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Development of recombinant protein based monoclonal ELISA for detection of early pregnancy in *Bubalus bubalis*

Kanisht Batra, Trilok Nanda, Sushila Maan and **Aman Kumar**
Lala Lajpat Rai University of Veterinary and Animal Sciences, India

Statement of the Problem: An early and accurate diagnosis of pregnancy in animals is crucial to better reproductive management in livestock. This plays important role in shortening the calving interval through early identification of open animals and their timely treatment and rebreeding so as to maintain a postpartum barren interval close to 60 days. A buffalo (*Bubalus bubalis*) is the most important dairy animal and well known for problems related to high calving interval, late puberty and high incidence of anestrus. Lack of pen side early pregnancy diagnostic methods further aggravates this situation. Therefore, present study was conducted to clone, express interferon stimulated genes for development of ELISA which can detect pregnancy at 18-20 days after Artificial Insemination (AI).

Methodology & Theoretical Orientation: The expression of ISGs (ISG15, Mx2 and OAS1) was seen during 18-21 day after artificial insemination by semi quantitative RT-PCR amplification of mRNA by specific primers. These proteins ORF regions were further cloned in pET 28a vector and in silico characterized for their different properties. Out of these proteins, ISG15 was transformed in *E. coli* BL 21 (DE3) expression host for both *in vitro* and *in vivo* studies. This expressed ISG15 was purified by Nickel NTA column and used for raising of hyperimmune sera in rabbit and for development of monoclonal antibody against it.

Findings: The ISGs were upregulated ($P<0.05$) in pregnant buffalo at 18 to 21 days of pregnancy. The anti ISG15 based monoclonal ELISA has an overall diagnostic accuracy of 75.0%.

Conclusion & Significance: The recombinant ISG15 retains the potential for detecting pregnancy in *B. bubalis* and can be used in ELISA kits for pregnancy detection. It may be generated in heterologous expression system and can be used for pregnancy detection in lateral flow devices. It will be a boon for different stakeholders.