentamicin+pFSH+ Estradiol 17-β), medium-II (control+5% ECS), medium-III (control+100µM/ml cysteamine), medium-IV (control+10ng/ml EGF) and medium-V (control+ 5% ECS+ 100µM/ml cysteamine+10ng/ml EGF). The rates of maturation based on cumulus cell expansion and nuclear maturation varied significantly (p<0.01) between media being the highest in the medium-IV (86.92% and 62.36%) containing epidermal growth factor (EGF) followed by medium-III (82.24% and 56.82%) containing cysteamine among all the media.

Keywords: *In-vitro* maturation, Bovine, Oocytes, TCM-199

1. Introduction
Oocyte maturation is the first and the most critical step towards successful *in-vitro* fertilization. *In-vitro* maturation (IVM) is a process during which oocytes are matured in the laboratory before being fertilised after their removal from the ovary when still at immature state. In this process the primary oocytes resume meiosis and progress from the diplotene stage of prophase of first meiotic division to metaphase of second meiotic division with breakdown of germinal vesicle, expansion of surrounding cumulus cells, formation of meiotic spindle and extrusion of first polar body. It is the most important step which determines the subsequent successful fertilisation, zygote formation, attainment of blastocyst stage, normal embryo growth and development, as well as implantation. Various attempts had been made to improve the IVM percentage of oocytes with the addition of hormone, growth factor, antioxidant, serum, and follicular fluid either independently or simultaneously (Anand *et al.*, 2007; Wang *et al.*, 2007; Farag *et al.*, 2009; Hammam *et al.*, 2010; Deneke *et al.*, 2011; Chandra *et al.*, 2012; Gottardi *et al.*, 2012) [2, 34, 10, 14]. However, the results had been divergent. Although *in-vitro* techniques for embryo production are well established for cattle, there is as yet, a sub-optimal oocyte maturation that limits its further development. It was reported that cysteamine an antioxidant when added to IVM media improved embryo developmental rates by eliminating deleterious effect of Reactive Oxygen Species (de Matos *et al.*, 2002; Deleuze and Goudet, 2010) [8, 7]. Improvement of maturation rate of bovine oocyte *in-vitro* was obtained by supplementation of epidermal growth factor (Lonergan *et al.*, 1996; Bastan *et al.*, 2010) [20, 3] as well as estrous cow serum (Cevik *et al.*, 2011) [4] in maturation medium. In this context it was felt necessary to study the combined effect of antioxidant like cysteamine and growth factor like epidermal growth factor along with estrous cow serum with a view to improving the efficacy of IVM medium for accelerating the rate of maturation of cattle oocytes.

Materials and Methods
A total of 209 cattle ovaries obtained from the slaughter house soon after sacrifice were utilized during the study. The ovaries were washed in the transportation medium and follicular diameter was measured using Vernier calipers. Follicles were classified based on diameter into three categories: small (<3mm), medium (3 to 8 mm) and large (>8 mm). Oocytes from the medium size follicles were only collected by aspiration technique and examined under a

stereozoom microscope and then under a compound microscope (150X) and classified into 4 grades (grade 'A' to 'D') based on the layers of cumulus cells adhered to the oocytes as per Ramsingh *et al.* (2013). Only grade A and B oocytes were washed 3-4 times in the washing medium and then 2 times in maturation medium. After washing, the oocytes were transferred to an IVM droplet for incubation and a total of 526 oocytes were subjected to IVM in five different maturation media prepared using TCM-199 as a basic medium along with additives. IVM of oocytes was done at 38.5 ^{0}C in humidified atmosphere

