International Journal on Veterinary Medicine

In Vitro Production of Sex-Specific Embryos to Get Desired Sex Animal

Opinion
Volume 3 Issue 1- 2024

Author Details

Anupama Karimpat[1], Ashish Mishra[2], SC Roy and IJ Reddy[2]*

[1]*PhD Scholar, Jain University, Bengaluru, India*

[2]*Animal Physiology Division, ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India*

*Corresponding author

Ashish Mishra, Animal Physiology Division, ICAR-National Institute of Animal Nutrition and Physiology, Adugodi, Bengaluru-560 030, India

Article History

Received: August 01, 2024 Accepted: August 14, 2024 Published: August 15, 2024

Introduction

With the ever-growing human population, demand for food is increasing with no such increase in cultivable lands. Hence, an urgent need is to improve livestock productivity by increasing the female population to contribute to National food security. Reproductive failure is a significant animal health and productive performance concern. Reproductive biotechnologies have made great progress for last three decades. Desired sex offspring production is the major challenge and primary requirement for profitable and sustainable livestock farming. Female offspring production is sought by herd owners while male offspring are conceived as an unwanted population. To address this, breeding management through desired sex offspring production is an appealing approach. Artificial insemination (AI) and embryo transfer technology have been approached to improve the breeding program. However, neither AI nor embryo transfer technologies guarantee the probability of sex of animals. Besides this, affordability and having indigenous technology remain a significant hurdle for mass adaptation. The current emphasis is limited to semen sorting to get desired sex calves; however, sorting procedures are yet to be validated. Hence, development of alternative strategies for sex selection is the need of the hour.

In vitro production of sexed embryos and their cryopreservation for subsequent transfer to get desired sex calf or *in vivo* production of desired sex animal are the available alternative approaches. The success of *in vitro* embryo development depends upon the oxidative status of culture condition and is influenced by factors like temperature, gas composition (O_2/CO_2), pH, media composition, and air quality. Ambiguity is that whether all these factors play role in the development/production of sex-specific embryos or not? Many reports have reflected the sex bias based on these factors (detail is discussed in the review of literature). The culture-dependent sex-specific developmental potential of embryos might be due to the production of different concentrations of ROS by different culture media. Our laboratory in sheep is able to alter the polarity of oocytes by modulating oxidative status of culture condition to favor X or Y sperm specific fertilization to result in desired sex embryos [1]; Indian patent filed on dt. 24. 11. 2020). This finding concluded that the level of oxidative status of culture condition keeps oocytes in respective polarized (depolarize/ repolarize) state to fertilize with charged specific sperm (X/Y) to produce sexed embryos. The sex ratio of the offspring at birth can be slightly skewed to either male or female due to numerous factors such as the weight of the mother, age of the parents, family size, stress, geographic and climatic conditions and environmental toxins [2].

Oxidative Stress (OS) and Embryo Survivability

Like other living aerobic cells, embryo and oocyte are major sources of ROS through mitochondrial oxidative phosphorylation. *in vitro* setup can never mimic the exact physiological conditions of *in vivo* system. Multiple factors putting effect on IVMFC set up to increase oxidative stress that leads to suboptimal outcome. The external environment that surrounds the IVMFC procedure also plays an important role in the development of OS. The most important external factor that affect viability of oocyte and embryo is oxygen concentration. Generated ROS cause DNA damage which is significantly increase in *in vitro* cultured embryos as compare to *in vivo* derived embryos [3]. Studies have demonstrated that there is more delay or arrest of *in vitro* embryo development as compared to *in vivo* development. The Main important factor contribute to this difference is oxygen concentration.

Oxygen concentration in uterine environment is 2-8% as compare to high atmospheric concentration of 20%. Detrimental effect of oxygen concentration on embryo development has already been studied in number of species [4]. The free radicals are produced continuously in mitochondria because of the leakage of high energy electrons along the electron transport chain. It is already reflected that increased ROS levels affect cell membranes, DNA, and mitochondria. Effects of ROS on sperm cause abnormal DNA that leads to produce poor quality embryo. Oocyte maturation and embryo development are also affected by increased ROS or decreased antioxidant defenses. ROS causing oxidative stress hamper the activity of the enzymes of energy generation within the embryo. Increased levels of ROS can inactivate glyceraldehyde-3 phosphate dehydrogenase and thus reduce adenosine triphosphate (ATP) generation [5]. The developmental competence of the oocyte is believed to be of utmost complexity. Evidence exists that OS may be one of the factors that controls follicular atresia, initial recruitment of the primordial follicular cohort, subsequent growth of follicles, and selection and dominance of the follicle for ovulation.

