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Insulin-like Growth Factor-1 Supplementation and Early Embryonic Developmental Competence of Immature Cattle Oocytes

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ABSTRACT

Background: The livestock industry is a growing field where assisted reproductive technologies like *in vitro* embryo production (IVEP) enhance production efficiency. However, *in vitro* embryos show lower developmental competence due to stress and suboptimal culture conditions. Supplementing culture media with growth factor, such as insulin-like growth factor-1 (IGF-1) can improve embryo development. This study investigates the effect of IGF-1 on the developmental competence of oocytes and early embryonic development.

Methods: In this study, cattle oocytes were aspirated from 3-8 mm ovarian follicles, washed and matured in maturation media supplemented with different concentrations of IGF-1 (0, 10, 25, 50, 100 ng/ml) for 24 hours at 38.5°C 5% CO_2. Frozen-thawed sperm were used for *in vitro* fertilization of matured oocytes in fertilization media supplemented with IGF-1. Presumptive zygotes were cultured in IGF-1-supplemented mCR2aa media and embryos were monitored for 8 days until the blastocyst stage. The experiment was replicated six times and data were statistically analyzed by one-way ANOVA followed by duncan multiple range test using SPSS. Results are presented as means±standard error with graphs generated by GraphPad Prism.

Result: IGF-1 @ 50 ng/ml significantly enhanced embryo development, with highest cleavage rates at 2-cell (85.35% ±2.24) and 4-cell (86.10% ±1.90) stages and improved morula (32.53% ±5.11) and blastocyst formation (15.81%±1.07) and IGF-1 @ 100 ng/ml showed moderate benefits, while at lower concentrations *i.e.* 0 ng/ml, 10 ng/ml and 25 ng/ml had minimal effects. Overall, @ 50 ng/ml IGF-1 was the most effective in promoting oocyte maturation and early embryonic development.

Key words: Blastocyst, Cattle oocytes, Cleavage, Embryo, IGF-1, IVM.

INTRODUCTION

The dairy sector is one of the fastest-growing industries, contributing significantly to the livelihood of dairy farmers. Among the available technologies, assisted reproductive techniques (ART) play a vital role in enhancing reproductive efficiency and accelerating production. *In vitro* embryo production (IVEP) associated with assisted reproductive techniques recently has shown significant progress in cattle. Instead of the significant progress in ART, the *in vitro* embryo production faces many challenges. *In vitro* developed embryos show fewer competencies as compared to naturally developed embryos because of scarcity of maternal signalling, oxidative stress and unavailability of balanced nutrients in culture media, which disrupt embryo development (Cagnone and Sirard, 2016). IVEP includes *in vitro* maturation (IVM) *in vitro* fertilization (IVF) and *in vitro* culture (IVC). Supplementing macro-molecules, growth factors, hormones and cytokines in culture media helps in achieving developmental competency under *in vitro* conditions. Previous studies indicate that growth factors exert regulatory effects on embryo development by acting through autocrine and paracrine pathways (Ahumada *et al*., 2013). It was reported that addition of estradiol, gonadotropins and granulosa cells in culture media helps in sheep oocytes maturation outside the follicle (LSS *et al.,* 2022; Nia *et al*., 2025 and Hirao, 2025). *In vitro* maturation media (IVM) supplemented with epidermal growth factor (EGF) positively affects follicular fluid levels and stimulates meiosis in many species. Lonergan *et al*. (1996) revealed that epidermal growth factor (EGF) attributes to oocyte maturation and development likewise in cattle. Zhang *et al*. (2010) reported that locally derived fibroblast growth factor (FGF) massively affects oocyte maturation in IVM. Umdor *et al*. (2021) studied the supplementation of platelet-derived growth factor (PDGF) alone and in combination with FGF and found significant improvements in developmental competence.

