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Research Article

EXPRESSION PROFILE OF INTERFERON STIMULATED GENES (MX-1 AND OAS-1) DURING EARLY PREGNANCY IN PLURIPAROUS JERSEY CROSSBRED COWS

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ABSTRACT

The present study aimed at studying the expression profiles of Myxovirus resistance (MX-1) and 2, 5-Oligo Adenylate Synthetase 1 (OAS-1), which are interferon stimulated genes (ISG) in peripheral blood mononuclear cells of pregnant pluriparous cows and non-bred cows and to correlate their expression pattern with early pregnancy. Twelve Jersey crossbred cows were selected and divided into 2 groups *viz.*, pregnant cows and non-bred control. Blood samples from all experimental animals categorized retrospectively after pregnancy verification and processed for expression studies of MX-1 and OAS-1 genes by quantitative real time PCR and the relative expression was calculated by $2^{-\Delta\Delta CT}$. A significant ($P<0.05$) high relative expression of MX-1 was noticed greater than 1.5 folds on day 20 in pregnant cows. MX-1 mRNA levels were not different ($P<0.05$) until day 20 in pregnant cows as compared to non-bred cows. A significantly ($P<0.01$) higher expression of OAS-1 was observed on days 17, 18, 19 and 20 of pregnant cows compared to control. The higher expression level of both the genes during early pregnancy indicates that the presence of viable embryo which produces interferon tau thus stimulate the expression of other developmentally competent genes for embryonic development.

Keywords: Cow, Early pregnancy, MX-1 gene, OAS-1 gene, qPCR

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Livestock sector is an integral component of Indian agriculture, contributing about 30 per cent of total agrarian economy of the country. India ranks first in the milk production in the world, but the average milk production of individual animal is very low. It may be due to various causes like genetics, hereditary and poor animal husbandry practices etc. Reproductive efficiency, feed costs associated with maintaining non-pregnant cows and annual milk production are major constraints for optimization of management in Indian dairy industries. Therefore, early and accurate detection of pregnancy is one of the critical methods to efficient cattle management and enhance the milk production. However, currently there is no rapid and reliable test available to detect early pregnancy in bovine. Another pregnancy specific marker, Early Pregnancy Factor (EPF) also has been called Early Conception Factor (ECF) was first described by its ability to inhibit rosette formation between T lymphocytes and red blood cells and this bioassay was used to detect pregnancy in ruminants, but never was developed fully into a useful diagnostic test, because the specific protein that had this unique activity was difficult to purify (Gandy *et al.*, 2001). Yet another putative pregnancy marker, Pregnancy-Specific Protein B, is reported to be secreted by binucleate cells of the trophoblast as early as day 21 of pregnancy in cows (Sasser *et al.*, 1986). In light of the criticality of early and accurate pregnancy determination for better dairy herd management, and the current lack of an early and accurate means of determining pregnancy based on biomarker in cattle, a need exists for the further characterization of interferon stimulated genes which are differentially expressed in pregnant bovine which may be helpful for identification of early pregnancy marker. Hence, the present study was conducted with the objective of studying the expression pattern of MX-1 and OAS-1 genes which are involved in the embryonic development during early pregnancy and may be used as biomarker if their encoded proteins identified for early pregnancy diagnosis in cattle.

MATERIALS AND METHODS

Animals: A total of 18 apparently healthy pluriparous Jersey crossbred cows between 2 and 8 years of age and in good body condition (BCS: 3 to 5) were selected for the present study based on the breeding history and rectal examination. All the cows in the study, irrespective of their stage of cycle were synchronized using intravaginal

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progesterone device TRIU-B® (Virbac animal health, Mumbai, India) on day 0 and kept in situ for 9 days and administered 500 µg of Inj. Cloprostenol sodium intramuscularly on day 8. On day 9, TRIU-B® was removed and the animals were inseminated with frozen Jersey crossbred semen straws at 72 and 96 h after administration of Inj. Cloprostenol sodium. Injection of Buserelin acetate, a synthetic analogue of gonadotropin releasing hormone (GnRH), 10µg was administered at the time of first insemination. Further, experimental cows were grouped into pregnant (n=6) and non-bred cows (n=6) retrospectively after the pregnancy diagnosis was carried out after 30-45 days post insemination by using 5-7.5 MHz B-mode transrectal ultrasonography (Sonoscape, China).

Collection of blood samples and RNA Extraction: Whole blood samples were collected on days 0 (day of insemination), 14, 15, 16, 17, 18, 19, 20 and 25 post insemination for harvesting Peripheral Blood Mononuclear Cells (PBMC). PBMC were harvested by a density gradient cell separation method using Histopaque-1077® solution. Separated fractions were applied to total RNA using Trizol according to the manufacturer's recommendations (Fig. 1). cDNA synthesis (reverse transcription) was performed using random hexamers with initial concentration of 500 ng of total RNA from each sample using the High-capacity cDNA synthesis kit (Applied Biosystems, USA) following manufacturer's instructions of 20 µl final volume. The cDNA samples were categorized in to pregnant (n=6) and non-bred control (n=6) retrospectively after pregnancy diagnosis.

