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Proteomics and transcriptomics study reveals the utility of ISGs as novel molecules for early pregnancy diagnosis in dairy cows

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ABSTRACT

An early and precise diagnosis of pregnancy in cows is critical to short the calving interval and to improve their reproductive efficiency. Neutrophils are the first blood cells to sensitize the embryo in the uterus and participate in maternal recognition of pregnancy after getting induced by interferon tau (IFNτ). To study the protein abundance ratio, blood samples were collected on 0th, 10th, 18th and 36th day post-artificial insemination (AI) from crossbred Karan Fries cows. Neutrophils were isolated through density gradient centrifugation and studied for protein abundance by high-performance liquid chromatography coupled with mass spectrometry (LC–MS). Protein abundance ratios for Myxovirus resistance (MX1 and MX2) were found to be higher ($P < 0.05$) on day 10 and day 18 post-AI, whereas Oligoadenylate synthetase-1 (OAS1) and Interferon stimulated gene-15 ubiquitin-like modifier (ISG15) proteins were more abundant on day 18 post-AI. The relative mRNA expressions of these molecules were also studied by qPCR. The gene expression of ISG15, MX1, MX2 and OAS1 was found to be higher ($P < 0.05$) on day 10th, 18th and 36th post-AI compared to day 0. The study indicates that ISGs on blood neutrophils are essential for the establishment of pregnancy and may be targeted as potential biomarkers for pregnancy diagnosis in cows.

1. Introduction

To achieve a plethora of economic success and high profitability in milk production, optimal reproductive efficiency of cows is pivotal. Precise detection of estrus, fertility at service and accurate early pregnancy determination are the crucial elements of it (Posthuma-Trumpie et al., 2009). Confirmation of non-pregnancy at an earliest possible time i.e. particularly prior to 17-19th days where luteinization of corpus luteum (CL) takes place, facilitates dairy farmers to allow open animals for a treatment or rebreeding at the very next opportunity i.e. during the upcoming heat (21st day). Failure in pregnancy establishment in cattle imparts a burden of around U.S. $300 in exotic cows (Galligan et al., 2009), though this value needs an update. Abdullah et al. (Abdullah et al., 2014) calculated that increase in a single open day resulted in a loss of rupees 281/d for Zebu and rupees 368/d for crossbred cattle due to decrease in total milk production and increase in maintenance costs of non-pregnant animals in Indian scenario.

Pregnancy demonstrates an immunological paradox state where dam accommodates the semiallogenic fetus. In pregnancy an intimate relationship exists between the dam and the implanting embryo. Immune cells present at the implantation site are tightly controlled to facilitate pregnancy. Out of all the immune cells, neutrophils are the first cells to recognize a foreign organism. Elucidating their role in pregnancy establishment, it is figured out that among all blood cells, neutrophils are the first blood cells to sensitize the embryo in the uterus (Kizaki et al., 2013). They participate in maternal recognition of pregnancy (MRP) after getting induced by interferon tau (IFNτ) which is exclusively secreted by trophoectodermal cells of developing embryo (Shirasuna et al., 2015). They not only help in recognition; but also assist in establishment and maintenance of pregnancy in dairy cows (Bhat et al., 2015; Manjari et al., 2016; Mohammed et al., 2017). Their results revealed that an alternative approach for pregnancy recognition could be made by measuring distinctive expressions of interferon-stimulated genes (ISGs) like Interferon-stimulated protein 15 kDa (ISG15), Myxovirus resistance-1 (MX1), Myxovirus resistance-2 (MX2), and Oligoadenylate synthetase-1 (OAS1) in neutrophils. Recently, Yoshino et al. (2018) documented a threshold value for the early pregnancy diagnosis in cows using the ISGs in granulocytes through quantitative

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PCR techniques. Thus, findings from these many researchers clearly demonstrate that mRNA abundances of ISGs in blood neutrophils of bovine during peri-implantation period might be useful for early pregnancy diagnosis. However, to further verify the diagnostic potentials of the above markers, we framed the present work with the aim that whether their mRNAs are translating into proteins or not and if so whether the abundant mRNA transcripts display same manifoldness at protein levels or not. Moreover, it is well known that genomics/transcriptomics investigations are inadequate to understand a complex immune-physiological state like pregnancy and proteomics may be one of the tools to clear the foggy air. A significant advancement in molecular techniques like proteomics and its demanding application in animal research unlock a new window to identify specific and sensitive biomarkers of unique criteria for early pregnancy diagnosis. Therefore the present work was undertaken to target neutrophil as the prime cell for pregnancy establishment (as it stamps its foot first during the process and clearly displays some distinct pregnancy-related gene expressions i.e. ISGs) for the first time we have tried to validate the predicted potential biomarkers for early pregnancy diagnosis in cows through targeted LC–MS proteomics approach.

