



Interferon Stimulated Genes (IsGs): Novel Pregnancy Specific Biomarker In Buffaloes (*Bubalus bubalis*)

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ABSTRACT

Early embryonic mortality (EEM) has been shown to be the prime cause of pregnancy failure in domestic species incurring severe economic losses in terms of milk production in dairy cows in most of the tropical countries including India. Despite of the availability of various diagnosis methods for the pregnancy detection the domestic animals are still prone to reproductive failure before the onset of implantation. Recently, a group of genes called as interferon stimulated genes (ISG) have been shown to be expressed during peri-implantation period which could serve as a potential diagnostic marker for early detection of pregnancy in domestic species. The present mini review highlights the differential expression dynamics of interferon stimulated genes (ISG) during early pregnancy period in buffalo.

Water buffaloes (*Bubalus bubalis*) are the principal milk producing domestic animal in many countries of Asia including India. Indian buffaloes produce 65% of world's total buffalo milk production thereby contributes greatly to Indian economy. In spite of the high productivity, buffalo's possess several integral reproductive problems i.e. silent heat, delayed puberty, long post partum anestrus period and long intercalving period which markedly reduce their reproductive efficiency. Therefore, pregnancy diagnosis at an early stage of gestation could be able to detect the embryonic loss and reproductive failure. Additionally, this might help to fertilize those non pregnant females again which may open up the chance of conception.

Nearly 30-40% of early embryonic mortality (EEM) was witnessed between days 8-17 following fertilization in small ruminants¹, cattle² and buffaloes³ resulting in reproductive failure and major economic losses. Therefore, we need to have some accurate method for the early detection of pregnancy which could identify the animals undergoing embryonic loss. Till date various diagnostic tools such as per-rectal palpation and trans-rectal ultrasonography are established but none could serve as a quintessential method for accurate diagnosis of pregnancy under field conditions⁴⁻⁶. Thus, it is utmost important to unmask the novel roles of signalling molecules responsible for conceptus-maternal communication during early pregnancy. Based on the above facts, the present mini-review focuses on the expression dynamics of aforementioned ISG family during peri-implantation period in domestic animals including buffaloes.

Interferon-tau (IFNT) seems to be a crucial signalling molecule between conceptus and mother, secreted from the trophectoderm cells of blastocysts during days 17 of pregnancy and increases with elongation of conceptus in ruminants⁷⁻⁹. The IFNT expression was elevated during day 16-17 of pregnancy in cattle¹⁰. In another study in sheep, the IFNT expression began to rise during days 8-9, found to be highest during days 16-17 followed by a sharp decline to basal level¹¹. Interestingly, some portion of IFNT secreted into the uterine cavity enters the uterine vein and directly triggers the expression of group of molecules known as interferon stimulated genes (ISGs) in both peripheral blood cells and the CL. The members of ISGs such as interferon-stimulated protein 15 (ISG15)¹², myxovirus resistance protein (MX) and 2'-5' oligoadenylate synthetase (OAS1) could play a paramount role in endometrial cells differentiation, implantation and conceptus development in early pregnancy¹³.

Interferon stimulated gene 15 (ISG15) ubiquitin-like modifier

ISG15 is popularly known as 17 kDa protein and type I interferon. ISG15 was found to be expressed in uterus of cow¹⁴, buffalo¹⁵, ewe¹⁶, sow¹⁷ and mare¹⁸. It has been indicated that, IFNT goes from uterine vein to systemic circulation and induces the ISGs expression in peripheral blood mononuclear cells (PBMCs)^{19,20}. It is well known that, ISGs expression was not only induce maternal recognition of pregnancy (MRP) but also helpful for early detection of pregnancy in ruminants²¹⁻²³. Likewise, administration of IFNT to uterine endometrium up surged ISG15 expression in PBMCs of ruminants²³.