Quantitative Real-Time PCR: Published oligonucleotide primers (Table 1) were used for real time, *viz.*, MX-1 (Gifford *et al.*, 2007), OAS1 (Pugliesi *et al.*, 2014) and Beta actin (Liu *et al.*, 2016) as endogenous control. Confirmation of MX-1 and Beta actin genes amplification was done by agarose gel electrophoresis and further analysed. The real time PCR was carried out using SYBR green based method for MX-1 and OAS1 in Applied Biosystem qPCR master cycler using SYBR Premix Ex Taq (Sigma, Invitrogen, USA). The reaction mix (10 µl) used for qPCR was composed of Template cDNA 2.0 µl, SYBR Premix Ex Taq 5.0 µl, MX1 forward primer (10 pmol/µL) 0.5 µl, MX-1 reverse primer (10 pmol/µL) 0.5 µl and Nuclease-free H_2O 2µl. In the negative control reactions, instead of cDNA, 2 µl of nuclease free water was added. The real time PCR machine was run with annealing temperature of 56.3 ºC. The assay was designed based on $^{\Delta\Delta}$Ct method and comprised of samples collected at different time point interval along with negative controls, having technical replicate wells (2 each) with endogenous control as beta actin. The Ct values were recorded for the target and the internal control genes. After each PCR cycle, melting curves were obtained to ensure single product amplification. The relative quantification and expression pattern of MX-1 on different days in pregnant and non-bred control experimental animals were analysed keeping Beta actin as endogenous control and 0 day as calibrator. Relative expression was analysed using $^{\Delta\Delta}$Ct method and relative quantification of expressed genes was given by $2^{-\Delta\Delta Ct}$ value (Schmittgen and Livak, 2008). The results were expressed as relative expression compared to day 0 of non-bred control and pregnant animals.

Statistical analyses: The relative expression data were analysed using independent t-test and ANOVA using SPSS statistics 20.0 (International Business Machine (IBM) corp., Chicago, USA)

RESULTS AND DISCUSSION

Expression of MX-1: The relative expression of MX-1 in control and pregnant animals is presented in Fig. 1. The conceptus produced IFNT causes an increase in the expression of ISG in the endometrium (Ott and Gifford, 2010). In the present study, a significant ($P<0.05$) high relative expression of MX-1 was noticed greater than 1.5 folds on day 20 in pregnant cows. Similar results were reported for dairy cows in which MX-1 and MX-2 gene expression increased in PBLs of pregnant, but not in bred non-pregnant cows (Gifford *et al.*, 2007). MX-1 mRNA levels were not different ($P<0.05$) until day 20 in pregnant cows as compared to non-bred cows. Charleston and Stewart (1993) were the first to show that MX1 mRNA was expressed in the ovine endometrium during pregnancy. The expression of MX1 in peripheral blood leukocytes (PBL) was investigated in pregnant sheep during early pregnancy (Yankey *et al.*, 2001). The IFNT stimulated certain genes like OAS-1, ISG-15, MX-1 and MX-2 transcripts between days 18 and 22 post AI not only in uterus but also in PBLs (Pugliesi *et al.*, 2014). ISGs like ISG15, MX1, MX2 and OAS1 could be detected in leukocytes and has been recently applied as marker for pregnancy diagnosis in cattle (Green *et al.*, 2010). Yankey *et al.* (2001) first reported systemic responses to IFNT by showing that MX1 mRNA was elevated in PBL from Day 15 through 30 after insemination in pregnant, compared to bred, non-pregnant ewes. The IFNT stimulates the expression of a large number of ISGs in the pregnant uterine endometrium during the pre-attachment period

Table 1

Primer sequences for Mx-1, OAS-1 and Beta actin.

S.No.	Primer	Sequence (5' to 3')	Product size	Reference
1	MX-1-FMX-1-R	GTACGAGCCGAGTTCTCCAAATGTCCACAG CAGGCTCTTC	197 bp	Gifford *et al.* (2007)
2	OAS1-FOAS1-R	TAGCCTGGAACATCAGGTCTTTGGTCTGGC TGGATTACC	104 bp	Pugliesi *et al.* (2014)
3	Beta actin-FBeta actin-R	CTGGACTTCGAGCAGGAGATGGATGTCGAC GTCACACTTC	203 bp	Liu *et al.* (2016)