2. Materials and methods

2.1. Ethics approval and consent to participate

Clearance of the present study was taken from the Institute's Animal Ethics Committee (Approval No. 41-IAEC-18-32) according to the article 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules, laid down by the Government of India.

2.2. Selection of animals

Multiparous lactating crossbred Karan Fries (KF) cows (n = 20), maintained at the Livestock Research Centre of National Dairy Research Institute, Karnal, Haryana, India were used for this experiment. The cows were managed as per the practices followed in the institute's herd and were offered *ad lib* green fodder, water and a calculated amount of concentrate mixture. Blood samples (10 mL) were collected from the jugular vein on a routine basison four different days i.e., 0th, 10th, 18th and 36th day post-AI (Day 0 = standing estrus and AI) of each cows and neutrophils were isolated and stored at −20 °C till confirmation of pregnancy. At 45 day after AI, pregnancy diagnoses were performed by ultrasonography and reconfirmed by progesterone assay. Out of 20 cows, 9 cows were confirmed as pregnant and these pregnant samples were used for further study. Day 0 was considered as non-pregnant.

2.3. Isolation of neutrophils

Blood neutrophils were isolated through density gradient centrifugation using Histopaque 1119 (Sigma Aldrich, Darmstadt, Germany) and Histopaque 1077 (Sigma Aldrich, Darmstadt, Germany) and cells were collected at the interface of the Histopaque 1119 and Histopaque 1077 layers. These cells were then washed 3 times in PBS (750 × g, 10 min, 4 °C) and stained using pure May–Grunwald (HiMedia Laboratories, Pennsylvania, USA) for 2 min and Giemsa solution (HiMedia Laboratories, Pennsylvania, USA) for 20 s to check the purity of neutrophils. Isolated neutrophils were divided into two parts: one part for gene expression study and other for mass spectrometry.

2.4. Relative mRNA expression

Total RNA extraction from isolated blood neutrophils (1×10^6cells/mL) was performed using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Briefly, neutrophils were lysed by adding 1 mL of TRIzol reagent and the cell lysate was passed several times through a pipette followed by vortex for 5 min at room temperature (RT). The samples were incubated for 5 min at RT, followed by addition of 0.2 mL of chloroform (HiMedia Laboratories, Pennsylvania, USA) per 1 mL of TRIzol and centrifuged at 12,000 g for 15 min at 2−8 °C. The aqueous phase was transferred to a new tube and 0.6 mL of isopropyl alcohol (Sigma Aldrich, Darmstadt, Germany) was added per ml TRIzol, mixed and incubated for 10 min at RT. The samples were centrifuged at 12,000 g for 10 min at 2−8 °C and the RNA pellets were washed with 1 mL of 75% ethanol, vortexed and centrifuged at 8500 g for 5 min at 2−8 °C. The ethanol was removed and the pellets were left to air dry for 15 min. The dried RNA pellet was dissolved in 25 µl RNase free water. The integrity of the RNA was checked by agarose gel electrophoresis (1.5% agarose), and the quantity and quality of RNA were examined by BioSpec-nano (serial no., A116449; Biotech). Complementary DNA (cDNA) was prepared from 1 µg of total RNA using the Novagen first strand cDNA synthesis kit (La Jolla, CA, USA) according to the manufacturer's protocol. Synthesized cDNA was kept at −80 °C till use. Optimization of the annealing temperature of each primer (including housekeeping β-actin and GAPDH primer) was done using gradient PCR followed by visualization of the band in agarose gel electrophoresis. Details of primers for specific bovine MX1, MX2, ISG15 and OAS1 genes have been shown in Table 1 (Sigma Chemicals Co., St. Louis, Missouri, USA).

