RESEARCH ARTICLE

# Expression of MX-1 and OAS-1 Genes in Peripheral Blood Mononuclear Cells during Early Pregnancy in Jersey Crossbred Heifers

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## Abstract

The present investigation was conducted to study Myxovirus resistance (MX-1) gene expression and 2,5-Oligo-Adenylate Synthetase 1 (OAS-1) gene, present in peripheral blood mononuclear cells and its correlation with early pregnancy in heifers. A total of 24 Jersey crossbred heifers were selected and synchronized using TRIU-B®, $PGF_2\alpha$, and GnRH. All the experimental animals were fixed time inseminated, and blood samples were collected on days 0, 14, 15, 16, 17, 18, 19, 20, and 25 post-insemination. The animals were divided into two groups retrospectively 30-45 days later by pregnancy verification. Blood samples from six pregnant and six non-pregnant heifers were processed for expression studies of MX-1 and OAS-1 genes by real-time 2-ΔΔCT calculated PCR and their relative expression. The results showed that MX-1 mRNA levels were not different ($p<0.05$) until day 19 in pregnant heifers compared to non-pregnant heifers. The expression of OAS-1 gene was two-fold higher than MX-1, indicating that OAS-1 gene might be more specific to early pregnancy than MX-1.

**Keywords:** Early pregnancy, Heifers, Gene expression, MX-1 gene, OAS-1 gene, RT-PCR.

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## Introduction

The growth rate of the livestock sector has been steady and is around 4–5 % despite receiving less investment compared to the manufacturing and service sectors (Ali, 2007). Accurate detection of early pregnancy is one of the precise methods to enhance efficient cattle management and milk production. The establishment of pregnancy in domestic ruminants requires maternal recognition of pregnancy (MRP), wherein the conceptus secretes interferon tau (IFNT) as a maternal recognition factor. IFNT acts in the uterus at about day 16 after insemination and prevents luteolysis by inhibiting $PGF_2\alpha$ release, resulting in the maintenance of the corpus luteum. IFNT induces the synthesis and secretion of IFNT stimulated genes (ISG) such as 2, 5 oligoadenylate synthetase (OAS-1), IFN regulatory factor 1, ISG-15, Myxovirus resistance gene (MX-1 and MX-2) within the uterus and also in blood cells in cows (Toji *et al*., 2017). The circulating leucocytes respond to IFNT by expressing interferon-stimulated genes whose expression pattern can be studied and correlated to early pregnancy (Toji *et al*., 2017). Such study may form a basis for the development of markers for early pregnancy diagnosis. Hence, the objective of the present study was to assess the expression of MX-1 and OAS-1 genes in the peripheral blood of early pregnant heifers to suggest their suitability as a potential marker for early pregnancy diagnosis in cattle.

## Materials And Methods

### *Selection of Animals and Blood Sampling*

A total of twenty-four healthy Jersey crossbred heifers, aged between 2 and 6 years with good body condition (BCS: 3 to 5), were included in the present study. They were selected based on the breeding history and without palpable abnormalities of reproductive organs. Irrespective of their stage of the estrous cycle, all the experimental heifers were synchronized using intravaginal progesterone device TRIU-B® (Virbac Animal Health, Mumbai, India) on day 0,

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which was kept *in situ* for 9 days and 500 µg of Cloprostenol sodium (Pragma, Intas Pharma) (I/M) on day 8 post-TRIU-B® insertion. On day 9, TRIU-B® device was removed, and fixed-time artificial insemination (FTAI) at 72 and 96 hr following $PGF_2\alpha$ injection was done. Buserelin acetate (Receptal Vet, MSD animal health) 10 µg was administered (I/M) at the time of first insemination. Whole blood samples were collected from all 24 animals by jugular vein puncture in EDTA vacutainers on days 0 (day of insemination), 14, 15, 16, 17, 18, 19, 20 and 25 post-insemination for harvesting Peripheral Blood Mononuclear Cells (PBMC) to study the expression pattern of MX-1 and OAS-1 genes using real-time PCR. Pregnancy diagnosis was performed using real-time transrectal ultrasonography (USG) 30-35 days post-FTAI. The experimental heifers were categorized into pregnant and non-pregnant heifers, retrospectively after pregnancy diagnosis by USG, and blood samples of six animals from each category (pregnant and non-pregnant control) were taken up for gene expression study.

### Harvesting of PBMC, Isolation of Total RNA and cDNA Synthesis

PBMC were harvested from each sample using a density gradient cell separation method using Histopaque-1077® solution as per Pradeep Nag *et al.* (2018). The pellet containing PBMC was suspended in 100 µL of RNAlater® solution and mixed well and was kept at -80°C until further use. The RNA was extracted from PBMC using TRIzol® LS reagent (Thermo Fisher Scientific, India). The RNA pellet was resuspended in 20 µL of nuclease-free water. The concentration of the RNA was estimated at $A_{260/280}$ in the spectrophotometer. cDNA synthesis (reverse transcription) was performed using random hexamers with an initial concentration of 500 ng of total RNA from each sample using the High capacity cDNA synthesis kit (Applied Biosystems, USA) in the final reaction mixture volume 20 µL following the manufacturer's instructions.