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Recently, Pramanik *et al*. (2023) demonstrated that supplementation of leukemia inhibitory factor (LIF) throughout the culture media results in higher developmental competency of oocytes and embryos. Different combinations of growth factors positively affect blastocyst formation rate in cultured bovine embryos (Sirisathien *et al*., 2003). Bera *et al*. (2024) reported that different concentrations and combinations of PDGF+IGF1, EGF+FGF and T3+T4 significantly enhance cleavage rate and blastocyst formation rate. Among these growth factors, insulin-like growth factor-1 (IGF-1) is widely used because IGF-1 belongs to the insulin-like growth factor family and plays a key role in promoting cellular growth, cell division and specialization (Neirijnck *et al*., 2019). The objective of this study is to investigate the effect of insulin-like growth factor-1 (IGF-1) on developmental competence of cattle oocytes and embryos.

MATERIALS AND METHODS

Materials and Supplement

All plastic ware used in this experimental study were purchased from Tarson products Pvt. Ltd. (Kolkata India). Chemicals, biochemical and mineral oil were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). Syringe filters having pore size of 0.22 µm were sourced from Millipore Corp., Bedford, MA, USA. Sterile, disposable, non-toxic, non-pyrogenic plastic syringes and 19-guage hypodermic needles (Dispovan, Kolkata, India). All the experimental works have been completed by 2025 in Animal Biotechnology Lab., Eastern Regional Station, ICAR-National Dairy Research Institute, Kalyani, India.

Oocytes Aspiration, washing and *In vitro* maturation

Fresh cattle ovaries and oviducts were collected from the abattoir (Kolkata, India) in a thermos flask, containing saline (0.15 M NaCl) solution (35-38°C) fortified with antibiotic (400 IU/ml penicillin) and carried to the laboratory within 2-3 hours. Ovaries were then trimmed for removal of adjacent tissues and washed 3-4 times in warm saline solution. Immature oocytes were aspirated from 3-8 mm follicle with the help of the 19-gauge hypodermic needle fitted with 5 ml disposable syringe containing 5 ml aspiration media (TCM-199, DPBS, 0.3% BSA and 60 µg/ml gentamycin solution). Collected oocytes were graded depending on the presence of cumulus cell layers covering. Total 855 immature oocytes were used for this experiment. Cumulus oocyte complexes (COCs) of Grade A (>3 layers) and B (2-3 layers) were given serial washing (4 to 5 times) in washing medium and then exposed to maturation medium (TCM-199 + 10% FBS + 5 µg/ml FSH-P + 0.33 mM sodium pyruvate + 50 µg/ml gentamicin sulfate). Approximately 25 to 30 COCs were kept in 100 µl droplet of maturation medium supplemented with different concentration of insulin like growth factor-1 (0, 10, 25, 50, 100 ng/ml) covered with 2.5 ml to 3 ml sterile mineral oil in 35 mm petri dishes and kept for maturation in 5% CO_2 incubator for 24 hours at 38.5°C with maximum humidity. Oocyte maturation was evaluated after 24 hours by examining cumulus cell layer expansion and the presence of a polar body.

Processing of sperm and *In vitro* fertilization with *In vitro* matured oocytes

The spermatozoa used for IVF throughout the study were from the same donor that had been tested for IVF earlier. The spermatozoa were prepared for fertilization as described earlier (Das *et al.,* 2013). Briefly, two straws of frozen-thawed cattle semen were suspended in 1 ml of Working Bracket Oliphant (WBO) medium (Brackett and Oliphant, 1975) with 10 µg/ml heparin, 0.55 mM caffeine sodium benzoate, 7.1 mg sodium private and incubated for swim-up at 38.5°C for 15 minutes. After incubation progressively motile sperm cells were taken by collecting 6 ml of WBO medium from the upper layer and centrifuged at 2500 rpm for 5 min. After that, the supernatant was removed and the pellet was dissolved in 1.2 ml of WBO medium and centrifuged at 2500 rpm for 5 min. Finally, the pellet was dissolved in l ml of Fertilization Bracket Oliphant (FBO). In the treatment group 100 µl droplet of FBO was supplemented with different concentration of insulin like growth factor-I (10 ng/ml, 25 ng/ml, 50 ng/ml and 100 ng/ml) with control group 0 ng/ml. The *in vitro* matured oocytes were washed twice with the FBO medium in the same maturation droplet and inseminated with 50 µl capacitated motile spermatozoa (1-2 million spermatozoa/ml) per droplet overlaid with pre-incubated light weight mineral oil of 35 mm culture dish and placed in 5% CO_2 incubator at 38.5°C for 14-18 hrs with maximum humidity in air.