Quantitative real-time PCR (qPCR) (Roche's Lightcycler 480) was performed using Thermo Scientific Maxima SYBR Green qPCR Master Mix kit (Thermo Scientific, USA) according to the manufacturer's instructions. Briefly, the reaction mix prepared was: 1 µl template; 5 µl (2×) SYBR green mixes, 0.5 µl each of reverse and forward primer, and 3 µl nuclease-free PCR grade water. The reaction was continued for 45 cycles at 95 °C for 15 s, annealing at 59 °C for 20 s, and performed the denaturation kinetics to assess the reaction product. The expression of each gene was analyzed in triplicate and normalized with β-actin and GAPDH which were used as housekeeping gene. The relative gene expression level was evaluated by the $2^{-\Delta\Delta Ct}$ method.

Table 1
Details of primers used in the study.

Genes	Sequence (5′→3′)	Acc. no.	Annealing Temp (°C)
ISG15	F: ACTCCATGACGGTATCCGAG R: ACCCTTGTCGTTCCTCAC	NM_174366	59
MX1	F: GTACGAGCCGAGTTCTCCAA R: ATGTCCACAGCAGGCTCTTC	AF_047692	59
MX2	F: GGGCAGCGGAATCATCAC R: CTCCCGCTTTGTCAGTTTCAG	NM_173941	59
OAS1	F: TCATCCGCCTGGTGAAGCACTGG R: TTGCTCCCAGGCATAGACCGTCAG	NM_001040606.1	59
ß-Actin	F: CATCGCGGACAGGATGCAGAAAGC R: GCGCGATGATCTTGATCTTCATTG	NM_173979.3	59
GAPDH	F: GGGTCATCATCTCTGCACCT R: GGTCATAAGTCCCTCCACGA	NM_001034034	59

2.5. Total protein extraction and digestion

To extract the total protein, 1×10^6 neutrophils were lysed in 500 µl of protein extraction buffer [1% CHAPS, 1% Triton X100, 50 mM TRIS (pH 7.5), 6 M Guanidinium thiocyanate, 1 M thiourea and 10% PMSF] and then quantified by Bradford assay. A total of 100 µg protein was cleaned up using BIO-RAD 2D clean up kit (California, USA) and the precipitated protein was dissolved in urea buffer (8 M urea, 50 mM TRIS, pH 8.0). The dissolved proteins were diluted by adding 50 mM TRIS-HCl (pH 8.0) to bring down urea concentration to 0.2 M, finally trypsin (Promega, Madison, USA) was added at 1 µg per 50 µg of protein and was incubated overnight at 37 ˚C. Reaction was stopped by adding 4 µl of 10% Trifluoro acetic acid (Sigma Chemicals Co., St. Louis, Missouri, USA) followed by assessment of digestion efficiency by analyzing 20 µg of each digested and undigested protein using SDS-PAGE. Bovine serum albumin (BSA) protein was kept as a control for all the steps.

2.6. Mass spectrometric analysis of peptide mixtures

The dried pellet was resuspended in buffer A (5% acetonitrile, 0.1% formic acid). All the experiment was performed using EASY-nLC 1000 system (Thermo Scientific, USA) coupled to Thermo Fisher-QExactive equipped with nanoelectrospray ion source. Peptide mixture (1.0 µg) was resolved using 25 cm PicoFrit column (360 µm outer diameter, 75 µm inner diameter, 10 µm tip) filled with 1.9 µm of C18-resin (Dr Maeisch, Germany). The peptides were loaded with buffer A and eluted with a 0–40% gradient of buffer B (95% acetonitrile, 0.1% formic acid) at a flow rate of 300 nl/min for 130 min. MS data was acquired using a data-dependent top10 method dynamically choosing the most abundant precursor ions from the survey scan.

2.7. Data processing

All samples were processed and RAW files generated were analyzed with Proteome Discoverer (v2.2) against the Uniprot *Bos taurus* reference proteome database. For Sequest search, the precursor and fragment mass tolerances were set at 10 ppm and 0.5 Da, respectively. The protease used to generate peptides, i.e. enzyme specificity was set for trypsin/P (cleavage at the C terminus of “K/R: unless followed by “P”) along with maximum missed cleavages value of two. Carbamidomethyl on cysteine as fixed modification and oxidation of methionine and N-terminal acetylation were considered as variable modifications for database search. Both peptide spectrum match and protein false discovery rate were set to 0.01 FDR.

