ECRONICON OPEN ACCESS

EC CLINICAL AND MEDICAL CASE REPORTS
Short Communication

Enhancing Embryo Survivality by CRISPR/Cas9 Mediated Editing of PTGFS Gene

S Mondal[1]*, IJ Reddy[1], S Nandi[1], PSP Gupta[1], DN Das[2] and D Malakar[3]

[1]ICAR-National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore, Karnataka, India

[2]ICAR-National Dairy Research Institute (SRS), Bangalore, Karnataka, India

[3]ICAR-National Dairy Research Institute, Karnal, Haryana, India

***Corresponding Author:** S Mondal, ICAR-National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore, Karnataka, India.

Received: April 16, 2020; **Published:** May 28, 2021

Early embryonic mortality is one of the major factors of reproductive failure that causes considerable challenge to the livestock industry [1-11]. Indian livestock economics is seriously affected by embryonic wastages and hence its control is the greatest concern to the scientists and policy makers. It has been observed that more than 40% of the total embryonic mortality occurs between days 8 and 17 of pregnancy in bovine [12]. Embryonic losses are reported to be 20-30% in sheep and more than 70 - 80% of the total embryonic mortality occurs between days 8 and 16 of pregnancy [13]. The survivality of embryo during early embryonic life in mammals is mostly dependent on the concerted events where the uterus and terminally differentiated embryos exchange signals culminating in maternal recognition of pregnancy (MRP) [14]. The major reason for embryonic mortality is failure of cellular and molecular dialogues at embryo-uterine interface [15]. Understanding and unraveling the secrets of implantation, embryo development and reciprocal signaling networks between the embryo and uterus will lead to alleviation of the problems of infertility. The recent advances in molecular biology and biotechnology particularly functional genomics (DNAarrays) allowed identifying embryonic and maternal genes potentially involved in embryo survival. Validation of the functional involvement of genes identified requires extensive *in vitro* studies before *in vivo* therapy can be applied.

Prostaglandins (PG) are key regulators of female reproductive function and are involved in ovulation, luteolysis, MRP, implantation and parturition. The candidate genes responsible for their biosynthesis, transport and signal transduction are among the first to consider for involvement in embryonic wastage. A comprehensive understanding and unraveling uterine function and embryo uterine dialogue will facilitate management strategies to improve embryonic survival. The first limiting step in the generation of PGs is the transformation of arachidonic acid by prostaglandin synthases 1 and 2 (PGHS-1, -2 or COX-1, -2). Downstream enzymes such as PGE synthase (PTGES) and PGF synthase (PTGFS) catalyze the conversion of PGH_2 to PGE_2 and $PGF_{2\alpha}$ respectively. The importance of PGs has been confirmed in the mouse where targeted disruption of COX-1 [16] or COX-2 genes [17] induced multiple failures in female reproductive processes. A review confirms that across species, $PGF_{2\alpha}$ and PGE_2 are universally important in the regulation of endometrial function [18]. Relatively little is known about the biosynthetic pathways leading to the formation of PGE_2 and $PGF_{2\alpha}$. Studies with COX-1 and COX-2 knockout mice demonstrated that COX-2, but not COX-1, is crucial for normal ovulation, implantation, and decidualization. Most prostaglandin $F_{2\alpha}$ synthases (PTGFS) identified to date are aldo-ketoreductases (AKRs). Aldoketoreductase 1B5 (AKR1B5) was the most likely PTGFS involved in the production of PGF2α in bovine endometrium at the time of luteolysis [19]. Recent advances in the development of novel, robust and efficient genome editing technologies based on programmable nucleases have substantially improved our ability to make precise changes in the genomes of eukaryotic cells. Generation of human endometrial knockout cell lines with CRISPR/Cas9 system confirmed prostaglandin $F_{2\alpha}$ synthase activity of AKR1B1 [20].