In vitro culture of early embryos

After completion of 14-18 hrs of incubation, the cumulus cells were separated by gentle pipetting in washing media and then presumptive zygotes were washed twice with modified Charles Rosenkrans 2 amino acid (mCR2aa) medium and cultured in 100 µl of mCR2aa medium supple--mented with different concentration of the Insulin like growth factor-1(10 ng/ml, 25 ng/ml, 50 ng/ml, 100 ng/ml). After 48 hrs the cleaved zygote/embryos were shifted in fresh 100 µl of mCR2aa blastocyst cultured medium in 5% CO_2 incubator at 38.5°C with maximum humidity for 7 to 8 days.

Experimental design and statistical analysis of data

The experiment was conducted for six replicates in different concentrations of IGF-1 (10 ng/ml, 25 ng/ml, 50 ng/ml and 100 ng/ml) with control (0 ng/ml). Experimental data analyzed by using simple One Way ANOVA. Means were compared using Duncan multiple range test (IBM R Statistical Package for the social sciences R (SPSS version 23). Graphs were made by using GraphPad Prism version 8.4.3 (686) and the values were expressed as means±standard error.

RESULTS AND DISCUSSION

Effect of the different concentration of IGF-1 on *in vitro* Maturation of oocytes

Supplementation of IGF-1 during *in vitro* maturation experiment resulted in a marked improvement as compared

to the control group. Total 855 immature oocytes were used for this experiment. In control group (0ng/ml), the oocyte maturation rate was 90.30±0.84%. Upon supplementation of IGF-1 @ 10 ng/ml, 25 ng/ml, 50 ng/ml and 100 ng/ml, the maturation rates increased to 92.06± 0.66%, 92.51±1.07%, 96.44±0.09% and 93.50±0.62%, respectively. Notably, @ 50 ng/ml group shown the highest maturation percentage which was statistically significant ($p<0.05$) as compared to the control group (Table 1, Fig 1A). The increase in maturation may be hallmark to IGF's role in enhancing mitochondrial function, energy metabolism and activation of key signaling pathways that promote both cytoplasmic and nuclear maturation and the up-regulation of IGF and activation of its receptor (IGF-1R) correlate with increased ATP production and heightened mitochondrial polarization in maturing oocytes (Biswas *et al.,* 2023). These cellular changes are critical for the progression of meiosis and acquisition of developmental competence (Jung *et al.,* 2023). These findings are consistent with reports in other species where IGF-1 supplementation facilitated the expansion of cumulus cells and enhanced nuclear maturation of oocytes by stimulating Ras/MAPK and PI3K/Akt pathways, leading to improve *in vitro* maturation outcomes (Sato *et al.,* 2018; Li *et al.,* 2016). Additionally, a dose-dependent effect was observed, with 50 ng/ml IGF-1 concentration have shown the positive effect on buffalo oocytes maturation (Badr and Zohery, 2011). Similar biphasic responses of IGF-1 have also been reported in mouse and buffalo oocytes, where optimal concentrations significantly improved maturation and developmental competence, whereas supra-optimal concentrations had no additional or negative effects (Palma *et al.,* 1997).

Effect of the different concentration of the IGF-1 on the early embryonic developmental stages