2.8. Statistical analysis

Statistical analyses were performed using the GraphPad Prism software. All the data were expressed as mean ± standard error of the mean (S.E.M.) and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests. A minimum of three independent experiments was performed for each experimental condition tested. The value of $P < 0.05$ was considered to be statistically significant.

3. Results

The results of plasma progesterone levels of pregnant cows have been presented in Fig. 1.

Plasma progesterone levels increased ($P < 0.05$) from 10th day onwards and reached maximum on 18th day and remained constant upto 36th day of the experimental study.

Fig. 2 shows the relative mRNA expression levels and protein abundance ratio of ISG15, MX1, MX2 and OAS1. The mRNA levels for ISG15, MX1, MX2 and OAS1 genes started to increase from day 10th and were higher ($P < 0.05$) on day 18th post-AI compared to day 0. On 36th day post-AI, mRNA levels of all the four genes fall back to their basal level as on day 0 of AI. On the other hand, the protein abundance ratios were higher ($P < 0.05$) on day 10 and day 18 post-AI for MX1 and MX2 proteins, whereas, OAS1 protein was more abundant on day 18 post-AI only as compared to day 0. The abundance ratios of ISG15 remain unchanged on day 10 and day 18 post-AI and decreased ($P < 0.05$) on day 36 compared to day 0.

4. Discussion

Early and accurate pregnancy diagnosis has been a long lasting saga so far as optimal reproductive efficiency of cows is concerned. Confirmation of non-pregnancy within 18 days of artificial insemination (AI) or breeding can lead to a better reproductive management in cows as approximately 80% of embryonic losses happen between 8–16 days post-insemination (Diskin and Morris, 2008). It critically illustrates that early method for pregnancy diagnosis is the need of the hour. In our study, we have performed the proteomics approach for the first time in bovine neutrophil in pregnant cows and subsequently validated the molecules at mRNA level. Per rectal examination, ultrasonography, plasma/milk progesterone assay have been widely used for pregnancy diagnosis in cows. Progesterone which is known to be the hormone of pregnancy is necessary for pregnancy maintenance as it nullifies myometrial contraction to abrogate abortion (Lewis, 2003) and more importantly protects semi allograft embryo against the maternal immune response (Soloff et al., 2011). Elucidating its pregnancy establishment role, Lonergan et al. (2013) reported that progesterone is also responsible for blastocyst development, histotroph synthesis, conceptus-maternal interactions, and uterine receptivity to implantation. Plasma progesterone assay in blood/milk around 19–24 days is a good method for pregnancy diagnosis (Pohler et al., 2015). Higher plasma progesterone levels on day 10, 18 and 36 compared to day of AI reveals pregnancy status of animals as mentioned in our result and correlated with the ultrasonography result. Ultrasonography method of pregnancy diagnosis has the highest accuracy as early as days 26–29 (Purohit, 2010).

MX1, MX2, OAS1 and ISG15 are the four markers which have been studied extensively in relation to early pregnancy diagnosis in dairy cows in peripheral blood leucocytes (Gifford et al., 2007), peripheral blood mono nuclear cell (Matsuyama et al., 2012), neutrophils (Sheikh et al., 2019; Manjari et al., 2018), hepatocytes (Ruhmann et al., 2017), cervico and vaginal mucosal membrane (Kunii et al., 2018) and endometrium (Forde et al., 2011). IFNτ, the primary key molecule of maternal recognition of pregnancy (MRP) could have been the ideal marker but its transitory secretive nature, meager detectable level and no accumulation in extra uterine tissue and body fluid hampered its candidature as a potential biomarker for early pregnancy diagnosis (Lucy et al., 2011). Thus, the alternative approach to measure its influence came to the limelight through the study of above biomarkers. In our study, we have targeted the blood neutrophils to measure the influence of IFNτ in terms of ISGs expression first at the proteomics level and second at the transcriptomics level and subsequently tried to correlate both.

