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A systematic review on activation of developmental genes and the developmental block in buffalo embryos

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Abstract
Providing desired in-vitro conditions for maturation of oocytes, fertilisation and embryo development are necessary to achieve greater success rate in in-vitro embryo production. However maintenance of these systems are more complicated and challenging than the standard in-vitro culture, this is mainly because of the difference in the environments where the gametes and embryos have to pass through during their *in vivo* development. With the improvement in various culture medium and culture conditions, the developmental rate of *in vitro* culture embryos are found to be high, but in majority of the cases zygotes stop their development within certain cell cycles after the initiation of cleavage. This is mainly because of the developmental block which arises mostly at the eight-cell stage and it varies with species. Developmental block is found to have a correlation with the quality of the cytoplasm in the oocyte. Oocyte is the principle source provider of all mRNAs and proteins that are required by the embryo to reach fourth or fifth cell cycle. Embryos that are failed to transcribe their own genome cannot develop further. The present review describes the developmental block, activation of the developmental genes in embryos and the factors that can assist proper development of embryo.

Keywords: Cell cycle; developmental genes; embryo transcription; genome activation; serum supplementation

Introduction
In-vitro embryo production advances the developmental of various technologies and these can be utilized for commercial applications. However the production of embryos is very difficult and frequency of *in vitro* embryos reaching the blastocyst stage is very less when compared to *in vivo* developed embryos. The main cause behind this includes disability of the embryo to undergo proper cleavage and developing into blastocyst stage [1]. There are several factors associated with the interference in embryo development, which causes the block in embryo cleavage, and it mostly occurs during fourth transition or in-between fourth to fifth transition of cell cycle [2].

The contribution of Zebu cattle with regard to in-vitro embryo production is highly appreciable in both beef and milk herds [3, 4]. *Bos indicus* can maintain their productive and reproductive status even in stressful environmental conditions and they are very well adapted to specific regions with high humidity and warm weather. Furthermore, *Bos indicus* cows generally tends to have an good recovery rate of oocytes ranging from 18-25 oocytes per Ovum Pick Up section, which is fourfold higher when compared to *Bos taurus* [5]. Also, Zebu cattle produces embryo which differ from taurine cattle that they have high tolerance capacity to cryopreservation and amount of lipid in embryos. In addition to the difference in number of antral follicles, both zebu and taurine cows differ in various aspects like

1. Size of the follicles [6],
2. Concentration of Insulin and Insulin-like growth factor 1(IGF-1) [7],
3. Concentration of Progesterone [6] and
4. Manifestation of oestrus [8].

Nowadays the success rate of production of IVF embryos is tremendous higher when compared to previous days. Still, the number of IVF embryos which are going to be implanted in the uterus (embryo implantation) are very less when compared to the actual number of embryos produced. The main reason behind this strategy may be a developmental block. Generally once when the embryo is formed, it has to go for various cell divisions, epigenetic

reprogramming, compaction, embryonic genome activation, compaction, differentiating onto the cell types and blastocoel cavity development [9, 10]. Among the above mentioned events, activation of embryonic genome is highly crucial and demanding. Once when the embryonic genome is activated, which occur primarily during eight – sixteen cell stage in case of bovines, embryo is totally dependent on the new transcripts which are produced by the nucleus to continue their development [11].

The embryonic genome activation is very much essential, which happens predominantly at particular transition period mentioned in the table I for particular species. At this stage, embryo solely depends upon the transcripts which are synthesized by the nucleus to continue their development [11]. Those embryos which fail to attain this stage, won't subsist beyond the eighth cell stage, this occurrence is called as Developmental block.

Table I: Developmental Block at Different stages in different Species

	Species	Transition stage- cell cycle	References
1	Bovine	Fourth (8-16)	[14]
2	Rabbits	Fifth	Reviewed by [2]
3	Humans	Third- Fourth (4-8)	[20]
4	Murine	Second(2)	[21]
5	Cats	Fourth	[22]
6	Porcine	Third (4)	[23]
7	Ovine	Fourth (8-16)	[24]
8	Bubaline	Fourth (8-16)	[25]

Proteins and mRNAs present within the oocyte are necessary for the early embryonic development. Development of the embryos is activated by fertilisation, which ensures cleavage. Up to 4 cell stage of cleavage, it is synchronous in nature. Decline in protein synthesis is noticed from 1- cell to the 8-cell stage. Activation of Ribosomal RNA synthesis is active by the last 8- cell stage [12]. Table II indicates the number of cell cycle with associated changes observed in embryos.