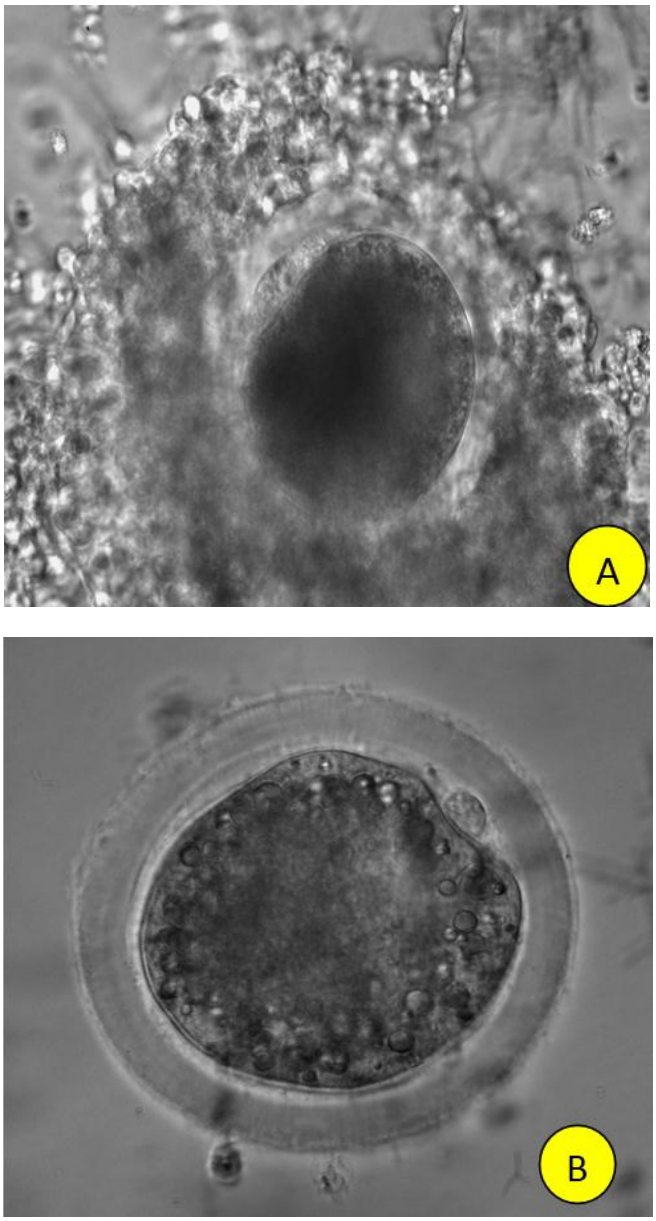

Fig 1: Expansion of cumulus cells (A) and Extrusion of polar body (B) under Inverted microscope

of 5% CO_2 tension for 24 hours and matured *in-vitro* in medium-I or control (TCM-199+10% FBS+L-glutamine+sodium pyruvate+Gentamicin+pFSH+ Estradiol 17-β), medium-II (control+5% ECS), medium-III (control+100µM/ml cysteamine), medium-IV (control+10ng/ml EGF) and medium-V (control+ 5% ECS+ 100µM/ml cysteamine+10ng/ml EGF). After 24 hours of incubation in IVM media, cumulus expansion and nuclear maturation of oocytes were determined microscopically. The percentage of oocyte maturation on the basis of cumulus expansion was calculated by recoding the number of oocytes showing expansion out of total number of oocytes incubated in different medium. The data were subjected to chi square test statistically. The percentage of nuclear maturation was calculated out of the oocytes showing cumulus expansion on the basis of polar body extrusion.

Statistical Analysis

Data obtained in the present experiment were analyzed statistically by SAS Enterprise Guide 4.3.

Results and Discussion

The extents of IVM of based on cumulus expansion and nuclear maturation in media-I to V are furnished in the tables 1 and 2 respectively. The Chi-square test revealed significant (P<0.01) difference on the percentage of oocyte maturation for both cumulus expansion and nuclear maturation between different maturation media.

Table 1: Rate of *in-vitro* maturation of oocytes based on cumulus cell expansion in different media

Media	Number of oocyte incubated	No. of oocyte showing cumulus expansion	Rate of maturation (%)	Chi-square value
Medium-I	98	72	73.46	**26.223****
Medium-II	107	70	65.42	
Medium-III	107	88	82.24	
Medium-IV	107	93	86.92	
Medium-V	107	81	75.7	

** P<0.01

Table 2: Rate of *in-vitro* maturation of oocytes based on nuclear maturation in different media

Media	Number of oocyte that expanded	Number of oocyte showing nuclear maturation	Rate of maturation (%)	Chi-square value
Medium-I	72	32	44.44	273.96**
Medium-II	70	26	37.14	
Medium-III	88	50	56.82	
Medium-IV	93	58	62.36	
Medium-V	81	38	46.91	