Proteomics involves extensive study of protein functions, protein expression, protein-protein interactions, or post-translational modifications in a particular cell, tissue, organ, or organism and enables identification of all the proteins present there (Graves and Haystead, 2002). Extensive characterization of proteins found in a specific condition or comparing their differential expressions in two or more situations become possible these days. Currently targeted proteomics using LC–MS has been one of the stands out and highly versatile perspectives to address the issue like early pregnancy diagnosis.

MX1 and MX2 proteins have anti-viral properties and have a role in regulation of endometrial secretion, uterine remodeling and anti-luteolytic activities (Hicks et al., 2003). Protein abundance ratio of MX1

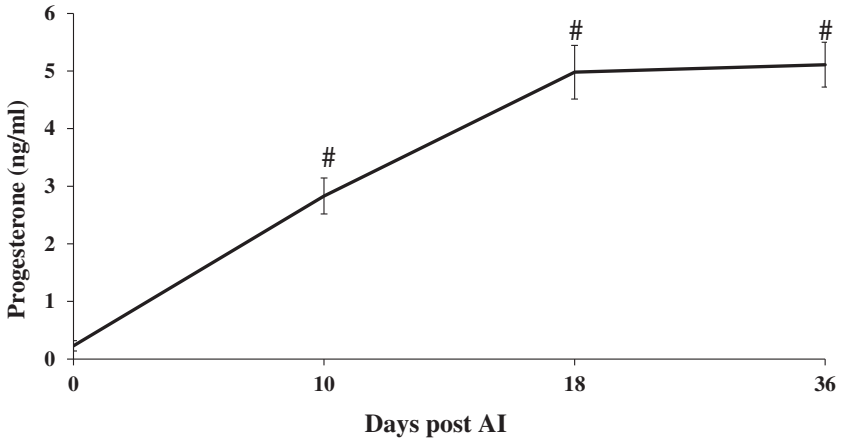

Fig. 1. Plasma progesterone levels in pregnant cows post-AI. (# Significant at $P < 0.05$ with respect to day 0).

and MX2 were higher ($P < 0.05$) on 10th and 18th days of gestation compared to day 0 (Fig. 2). The probable reason might be to impart the migration of maternal neutrophil towards the developing embryo as MX proteins have GTPase activity (Haller et al., 2015; Pantarelli and Welch, 2018) or it might be to protect the dam against viral infections during pregnancy by boosting innate immunity. OAS1 is an enzyme encoded by the OAS1 gene which is essential to combat viral replication by degrading viral RNAs (Dong and Silverman, 1995). It affects the PGF2α secretion in the endometrial epithelium by altering the arachidonic acid metabolism (Schmitt et al., 1993). In our current study, protein abundance of OAS1 was higher ($P < 0.05$) on 18th day of gestation when compared to day 0 (Fig. 2). LC–MS based study portrayed that protein abundance ratio of ISG15 was greater ($P < 0.05$) particularly on day 18 compared to day 0 (Fig. 2). ISG15 covalently conjugates with different proteins through ISGylation. ISGylation is pivotal for maintenance of pregnancy across all mammalian species (Hansen and Pru, 2014). It may do a balancing act by shifting the immune system from infection towards pregnancy establishment when the animals inculcate some infection during pregnancy. It is known that ISG15 behaves as a potent chemo-attractant for neutrophils during infection (Owhashi et al., 2003). It may allow the neutrophils to detect the semiallogenic embryo as self and to work at the intersection of innate and adaptive immune system during pregnancy. However, neutrophils contain ISG15 in its secretory granules during unstimulated state (Bogunovic et al., 2012). Moreover, ISGylation enables its linking to different endometrial proteins which are necessary for pregnancy establishment and maintenance (Austin et al., 2004).

Quantitative real time PCR (qPCR) has been a powerful technique to provide a precise quantitative result at the mRNA level. The relative mRNA expression of MX1, MX2, OAS1 and ISG15 was higher ($P < 0.05$) on day 10, 18, 36 in pregnant neutrophil compared to day 0 and it was maximum on day 18 (Fig. 2). Our results are in agreement with Manjari