Table II: Cell cycle with associated changes in embryos

Cell Cycle	Changes observed
1st cell cycle	Long with distinct G1 and G2 phases
2nd cell cycle	Fails to demonstrate any appreciable gap phase
3rd cell cycle	Cleavage becomes asynchronous Embryonic transcription begins
4th cell cycle	Major change in protein pattern

Activation of Developmental genes in Buffalo Embryo genome

For the initiation of transcriptional activation of genome, embryos must overcome a transcription repression state that is mainly because of chromatin. The process of earlier phase of transcription should takes place in a minor sections of chromatin that are remodelled, this phenomenon can be observed in de-condensation for the substitution of protamines by histones in sperm chromosome [13]. As discussed before, the major transition of maternal-embryo side is mostly occurring in fourth stage of cell cycle in case of bovine embryos. Hence, various genomic techniques can be used at this stage to characterise the activated genomic products. Various group of scientists are ding extensive research at this stage with various techniques like real-time PCR, array technologies, sequencing and subtractive hybridisation and sequencing and various other techniques. The principle aim of doing all this research activities is to identify gene transcription pattern that are necessary for the activation of genome. With the advances in biotechnology, various other advanced approached are being handled now that includes transcriptomics etc.

The identification of candidate genes that follow the specific genome activation has to be further validated with the necessary techniques like quantitative real time PCR during respective cell cycles, so that the specific time period of transcription activation can be assessed. If the candidate gene or transcript is found to have some relationship with the embryo quality, then the association between variations in the amount of the specific transcript and the developmental potential of embryo has to be demonstrated. Currently, a number of potential genes have been identified that are having relation to early embryonic development and genome activation by different group of scientific researchers.

Factors associated with proper development of buffalo embryos

Care should be taken during preparation of media, so that the components of media should regulate the proper osmolarity and desired ionic content, also use purified cell culture grade water for media preparation. This helps in overcoming or reducing the developmental block. Incompetence to overcome the repression of chromatin, transcriptional activation of essential developmental genes and to respond to environmental created injuries are also responsible for causing developmental block [14]. During follicular growth of the ovaries, the accumulation of maternal reserves in the oocyte and further loss of these maternal reserves during aging of oocyte and embryo cleavages causes tremendous changes in developmental capacity. Embryos which are co-cultured have very slower developmental rates, however in case of embryos which are having prompt developmental rates have superior developmental potential. This specific phenomenon is mainly because of the inherited cytoplasm quality and having some correlation with maternal zygotic transition.

Effect of serum supplementation on embryonic development

Fetal Calf Serum (FCS) can be added to the culture medium and the extensive studies give an indication that FCS can affect the viability of embryos. Although it has got a negative impact associated with the embryo viability, FCS is used extensively in in-vitro embryo production, because it provides higher degree of blastocyst formation [15].

The adverse deleterious effects caused by FCS to embryos include

a. Modifications in expression of various genes [16]
b. Induction of apoptosis or Programmed cell death [17]
c. Altered structure of mitochondria [16] and
d. Accumulation of lipids [17].

The above-mentioned findings indicate the involvement of FCS in DNA fragmentation and blastomere apoptosis, and possibly the developmental blockage of embryos. Another possibility is that the culture medium which is supplemented with FCS can stimulate various factors which are associated with programmed cell death or apoptosis which includes apoptosis- inducing factor (AIF), which is highly required for the formation of blastocoel [18]. Because of this apoptosis initiated by FCS, development of complete blastocyst is hampered. In relation to FCS, utilization of Bovine Serum Albumin (BSA) in culture medium can lead to the production of good quality embryos, since BSA is an essential protein

which is derived from serum. Thus, in a comparison, FCS can cause deleterious effects to the developing embryos; both the quantity and quality of developing embryos will be hampered. Supplementation with 10% FCS during the culture period decreases blastocyst rates and the quality of the embryos produced, thus reducing the number of cell cycles completed as well as hatching ability. Lastly, fast-cleaving embryos exhibit higher blastocyst rates, and the speed of development appears to be negatively associated with programmed cell death and the blocking of embryo development [19]. These type of studies will further help in clear understanding of developmental block in embryos that aids in better production of quality *in vitro* embryos.

Conclusions

As per the discussion in the review, genomic techniques play an crucial role in understanding the various molecular mechanism that are associated with the activation of genome in the developing embryos. These genomic techniques can be combined with the different recent developmental approaches of reproductive biotechnologies like transcriptomics, gene slicing etc. which will help in the remodelling of normal embryo physiology. These research activities will help in finding out various genes or transcripts and mechanisms that are responsible for proper development of embryos. This further helps in the conservation of various crucial species that are critically affected with this developmental block.