From the present study it has been observed that different concentrations of IGF-1 affected embryonic development at various stages with notable differences. At the 2-cell stage with 50 ng/ml showed a significantly higher cleavage rate (85.35%±2.24) as compared to the control group (72.20%±2.74), while @ 25 ng/ml (79.07%±3.10) and @ 100 ng/ml (80.80%±2.22) groups showed lower rate without any significant differences. At the 4-cell embryonic stage @ 50 ng/ml IGF-1 resulted in a significantly higher percentage development (86.10%±1.90) than the control group (79.55%±2.64), whereas 100 ng/ml (79.83% ±3.70), 10 ng/ml (75.80% ±3.16) and 25 ng/ml (83.73% ±3.14) showed no significant differences. Though 8-cell stage did not exhibited any statistical difference ($P>0.05$) shown in Table 2, the 50 ng/ml IGF-1 showed the highest percentage of 8- cell stage embryo (64.00% ±3.67) and the lowest was recorded for the control group (51.48% ±3.72). At the 16-cell stage, 50 ng/ml IGF-1 supplementation increased developmental rate significantly (46.34% ±4.00) as compared to the control group (30.89% ±4.42), while other concentrations showing intermediate results. In the category of morula formation, 50 ng/ml (32.53% ±5.11) and 100 ng/ml (31.51% ±5.29) were showing significantly higher rate than the control group (17.93% ±3.71), while low concentration *viz.*10 ng/ml (17.02% ±3.04) and 25 ng/ml (20.34% ±2.68) did not show any significant difference. In the blastocyst development 50 ng/ml IGF-1 markedly improved (15.81% ± 1.07) as compared to the control group (6.01% ±1.24) and with 100 ng/ml (10.63% ±1.04). In the present study overall @ 50 ng/ml IGF-1 consistently promoted significantly higher ($p<0.05$) embryonic development at most of the stages, while other concentrations exhibited limited or intermediate effects (Table 2, Fig 1B to G). The beneficial effect of IGF-1 on embryo development is supported by prior studies, which reported increased cleavage rate, blastomere viability and blastocyst yield following IGF supplementation (Velazquez *et al.,* 2008). Spanos *et al.* (2000) reported in human preimplantation embryos that IGF-1 increased the proportion of embryos

Table 1: Effect of the different concentration of IGF-1 on maturation of oocytes.

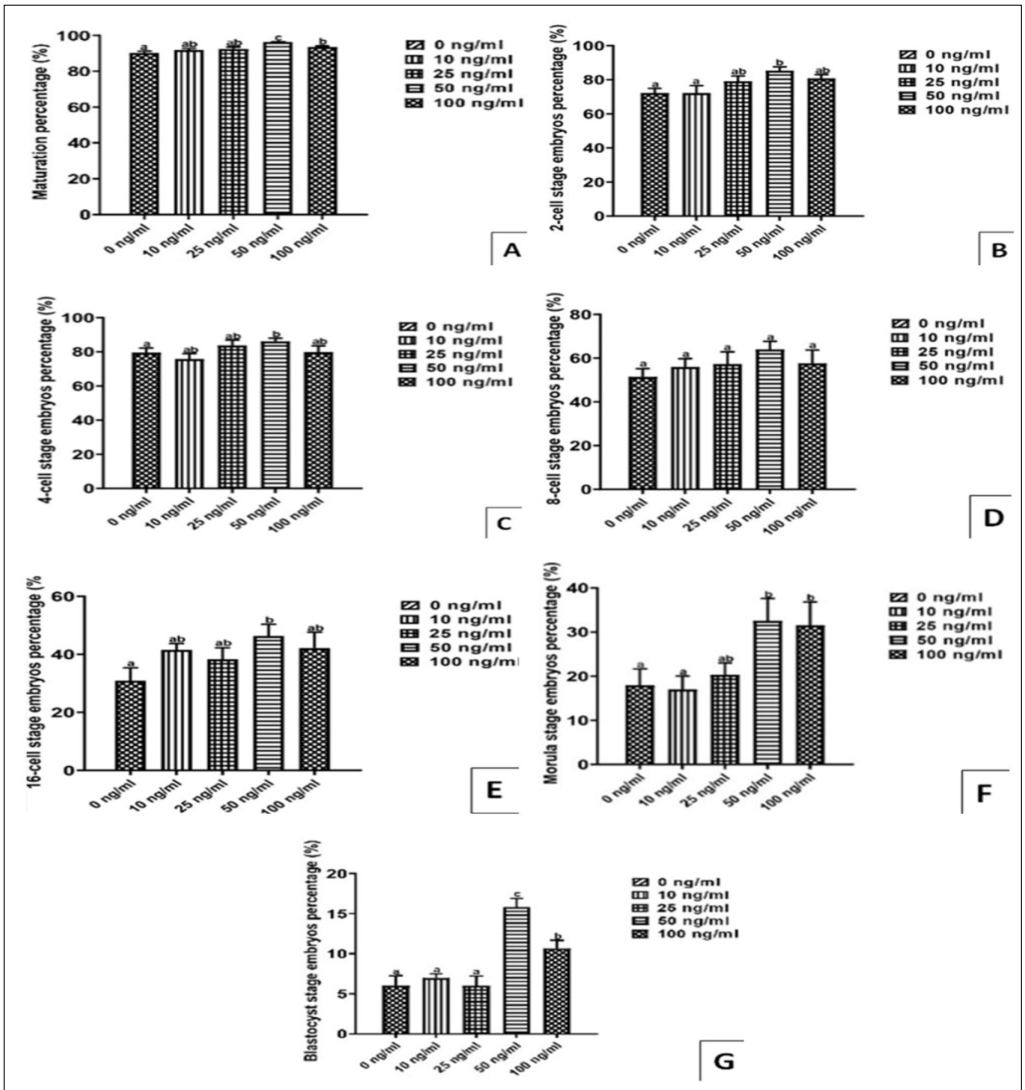
Treatment group	Total no. of oocytes used	No. of oocytes used	No. of matured oocytes	Maturation (%)
0 ng/ml	166	27.67^{a} ±1.05	25.00^{a}±1.06	90.30^{a}±0.84
10 ng/ml	176	29.33^{a} ±.42	27.00^{a}±.36	92.06^{ab}±0.66
25 ng/ml	174	29.00^{a} ±.25	26.83^{a}±.47	92.51^{ab}±1.07
50 ng/ml	169	28.17^{a} ±.70	27.17^{a}±.70	96.44^{c}±0.09
100 ng/ml	170	28.33^{a} ±.49	26.50^{a}±.56	93.50^{b}±0.62