Sex-Specific Embryo Survivability

Though globally scientific importance has been given to find out the impact of OS on embryo survivability but there is no such report to find out the impact of OS on sexual differentiation. Many reports have reflected the sex bias on the basis of these factors. Ambiguity is that whether all factors affecting developmental potential of embryos play role on sex of embryo or not? Though there are more numbers of case reports are available in human and mice studies but scanty reports are available on animal studies. High O_2 concentration has a greater detrimental effect on female embryos than male. Male embryos develop faster than female embryos *in vitro but reverse are in vivo* [6]. In mice it has been observed that sex ratios skewed towards males in summer and females in winter [7] The growth of particular sex of embryo is better at particular condition (*in vitro*/*in vivo*) may be speculated that comparing to *in vivo*, *in vitro* culture system have more free radicals which might be hindering the female embryos to grow better than male embryos. All these conditions discussed above for sex bias either due to O_2 concentration or temperature or culture conditions etc. are might be due to production of more free radicals called ROS and that might be hindering the female embryos to grow. From the above discussion it can be hypothesized that ROS must have role on skewing of sex towards either male or female.

The total glucose metabolism is two-fold higher in males relative to females and the activity of the pentose phosphate pathway to be four times greater in female than in male blastocysts [8]. Increased levels of ROS inactivate glyceraldehyde-3 phosphate dehydrogenase (GAPDH) and thus reduce adenosine triphosphate (ATP) generation [5]. Increased levels of ROS inactivate Glucose-6-phosphate dehydrogenase (G6PD) a rate limiting enzyme in the pentose phosphate pathway and antioxidants {hypoxanthine phosphoribosyl transferase (HPRT)} located on the X chromosome lead to energy metabolism and growth rate differences with respect to male and female embryos [9]. Atmospheric O_2 induced significant delays in female embryo development through blastocyst formation has already been observed [6]. It is also well known that female embryos exhibit a 4-fold higher activity of the PPP as compared to male embryos [8]. All the genes of energy metabolism are X chromosome linked and ROS inhibits energy metabolism [9]. The expression level of most of the X-linked gene expression is significantly higher in female than in male blastocysts (O_2 et al., 2002). Most X-linked genes display not only sex-related transcriptional differences but are also involved in the regulation of autosomal gene expression in preimplantation embryos [10].

Therefore, it might have suggested that high oxidative status might affect the chromatin of X chromosome in female embryos as compare to male embryos because of the double gene dose due to 2 X chromosomes in female embryos than male which contains single X chromosome. ROS induced damage or inactivation of X chromosome hindering the developmental potential of female embryos. Therefore, double dose activity of energy metabolism genes in female embryos due to their location on the X chromosome, there is growth rate differences in male and female embryos [9]. Human embryo culture *in vitro* in atmospheric O_2 and blastocyst transfer, gave birth to significantly more males. However, when human embryos were cultured in 5% O_2, the sex ratio at birth was more females [11]. Our study is in agreement with different studies which have resulted more male production at higher O_2 level i.e atmospheric O_2, by significant delays in female embryo development through blastocyst formation [1].

In vitro Production of Sex-Specific Embryos

Oxidative status-mediated change in polarity of oocytes, for subsequent fertilization of sex-specific sperm to produce desired sex embryos and their development is the new biological intervention approached. Our study has resulted in production of more female embryos in low oxidative status and high percentage of male embryos in high oxidative status of culture condition. Fertilization is associated with a change of resting membrane potential, referred as the "fertilization potential" and compared to the action potential in neurons. The egg membrane potential undergoes a different state of polarity [12] and oxidative status mediated alteration in polarity of the oocytes might be the reason of sex-specific (X or Y) charged sperm fertilization to the oocytes at corresponding state of polarity to produce sexed embryos. Potential changes in the membrane of the oocytes facilitate the binding of X or Y sperm to them [13]. In low oxidative status oocytes were in depolarized state and fertilized with negatively charged X sperm resulting significantly ($P<0.05$) more female embryos. In the same fashion a greater number of oocytes were in repolarized state at high oxidative status and fertilized with positively charged Y sperm resulting significantly ($P<0.05$) more male embryos [1].