### Real-Time PCR

Published oligonucleotide primers (Table 1) were used for real-time PCR, viz. MX-1 (Gifford *et al*., 2007), OAS-1 (Pugliesi *et al*., 2014) and Beta-actin (Lixin *et al*., 2016) as endogenous control. Confirmation of MX-1, OAS-1 and Beta-actin genes amplification was done by agarose gel electrophoresis and was further analyzed. The real-time PCR was carried out using SYBR green chemistry for the ISGs in Applied Biosystem qPCR master cycler using SYBR Premix Ex Taq (Sigma, Invitrogen, USA). The assay was designed based on the ΔΔCt method and comprised samples collected at different time intervals and 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. The relative quantification and expression pattern of MX-1 and OAS-1 on different days in pregnant and non-pregnant control were analyzed, keeping Beta-actin as endogenous control and 0 days as calibrator. Relative expression was analyzed using ΔΔCt 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-pregnant control and pregnant animals (n=6 each).

The relative gene expression data were analyzed using an independent 't' test and ANOVA using SPSS statistics 20.0 (International Business Machine (IBM) Corporation., Chicago, USA).

## Results And Discussion

### Expression of MX-1 gene

The mean relative expression of MX-1 in non-pregnant control and pregnant heifers is presented in Fig. 1. The expression of MX-1 was more significant than two folds on day 19 and day 20 in pregnant heifers. The difference in the relative expression on day 20 was significantly higher ($p<0.05$) than the non-pregnant heifers. There was no significant difference in the relative expression of MX-1 gene between different days till day 19 post-AI in both pregnant and non-pregnant control animals. Significantly higher expression of ISG-15, MX-1, and MX-2 mRNA in PBLs on day 18 than day 14 of pregnant cows was observed, and significantly higher expression ($p<0.05$) was also noticed on day 20 in primiparous heifers when compared to pleuriparous cows (Sakumoto *et al.,* 2018). Similar results were reported in 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).

### Expression of OAS-1 gene

The mean relative expression of OAS-1 in non-pregnant control and pregnant heifers is presented in Fig. 2. In non-pregnant control animals, no significant difference was

**Table 1:** Primer sequences for Mx-1, OAS-1, and Beta-actin

| *S. No.* | *Primer* | *Sequence (5' to 3')* | *Product size* | *Reference* |
|---|---|---|---|---|
| 1 | MX1-Forward<br>MX1-Reverse | GTACGAGCCGAGTTCTCCAA<br>ATGTCCACAGCAGGCTCTTC | 197 bp | Gifford *et al*. (2007) |
| 2 | OAS1-Forward<br>OAS1- Reverse | TAGCCTGGAACATCAGGTC<br>TTTGGTCTGGCTGGATTACC | 104 bp | Pugliesi *et al*. (2014) |
| 3 | Beta-actin- Forward<br>Beta-actin- Reverse | CTGGACTTCGAGCAGGAGAT<br>GGATGTCGACGTCACACTTC | 203 bp | Lixin *et al*. (2016) |

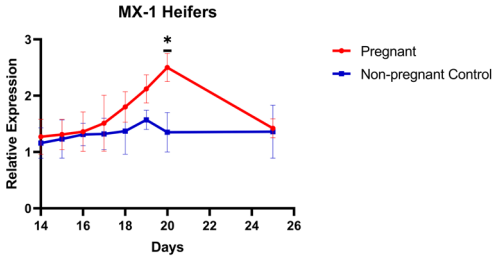


**Figure 1:** Relative expression pattern of MX-1 gene in early pregnant and non-pregnant control heifers (*p < 0.05)

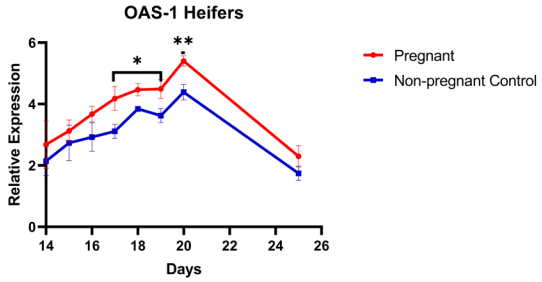


**Figure 2:** Relative expression pattern of OAS-1 gene in early pregnant and non-pregnant control heifers (*p < 0.05; **(p ≤ 0.01).

observed in the relative expression of OAS-1 between different days. In pregnant animals, significantly ($p \leq 0.01$) higher relative expression of OAS-1 was noticed on days 17, 18, 19, and 20 when compared with all other days. Similar findings have been reported earlier by Pugliesi *et al*. (2014). In the present study, recorded activation of OAS-1 gene in PBMC was recorded and was following the earlier reports by Manjari *et al*. (2016), wherein dynamic changes in the expression of various ISG's both in the endometrium as well as immune cells. OAS-1 gene expression increased by 5.4 folds on day 20 which agrees with Shirasuna et al. (2012), who reported higher OAS-1 mRNA expression in PBMC in pregnant animals than non-pregnant animals on day 8, which increased up to 5.7 folds on day 21 post-estrus. The OAS-1 is hypothesized to affect $PGF_2\alpha$ secretion by endometrial epithelium, possibly by altering arachidonic acid metabolism (Spencer and Bazer, 2004).

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 and during the period of luteolysis blockage in pregnant cow (Miagawa *et al.,* 2013). The higher expression of OAS-1 gene than MX-1, at early pregnancy, might have translated to protein which, if identified, could be used to develop a cow side early pregnancy diagnosis tools.

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