References

1. Lequarre AS, Marchandise J, Moreau B, Massip A, Donnay I. Cell cycle duration at the time of maternal zygotic transition for *in vitro* produced bovine embryos: effect of oxygen tension and transcription inhibition. Biology of Reproduction. 2003; 69:1707-1713.
2. Memili E, First NL. Zygotic and embryonic gene expression in cow: a review of timing and mechanisms of early gene expression as compared with other species. Zygote. 2000; 8:87-96.
3. Pontes JHF, Silva KCF, Basso AC, Ferreira CR, Rigo A. G *et al*. Large-scale *in vitro* embryo production and pregnancy rates from Bos taurus, Bos indicus, and indicus taurus dairy cows using sexed sperm. Theriogenology. 2010; 74:1349-1355.
4. Pontes JHF, Sterza FAM, Basso AC, Ferreira CR, Sanches BV, Rubin KC. Ovum pick up, *in vitro* embryo production, and pregnancy rates from a large-scale commercial program using Nellore cattle (Bos indicus) donors. Theriogenology. 2011; 75:1640-1646.
5. Pontes JHF, Nonato-Junior I, Sanches BV, Ereno-Junior JC, Uvo S, Barreiros TR. Comparison of embryo yield and pregnancy rate between *in vivo* and *in vitro* methods in the same Nelore (*Bos indicus*) donor cows. Theriogenology. 2009; 71:690-697.
6. Hwang IS, Bae HK, Cheong HT. Mitochondrial and DNA damage in bovine somatic cell nuclear transfer embryos. Journal of Veterinary Sciences. 2013; 14:235-240.
7. Chaudhari M, Jayaraj R, Bhaskar ASB, Lakshmana Rao PV. Oxidative stress induction by T-2 toxin causes DNA damage and triggers apoptosis via caspase pathway in human cervical cancer cells. Toxicology. 2009; 262:153-161.
8. Rose S, Frye RE, Slattery J, Wynne R, Tippett M, Pavliv O. Oxidative stress induces mitochondrial dysfunction in a subset of autism lymphoblastoid cell lines in a well-matched case control cohort. PLos One. 2014; 9:e85436.
9. Zheng W, Liu K. Maternal control of mouse preimplantation development. Results and Problems in Cell Differentiation. 2012; 55:115-139.
10. Cantone I, Fisher AG. Epigenetic programming and reprogramming during development. Nature Structural & Molecular Biology. 2013; 20:282-289.
11. Wasler CB, Lipshitz HD. Transcript clearance during the maternal-to-zygotic transition. Current Opinion in Genetics and Development. 2011; 21:431-443.
12. Barnes FL, Eyestone WH. Early cleavage and the maternal zygotic transition in bovine embryos. Theriogenology. 1990; 33(1):141-152.
13. Ao A, Erickson RP, Winston RM, Handyside AH. Transcription of paternal Y-linked genes in the human zygote as early as the pronucleate stage. Zygote. 1994; 2:281-287.
14. Betts DH, King WA. Genetic regulation of embryo death and senescence. Theriogenology. 2001; 55:171-191.
15. Leivas FG, Brum DS, Fialho SS, Saliba WP, Alvim MTT, Bernardi MI. Fetal calf serum enhances *in vitro* production of Bos taurus indicus embryos. Theriogenology. 2011; 75:429-433.
16. Crosier AE, Farin PW, Dykstra MJ, Alexander JE, Farin CE. Ultrastructural morphometry of bovine compact morulae produced *in vivo* or *in vitro*. Biology of Reproduction. 2000; 62:1359-1365.
17. Sudano MJ, Paschoal DM, Rascado TS, Magalhães LC, Crocomo LF, Neto JFL. Lipid content and apoptosis of *in vitro*-produced bovine embryos as determinants of susceptibility to vitrification. Theriogenology. 2011; 75:1211-1220.
18. Pers-Kamczyc E, Pawlak P, Rubes J, Lechniak D. Early cleaved bovine embryos show reduced incidence of chromosomal aberrations and higher developmental potential on day 4.5 post-insemination. Reproduction in Domestic Animals. 2012; 47:899-906.
19. Garcia SM, Marinho LSR, Lunardelli PA, Seneda MM, Meirelles FV. Developmental Block and Programmed Cell Death in Bos indicus Embryos: Effects of Protein Supplementation Source and Developmental Kinetics. PLoS ONE. 2013; 10(3):e0119463.
20. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight- cell stages of preimplantation development. Nature. 1988; 332:459-461.
21. Telford NA, Watson AJ, Schultz GA. Transition from maternal to embryonic control in early mammalian development: A comparison of several species. Molecular Reproduction and Development. 1990; 26(1):90-100.
22. Hoffert KA, Anderson GB, Wildt DE, Roth TL. Transition from maternal to embryonic control of development in IVM/IVF domestic cat embryos. Molecular Reproduction and Development. 1997; 48:208-215.
23. Davis DL. Culture and storage of pig embryos. J Reprod Fert. 1985; 33:115-124.
24. Crosby IM, Gandolfi F, Noor RM. Control of protein synthesis during early cleavage of sheep embryos. Journal of Reproduction and Fertility. 1988; 82:769-775.
25. Chauhan MS, Singla SK, Palta P. *In vitro* maturation and fertilization, and subsequent development of buffalo (Bubalus bubalis) embryos, effects of oocyte quality and type of serum. Reproduction Fertility and Development. 1998; 10:173-177.