The sex biasness of the embryos produced at different levels of oxidative status must have been influenced by the pH-mediated ionic exchange of the oocyte membrane to change its polarity for subsequent fertilization of respective charged spermatozoa resulting in sex-specific embryos. The sexual dimorphism in the developmental potential of embryos in relation to the oxidative status of culture condition was compared by intracellular ROS level. Subsequently, relative expression levels of developmental genes were compared in sex-specific embryos (blastocysts) for antioxidant, glucose metabolism and apoptotic pathway. In conclusion the culture mediated modulation in intra cellular oxidative status of oocytes alters the polarity of oocytes for sex-specific sperm binding during fertilization to produce sex-specific embryos. Low oxidative status upregulated the expression of developmental genes to improve the developmental potential of female embryos. The approach to produce sex-specific embryos will definitely increase the female population and add a contribution to academic, research, and commercial systems as well as it will be competitive to semen sorting to get desired sex calf.

Conclusion

In light of the intricate relationship between oxidative status of culture condition and sex-specific embryo production and development, the biological intervention involves altering the polarity of oocytes for sex-specific sperm fertilization to produce desired sex embryos which can subsequently applicable to *in vivo* system to produce desired sex animals and can be competitive to semen sorting for sex specific animal production.

References

1. Ramesh Kumar G, Mishra Ashish, Dhali A, Reddy IJ, Dey DK, et al. (2022) *in vitro* production of desired sex ovine embryos modulating polarity of oocytes for sex-specific sperm binding during fertilization. Scientific Reports 12: 5845.
2. James WH (2006) Offspring sex ratios at birth as markers of paternal endocrine disruption. Environ Res100(1): 77-85.
3. Goto Y, Noda Y, Mori T, Nakan M (1993) Increased generation of reactive species in embryo cultured *in vitro*. Free Radic Biol Med 15(1) : 69-75.
4. Takahashi M (2012) Oxidative stress and redox regulation on *in vitro* development of mammalian embryos. J Reprod Dev 58(1): 1-9.
5. Halliwell B (1989) Free radicals reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. Br J Exp Pathol 70(6): 737-757.
6. Gardner DK, Kelley RL (2013) Male and female embryos differ in their response to oxygen concentration. Fertil Steril 100(3): S242.
7. Drickamer L (1990) Seasonal variation in fertility, fecundity and litter sex ratio in laboratory and wild stocks of house mice (Mus domesticus). Lab Anim Sci 40(3): 284-288.

8. Tiffin GJ, Rieger D, Betteridge KJ, Yadav BR, King WA (1991) Glucose and glutamine metabolism in pre-attachment cattle embryos in relation to sex and stage of development. J Reprod Fertil 93(1): 125-132.

9. Gutierrez Adan A, Oter M, Martinez Madrid B, Pintado B, De La Fuente J (2000) Differential expression of two genes located on the X chromosome between male and female *in vitro* produced bovine embryos at the blastocyst stage. Mol Reprod Dev 55(2): 146-151.

10. Meintjes M, Chantilis S, Guerami A, Dougals J, Rodrieguez A, et al. (2009) Normalization of the live-birth sex ratio after human blastocyst transfer from optimized culture conditions. Fertil Steril 92(3): S229-S230.

11. Bermejo Alvarez P, Rizos D, Rath D, Lonergan P, Gutierrez Adan A (2010) Sex determines the expression level of one third of the actively expressed genes in bovine blastocysts. Proc Natl Acad Sci USA 107(8): 3394-3399.

12. Jaffe LA, Cross NL (1984) Electrical properties of vertebrate oocyte membranes. Biol Reprod 30(1): 50-54.

13. Arangasamy A, Selvaraju S, Parthipan S, Somashekar L, Rajendran D, et al. (2015) Role of calcium and magnesium administration on sex ratio skewing, follicular fluid protein profiles and steroid hormone level and oocyte transcripts expression pattern in Wistar rat. Ind J Anim Sci 85(11): 1190-1194.