The discovery of CRISPR/Cas9 has revolutionized the field of animal agriculture and served as a potential gene editing tool, producing great excitement to the molecular scientists for the improved genetic manipulations. The major advantages of CRISPR/Cas9 over other genome editing technologies are its simplicity, high efficiency and low cost. This will allow breeders to improve animal welfare, performance and efficiency, paving the way to a more sustainable future for livestock agriculture. Traditional livestock breeding is restricted by genetic linkage and the available genetic variation within a breed. Genome editing allows animal breeders to overcome these biological impediments and introduce polymorphisms that are not present in the gene pool of elite brood stock, or even create novel changes predicted to result in improved gain. This technology has provided researchers with an invaluable tool to accelerate the generation of mouse models for biomedical *in vivo* research. CRISPR/Cas9 technologies have tremendous potential in understanding the biological processes involved in establishment of pregnancy in order to evolve potent gene based therapy for enhancing reproduction and production in buffalo. Targeted genome editing by CRISPR/Cas9 will facilitate in understanding the molecular mechanisms of implantation and will assist in improving fertilization success and early embryonic survival. Our innovative approach of CRISPR/Cas9 guided editing of PTGFS gene will enhance early embryonic survivality in buffalo which in turn will result in improved pregnancy rate, faster genetic improvement and substantial economic gain to the farmers.

Bibliography

1. Mondal S and Prakash BS. "Comparison of luteal function between cows and buffaloes during estrous cycle". *Indian Journal of Dairy Sciences* 55.3 (2002): 142-144.

2. Mondal S., *et al*. "Progesterone and nucleic acid contents of buffalo corpus luteum in relation to stages of estrous cycle". *Indian Journal of Animal Sciences* 74.6 (2004): 710-712.

3. Mondal S., *et al*. "Isolation and characterization of luteal cells in buffalo (Bubalus bubalis)". *Indian Journal of Physiology Pharmacology* 57.1 (2013): 1-6.

4. Mondal S., *et al*. "Isolation, culture and characterization of endometrial epithelial cells in buffalo (Bubalus bubalis)". *Buffalo Bulletin* 28.2 (2009): 101-106.

5. Mondal S., *et al*. "Isolation, culture and characterization of endometrial stromal cells in buffalo (Bubalus bubalis)". *Indian Journal of Animal Sciences* 79.12 (2010): 24-26.

6. Nandi S., *et al*. "Effect of prostaglandin producing modulators on in vitro growth characteristics in buffalo endometrial epithelial cells". *Theriogenology* 77 (2012): 1014-1020.

7. Mondal S., *et al*. "Molecular characterization of FSH receptor (FSHR) gene in Indian river buffalo". *General and Comparative Endocrinology* 158 (2008): 147-153.

8. Palta P., *et al*. "Peripheral inhibin levels in relation to climatic variations and stage of estrous cycle in buffaloes (Bubalus bubalis)". *Theriogenology* 47.5 (1997): 989-995.

9. Mondal S., *et al*. "Endocrine aspects of estrous cycle in buffalo: an overview". *Asian Australasian Journal of Animal Science* 20.1 (2007): 124-131.

10. Ghosh J and Mondal S. "Nucleic acids and protein content in relation to growth and regression of buffalo corpora lutea". *Animal Reproduction Science* 93 (2006): 316-327.

11. Suresh K P., *et al*. "Factors affecting laboratory production of buffalo embryos: A meta-analysis". *Theriogenology* 72 (2009): 978-985.

12. Humblot P. "Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants". *Theriogenology* 56 (2001): 1417-1433.

13. Michels H., *et al*. "Genetic variation of prenatal survival in relation to ovulation rate in sheep: a review". *Small Ruminant Research* 29 (1998): 129-142.

14. Mondal S., *et al*. "Genes Regulating Maternal Recognition of Pregnancy in Domestic Animals: an Update". *Brazilian Archives of Biology and Technology* 58.6 (2015): 854-863.

15. Suresh A., *et al*. "Suppression of COX-2 mRNA abundance in in vitro cultured goat (Capra hircus) endometrial cells by RNA interference and effect on PGF2α and PGE2 concentrations". *Animal Reproduction Science* 209 (2019): 1061-1066.

16. Langenbach R., *et al*. "Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration". *Cell* 83 (1995): 483-492.

17. Morham SG., *et al*. "Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse". *Cell* 83 (1995): 473-482.

18. Narumiya S and FitzGerald GA. "Genetic and pharmacological analysis of prostanoid receptor function". *Journal of Clinical Investigation* 108 (2001): 25-30.

19. Madore E., *et al*. "An aldose reductase with 20μ hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin F2μ in the bovine endometrium". *Journal of Biological Chemistry* 278 (2003): 11205-11212.

20. Lacroix Pepin N., *et al*. "Generation of human endometrial knockout cell lines with the CRISPR/Cas9 system confirms the prostaglandin F2α synthase activity of aldo-ketoreductase 1B1". *Molecular Human Reproduction* 20 (2014): 650-663.

Volume 4 Issue 6 June 2021