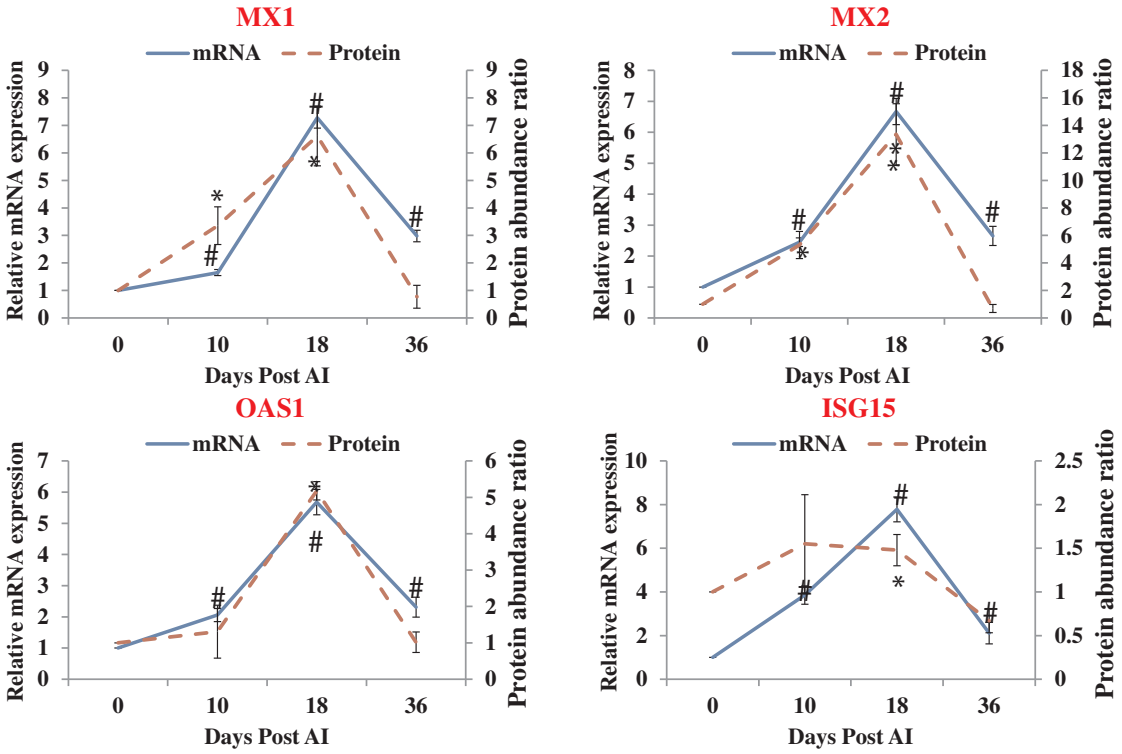

Fig. 2. Relative mRNA expression and protein abundance ratio of MX1, MX2, ISG15 and OAS1 in blood neutrophils of pregnant cows post-AI. All the values represent the relative fold change and protein abundance compared with the value of day 0 (AI) of pregnant cows. (# significant at $P < 0.05$ for mRNA; * significant at $P < 0.05$ for protein abundance ratio with respect to day 0).

et al. (2016), Sheikh et al. (2019) and Alhussien et al. (2018). The high expression may be subjected to IFNτ stimulation as it is well established that maternal recognition of pregnancy in cows occurs between days 16 to day 18 post-AI because of maximum level of IFNτ secretion at that time. In bovine embryo, its synthesis initiates around the time of blastocoel formation (around 8 days) and goes up to 21–25 days with the peak around 14–18 days post-fertilization (Mor et al., 2015). After conducting the LC–MS study followed by qPCR analysis, we found that out of all the ISGs, the abundance peaks of ISG15 are different between mRNA and protein. The probable reasons might be that there is often a poor harmony between mRNA transcripts and their translated protein levels. Moreover, ISG15 protein takes part in ISGylation process by conjugating with different proteins and remains in bound form whereas, the free ISG15 is being secretive in nature behaves as a cytokine for other immune cells and can be detected in blood as well as in urine (Zhang and Zhang, 2011).