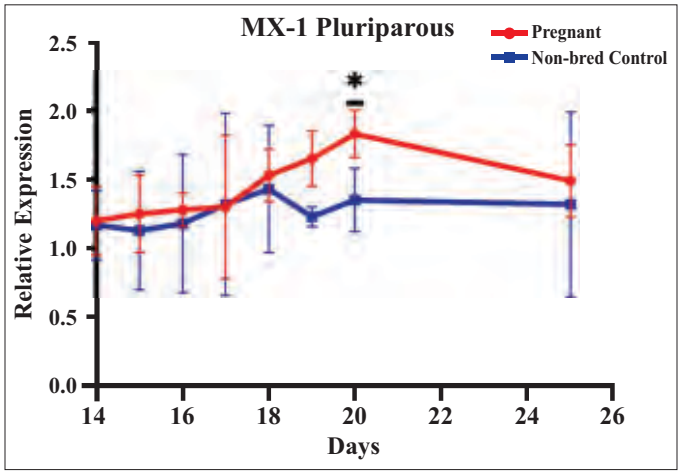

Fig. 1. Relative expression pattern of MX-1 gene in early pregnant pluriparous cows and non-bred control

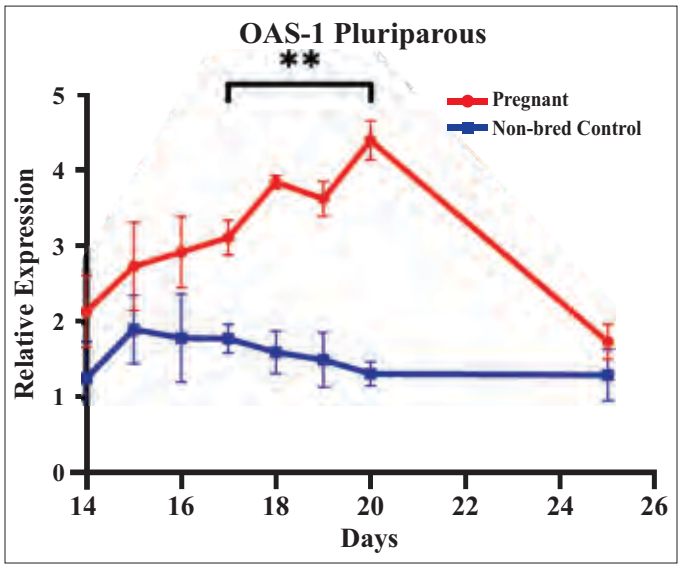

Fig. 2. Relative expression pattern of OAS-1 gene in early pregnant pluriparous cows and non-bred control

(Day 13-15 in cattle) of pregnancy (Gray *et al.*, 2006; Chen *et al.*, 2007). These IFNT-stimulated genes are hypothesized to regulate uterine receptivity to attachment and conceptus development (Hansen *et al.*, 1999; Bauersachs *et al.*, 2008).

Expression of OAS-1: The mean relative expression of OAS-1 in control and pregnant cows has been presented in Fig. 2. In the present study, activation of OAS-1 gene in PBMC is observed and is in accordance with the study conducted by Manjari *et al.* (2016) who reported the dynamic changes in expression of various ISG's both in endometrium as well as immune cells. A significantly ($P<0.01$) higher expression of OAS-1 was observed on days 17, 18, 19 and 20 post insemination or of pregnancy in pregnant cows compared to control which is supported by Pugliesi *et al.* (2014) who has reported increased OAS-1 expression in pregnant cows compared to non-pregnant cows with a functional CL on day 20. A peak expression of 5.4 folds increase in OAS-1 was observed on day 20 in the present study which is in agreement with Shirasuna *et al.* (2012) who reported that IFNT stimulated the expression of OAS-1 in PBMC that was higher in pregnant animals than in non-pregnant animals on day 8 and was increased up to 5.7 folds on day 21 post estrus. The abundance of ISG transcripts until day 22 was consistent, which was correlated to the profile of embryonic IFNT secretion in ruminants indicating that the presence of viable conceptus stimulated expression of ISGs in PBMC in a rapid fashion following the initiation of conceptus elongation and during the period of luteolysis blockage in pregnant cow (Miagawa *et al.*, 2013). The higher expression level of both the genes during early pregnancy indicates that the presence of viable embryo which produces IFNT thus stimulate the expression of other developmentally competent genes for embryonic development.

It is concluded from the present study that MX-1 and OAS-1 genes are stimulated by the IFNT secreted by the conceptus during elongation on days 14-19 of pregnancy in cows. The higher expression of MX-1 and OAS-1 at early pregnancy corresponds to the translation of related proteins, which if identified could be used as a biomarker for early pregnancy diagnosis. Similarly, higher expression of OAS-1 was observed at early pregnancy and their down regulation in later stages suggesting its abundance in early pregnancy, further indicating it as a good candidate for early pregnancy detection.

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