** P<0.01

The rate of IVM based on both cumulus cell expansion and nuclear maturation was observed to be the highest in medium IV supplemented with epidermal growth factor (86.92% & 62.36%) followed by medium III containing cysteamine (82.24% & 56.82%) and that had epidermal EGF, cysteamine and ECS (75.70% & 46.91%) and medium I or control (73.46% & 44.44%) respectively. The medium II supplemented with ECS (65.42% & 37.14%) recorded to be the lowest. The result of the present study was in agreement with the findings of Cevik *et al.* (2011) [4] who recorded 87.5 percent in cattle using TCM-199 medium supplemented with

EGF. The present finding in respect of percentage of nuclear maturation of oocytes in medium IV was similar that of Purohit *et al.* (2005) and Kumar and Purohit, (2004), [28, 18] who observed the nuclear maturation rate of 63.2 and 63.6 per cent respectively, however, it was lower than that recorded (91.5%) by Bastan *et al.* (2010) [3] in cattle oocytes. Sofi *et al.* (2011) [32] in ovine and Kumar and Purohit (2004) [18] in buffalo oocytes also reported similar values for cumulus expansion of 82.90 and 81.4 per cent respectively in medium supplemented with EGF. It was demonstrated that the presence of EGF stimulated cumulus cell expansion and significantly increased the proportion of oocyte attaining M-II (Park and Lin, 1993; Keefer *et al*., 1994; Lorenzo *et al*., 1994; Lorenzo *et al*., 1995 and Lonergan *et al.*, 1996) [26]. Lorenzo *et al.* (1994) [21] reported that nuclear maturation was not affected when denuded oocytes were cultured in presence of EGF, indicating mediation by cumulus cell in cattle. Bastan *et al.* (2010) [3] reported that regardless of concentration, EGF addition to the media had a positive effect on the percentages of cumulus expansion and nuclear maturation. The possible mechanisms of EGF action on oocyte maturation are either disruption of oocyte communication with cumulus cells (Decke and Sherizly, 1985) [6], creation of a positive maturational signal (Downs, 1989) and mediation of effect via a tyrosine kinase dependent intracellular mechanism (Lorenzo *et al*., 2001) [23]. Nagar and Purohit (2005) [25] reported that EGF at concentrations of 10 to 50 ng/ml increased the *in vitro* maturation and fertilization of goat oocytes. Kobayashi *et al.* (1994) [27] reported that the addition of epidermal growth factor to the maturation medium increased the number of fertilized ova that developed to the blastocyst stage in cattle.

In the present study maturation percentage of oocytes was also found to be higher in medium-III containing cysteamine at the rate of 100 µM (82.24%, 56.82%). De Matos *et al.* (1997) reported that addition of cysteamine to IVM medium increased glutathione (GSH) level in cumulus-oocyte-complexes. Supplementation of 100 µM cysteamine to the medium resulted in significantly (P<0.05) higher blastocyst yield as compared to that of control and also with higher number of inner cell mass (Lojkic *et al.*, 2012) [19]. The intracellular synthesis of GSH was found to be very important in oocyte cytoplasmic maturation (Gordon, 2003). Addition of IVM medium with either cysteine or cysteamine caused increases of oocytes GSH content (Gasparrini *et al.*, 2006). Sagara *et al.* (1993) [11, 30] demonstrated that the presence of cysteine in the extracellular environment was necessary for GSH biosynthesis. The higher *in-vitro* expansion of cumulus cells and nuclear maturation of oocytes recorded in cysteamine supplemented medium, compared to control medium in the present study might be attributed to the protection of oocytes from oxidative stress through elevated GSH level during IVM. The percentage of oocytes matured *in-vitro* on the basis of cumulus cell expansion and nuclear maturation (65.42% and 37.14% respectively) was the lowest in medium-II supplemented with 5% ECS which might be due to the lack of a positive effect of serum supplementation. The observed variation in the percentage of maturation of oocytes in different media also might have been influenced by the factors like age of the animal (Thibault, 1972) [33], season (Smith *et al.*, 1978) [31], type of follicle (Moor and Truson, 1977) and oocyte (Homa *et al.*, 1988) [24, 15], stage of oestrous cycle (Pattabiraman and Kathiresan, 1993) [27] and culture condition (Yadav *et al.*, 1993; Agrawal, 1992; Jhon *et al.*, 2015) [35, 1] that were followed during *in-vitro* incubation of oocytes.