ISG15 gene expression was up-regulated from day 18 to 23 and down-regulated from day 23 to 45 and did not register any change on day 50 of pregnancy indicating higher expression of ISG15 in pregnant as compared to non-pregnant cows^{24,14}. In contrast, Shirasuna et al. (2012) did not observe any change in expression pattern of ISG15 in pregnant and non-pregnant cows²⁵. In a study conducted in cow, the ISG15 expression was shown to be significantly up-regulated on day 17 of the pregnancy²¹. The expression ISG15 transcript was noticed significantly higher during first 24-48 hours in pregnant sheep^{16,19}, reached peak between days 13 to 19 and reverted to basal level on days 21 in uterine endometrium of pregnant sheep²⁶. It has also been revealed that, the ISG15 transcript was consistently expressed throughout pregnancy followed by a dip in ISG15 transcript during late pregnancy²⁷. In yet another study in sheep, the ISG15 expression was up-regulated on day 15, remained elevated till day 21 and its expression was dwindled on days 23 and 25 in PBMCs²⁸. Most recently, our study in buffalo demonstrated that, the ISG15 expression was increased between days 14 to 20 and then declined¹⁵. Taken together, ISG15 has been shown to modulate

endometrial proteins thereby promote establishment and maintenance of pregnancy in ruminants²⁹.

Myxovirus resistance protein (MX)

The IFNT intensifies the MX1 and MX2 expression in PBMCs of ruminants and could able to signal MRP²³. The MX gene was known to be expressed in the uterus of pregnant cow, sheep, pig and mare^{30,31}. In another study in pregnant ewes, the MX1 transcript levels was significantly upturned on days 15, remained consistent till days 23 and then declined on days 25-30²⁸. Furthermore, the MX1 expression was also noted to be elevated on days 15 in PBMCs of pregnant ewe³² and cow³³. In our recent study, the MX1 gene registered greatest expression on days 14, then gradually down-regulated up to days 20 and again increased on days 22 in pregnant buffaloes¹⁵. We have also witnessed that, the MX2 expression was up-regulated between days 14-20 with a significant peak on days 18 and then gradually ware off in pregnant buffaloes¹⁵. The expression of MX2 was also found to be higher between 24-48 h in pregnancy sheep¹⁶ and days 0-18 in pregnant heifers²². Additionally, the MX2 expression was stimulated by IFNT during days 12-17 and 15-21 in ewe and cow respectively³⁴. Moreover, IFNT was shown to stimulate MX2 expression in PBMCs of ewe³⁵.

2'-5' oligoadenylate synthetase (OAS1)

The IFNT has been shown to stimulate the OAS1 transcripts in PBMCs in pregnant ruminants²¹. The OAS1 expression was amplified during days 15-18 in heifers³⁶ and cow³⁷. Shirasuna et al. (2012) documented that, the OAS1 expression did not vary in pregnant and non-pregnant cows²⁵. The OAS1 mRNA expression was elevated within 24-48 h in PBMCs after initial signalling of IFNT in pregnant ewes¹⁶. However, the OAS1 protein expression was increased on days 15, maintained up to days 17 and then decreased on days 19 in pregnant ewes¹⁶. It has been illustrated that, interferons produced from bovine and ovine conceptus in early pregnancy i.e. bTP-1 and oTP-1 respectively, accentuates OAS1 expression in endometrium of pregnant cow and ewe³⁸. In sow, the OAS1 expression did not reveal any significant change suggesting that the maintenance of pregnancy might be independent of OAS1 gene³⁹. In our recent study in buffaloes, we reported highest expression of OAS1 transcript on days 18 suggesting that OAS1 might serve as ideal pregnancy marker¹⁵.

Conclusion

A plethora of signalling molecules known as ISGs secreted from the conceptus are required for conceptus-mother cross talk and induction of MRP following fertilization. Prominent expression of ISGs in uterine endometrium as well as in PBMCs during early pregnancy period may be indispensable for implantation and maintenance of pregnancy and may play a significant

role as early pregnancy biomarkers. There is a dearth of information in this field of study and further research investigations should be envisaged to explore the exact molecular signalling and function of these ISGs which might alleviate EEM and could augment reproductive efficiency in domestic species.

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