Values (Mean±SE) with different superscript (a, b, c) within a column differ statistically significant ($p<0.05$).

Table 2: Effect of different concentration of IGF-1 on early embryonic development

Treatment group	2-cell%	4-cell%	8-cell %	16-cell%	Morula%	Blastocyst%
0 ng/ml	72.20^{a}±2.74	79.55^{a}±2.64	51.48^{a}±3.72	30.89±4.42^{a}	17.93^{a}±3.71	6.01^{a}±1.24
10 ng/ml	72.17^{a}±4.45	75.80^{ab}±3.16	55.99^{a}±3.75	41.53^{ab}± 2.16	17.02^{a}±3.04	6.95^{a}±0.55
25 ng/ml	79.07^{ab}±3.10	83.73^{ab}±3.14	57.27^{a}±5.58	38.28^{ab}±3.99	20.34^{ab}±2.68	5.99^{a}±1.22
50 ng/ml	85.35^{b}±2.24	86.10^{b} ±1.90	64.00^{a}±3.67	46.34^{b}±4.00	32.53^{b}±5.11	15.81^{c}±1.07
100 ng/ml	80.80^{ab}±2.22	79.83^{ab}±3.70	57.63^{a}±6.10	42.14^{ab}±5.48	31.51^{b}±5.29	10.63^{b}±1.04

Values (Mean±SE) with different superscript (a, b, c) within a column differ statistically significant ($p<0.05$).



A. Oocyte Maturation percentage, B. 2-cells stage, C. 4-cells stage, D. 8-cells stage, E.16-cells stage, F. Morula stage, G. Blastocyst stage. (* above the bars indicate statistically significant differences from the control group at P<0.05).

Fig 1: Effect of different concentrations of IGF-1 supplementation on Oocyte maturation and different stages of early embryonic development.

reaching the blastocyst stage (from 49% to 74%) and significantly decreased apoptotic nuclei (from 16.3% to 8.7%). IGF-1 appears to exert its effect by promoting cell survival, cell division, reducing apoptosis and enhancing metabolic activity in early embryos (Sirard *et al.,* 2006). This strongly suggests that IGF-1 acts as a survival factor during early embryonic development.

CONCLUSION

The present study demonstrated that supplementation with IGF-1 @ 50 ng/ml during *in vitro* maturation and culture of cattle oocytes and embryos enhanced the maturation of immature oocytes and promoted the developmental competence of early-stage embryos.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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