Conclusion

From the present study, it could be concluded that rate of maturation of oocyte based on cumulus cell expansion and nuclear maturation differed significantly between different media and recorded to be the highest in medium-IV containing TCM-199 supplemented with 10% FBS, 10ng/ml EGF, sodium pyruvate, L-glutamine, Gentamicin, 1µg/ml 17β-estradiol and 5µg/ml pFSH.

Reference

1. Agrawal KP. *In-vitro* maturation of caprine oocytes. Indian J. Anim. Reprod. 1992; 13(2):195-197.
2. Anand T, Kumar D, Chauhan MS, Manik RS, Palta P. Cysteamine supplementation of *in-vitro* maturation medium, *in-vitro* culture medium or both media promotes *in-vitro* development of buffalo (*Bubalus bubalis)* embryos. Reprod. Fer. and Dev. 2007; 20(2):253-257.
3. Bastan A, Polat B, Acar DB, Korkmaz O, Colak A. Determination of optimal dose of EGF for bovine oocyte maturation and subsequent *in-vitro* fertilization and culture in two media. Turk. J. Anlm. Sci. 2010; 34(1):33-38.
4. Cevik M, Sen U, Kocyigit A, Soydan E, Kuran M. Effect of serum, gonadotropins, epidermal growth factor and estradiol 17-beta on cumulus expansion and nuclear maturation of bovine oocytes. Kafkas Univ. Vet. Fak. Derg. 2011; 17 (6): 1009-1014.
5. Chandra V, Mishra A, Sharma GT. Effect of growth factors (epidermal growth factor, platelet derived growth factor, and insulin-like growth factor-1) on buffalo embryos produced *in-vitro*. Ind. J. of Anim. Sci. 2012; 82 (12): 1510-1514.
6. Dakel N, Sherizly I. Epidermal growth factor induces maturation of rat follicle-enclosed oocytes. Endocrin. 1985; 116: 406-409.
7. Deleuze S, Goudet G. Cysteamine supplementation of *in-vitro* maturation media: a review. Reprod. Dom. Anim. 2010; 45: 476-482.
8. De Matos DG, Gasparrini B, Pasqualini SR, Thompson JG. Effect of glutathione synthesis stimulation during *in-vitro* maturation of ovine oocytes on embryo development and intracellular peroxide content. Therio. 2002; 57: 1443-1451.
9. Downs SM. Specificity of epidermal growth factor action on maturation of the murine oocyte and cumulus oophorus *in-vitro*. Bio. of Repro. 1989; 41: 371-379.
10. Farag IM, Giris SM, Khalil WKB, Hassan NHA, Sakr AAM, Abu Allah SM, Ali NI. Effect of hormones, culture media and oocyte quality on *in-vitro* maturation of Egyptian Sheep oocytes. J. Appl. Biosci. 2009; 24:1520-1534.
11. Gasparrini B, Boccia L, Marchandise J, Palo RD, Geotge F, Donnay I, Zicarelli L. Enrichment of *in-vitro* maturation medium for buffalo (*Buffalus bubalis)* oocytes with thiol compound: effects of cysteine on glutathione synthesis and embryo development. Therio. 2006; 65:275-287.
12. Gogdon I. Laboratory production of cattle embryos. CAB inter., Wallingford, Oxon Ox 108 DE, UK, 2nd edi. 2003; 81.
13. Gottardi FP, Barretto FS, Goncalves FS, Perri SHV, Mingotti GZ. Effects of cumulus cells and cysteamine during bovine oocyte *in-vitro* maturation on meiosis progression and acquisition of developmental

competence. Arq. Bras. Med. Zootec. 2012; 64(2):245-252.
14. Hammam AM, Whisnant A, Elias A, Zaabel SM, Hagab AO, Naga EMA. Effect of media, sera and hormones on *in-vitro* maturation and fertilization of Water buffalos (*Bubalus bubalis).* J. of Anim. and Vet. Adv. 2010; 9(1):27-31.
15. Homa ST, Mcgaughey RW, Racowsky C. Isolated subpopulation of mass harvested pig oocytes and their characterization by size, incidence of atresia and competence to mature in culture. J. Reprod. Fert. 1988; 49:101-109.
16. John A, Joseph M, Vijayakumaran V, Manoj CJ. Effect of oocyte retrieval techniques on yield and quality of caprine oocytes. IOSG J. of Agri. and Vet. Sci. (IOSG-JAVS) 2015; 8(4): 50-52.
17. Kobayashi K, Yamashita S, Hoshi H. Influence of epidermal growth factor and transforming growth factor-α on *in-vitro* maturation of cumulus cell-enclosed bovine oocytes in a defined medium. Jour. of Reprod. and Fert. 1994; 100: 439-446.
18. Kumar D, Purohit GN. 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. Veterinarski Arh. 2004; 74(1):13-25.
19. Lojkic M, Getz I, Samardzija M, Matkovic M, Bacic G, Karadjole T *et al* . Effect of cysteamine supplementation during *in-vitro* culture of early stage bovine embryos on blastocyst rate and quality. Acta Vet. Brno. 2012; 81: 229-234.
20. Lonergan P, Carolan C, Van Langendonckt A, Donnv I, Khatir H, Mermillod O. Role of epidermal growth factors in bovine oocyte maturation and preimplantation embryo development. Biology of Reprod. 1996; 54: 1420-1429.
21. Lorenzo PL, Illera MJ, Illera JC, Illera M. Enhancement of cumulus expansion and nuclear maturation during bovine oocyte maturation *in-vitro* by the addition of epidermal growth factor and insulin-like growth factor-I. J. of Reprod. and Fertil. 1994; 101: 697-701.
22. Lorenzo PL, Illera MJ, Illera JC Illera M. Role of EGF, IGF-I, sera and cumulus cells on maturation *in-vitro* of bovine oocyte. Therio. 1995; 44: 109-118.
23. Lorenzo PL, Liu IK, Illera JC, Picazo RA, Carneior GF, Illera MJ *et al* . Influence of epidermal growth factor on mammalian oocyte maturation via tyrosine kinase pathway. J. Physiol. Biochem. 2001; 57: 15-22.
24. Moor RM, Trounson AO. Hormonal and follicular factors affecting maturation of sheep oocytes *in-vitro* and their subsequent development capacity. J. Reprod. Fert. 1977; 49:101-109.
25. Nagar D, Purohit N. 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. 2005, 459-467.
26. Park YS, Lin YC. Effect of epidermal growth factor (EGF) and defined simple media on *in-vitro* bovine oocyte maturation and early embryonic development. Therio. 1993; 39: 475-484.
27. Pattabiraman, SR, Kathiresan D. Effect of functional status of the ovaries on the characteristics of follicles and oocytes in buffaloes. National symposium on role of theriogenology for augmenting fertility in domestic animal convention of Indian society for the study of Animal Reproduction, Salt Lake city, Calcutta, December 25-27, 1993.
28. Purohit GN, Brandy MS, Sharma SS. 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.* Anim. Reprod. Sci. 2005; 87: 229-239.
29. Ramsingh L, Sadasivarao K, Muralimohan K. Ovarian Biometrics and oocyte grading percentage of yield in local goats of Andhra Pradesh. Iosr J. of Phar. 2013; 3(1):47-49.
30. Sagara J, Miura K, Bannai S. Cystine uptake and glutathione level in fetal brain cells in primary culture and in suspension. J. of Neuroche. 1993; 61:1667-1671.
31. Smith DM, Conaway CH, Kerber WT. Influence of season and age on maturation *in-vitro* of rhesus monkey oocytes. J. Reprod. Fert. 1978; 54:91-95.
32. Sofi KA, Khan MZ, Islam R, Lone FA. Effect of cysteamine and epidermal growth factor supplementation on the *in-vitro* maturation rate of ovine oocytes. Small Ruminant Res. 2011; 96:191-194.
33. Thibault C. Final stage of mammalian oocyte maturation. In: oogenesis (J. D. Biggers and A.W. Schuetz, eds), University park press, Baltimore. 1972, 97-411.
34. Wang ZG, Xu ZR, Yu SD. Effects of oocyte collection techniques and maturation media on *in-vitro* maturation and subsequent embryo development in Boer goat. Czech J. Anim. Sci. 2007; 52(1):21-25.
35. Yadav PS, Dhanda OP, Razdan MN. The effect of incubation period on capacitation of buck sperm. Int. J. Anim. Sci. 1993; 267-268.