Regardless of pregnancy status, expression of ISGs (i.e. MX1, MX2, OAS1 and ISG15) is aberrant and delusive to grant them as molecules of early pregnancy determination according to some researchers. There are reports condemning their usage in cattle and ewe because of poor accuracy when they found exceptionally higher mRNA of ISGs in non-pregnant whereas relatively undetectable level in multiparous pregnant animals (Stevenson et al., 2007; Green et al., 2010; Pugliesi et al., 2014; Mauffré et al., 2016). According to Green et al. (2010) parity of animals also influences their expressions and primiparous or heifers display higher sensitivity than multiparous animals. Moreover, ISGs can be induced by conceptus derived IFNτ as well as by other interferons and in some studies scientists have documented the higher expression levels of ISGs during viral infections in cattle and ewe (Weiner et al., 2012; Rodrigues Hoffmann et al., 2013). Interestingly, all those above experiments have been conducted in PBMC and neutrophils which unveil more responsiveness to IFNτ (Shirasuna et al., 2011). According to Toji et al. (2017), granulocytes and other cell types respond to IFNτ by same signaling pathway but why granulocytes are more sensitive to IFNτ is still unclear. However, neutrophils of multiparous animals have no disparity with the ISGs expressions (Manjari et al., 2016; Sheikh et al., 2018a;, 2018b) and up to 4th parity; the defensive abilities of neutrophils are same in cattle (Alhussien and Dang, 2018). Moreover, few recent reports suggest that targeting ISGs expression on blood neutrophil may be encouraging for early pregnancy diagnosis in cows. By estimating ISG15 and MX2 levels on granulocytes about 80% accuracy can be achieved in pregnancy diagnosis during days 20–22 of gestation (Yoshino et al., 2018). Recently, an attempt has been made to develop a neutrophil lysate based immunoassay by using ISG15 as target molecule for early detection of pregnancy (Sheikh et al., 2018a). The proteins translated from ISGs (i.e. MX1, MX2, OAS1 and ISG15) due to stimulation from different interferons are evolutionary conserved and are assumed to be the arsenals within the genome for a strong first line of defense to combat viruses, bacteria, and parasites (Schneider et al., 2014). Whatever may be the type of cells or interferons, the cells have to express ISGs. There is always a basal level of ISGs expression not only in pathological state, but also in normal physiological state which is because of secretion of tonic/constitutive interferons at a low level (Gough et al., 2012). This may be a possible reason for having ISGs expression even in nonpregnant animal (provided that animal is in non-pathological state). Thus, we may hypothesize that out of all interferons, ISGs may be more responsive towards IFNτ as a topmost priority towards species conservation during peri-implantation stage even if animals sustain an inflammatory state because IFNτ is evolutionary evolved in ruminant and is known to be the only molecule for MRP.

5. Conclusions

The study highlights the role played by various ISGs on the blood neutrophils of pregnant cows during the implantation window. A positive correlation between protein abundance and mRNA expression clearly indicates that molecules like OAS1, MX1 and MX2 can be used for early pregnancy diagnosis after validation in large number of cows.

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Declaration of Competing Interest

The authors declare no conflict of interest, financial or otherwise.

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References

Abdullah, M., et al., 2014. Early pregnancy diagnosis in dairy cattle: economic importance and accuracy of ultrasonography. Adv. Anim. Vet. Sci. 2, 464–467.
Alhussien, M.N., Dang, A.K., 2018. Diurnal rhythm in the counts and types of milk somatic cells, neutrophil phagocytosis and plasma cortisol levels in Karan Fries cows during different seasons and parity. Biol. Rhythm Res. 49, 187–199.
Alhussien, M.N., et al., 2018. Effect of tropical thermal stress on peri-implantation immune responses in cows. Theriogenology 114, 149–158.
Austin, K.J., et al., 2004. Localization of ISG15 and conjugated proteins in bovine endometrium using immunohistochemistry and electron microscopy. Endocrinology 145, 967–975.
Bhat, I.A., et al., 2015. Changes occurring in the receptors of blood neutrophils during implantation in Sahiwal cows. Indian J. Dairy Sc. 68, 3.
Bogunovic, D., et al., 2012. Mycobacterial disease and impaired IFN-γ immunity in humans with inherited ISG15 deficiency. Science 337, 1684–1688.
Diskin, M.G., Morris, D.G., 2008. Embryonic and early foetal losses in cattle and other ruminants. Reprod. Domest. Anim. 43, 260–267.
Dong, B., Silverman, R.H., 1995. 2-5A-dependent RNase molecules dimerize during activation by 2-5A. J. Biol. Chem. 270, 4133–4137.
Forde, N., et al., 2011. Conceptus-induced changes in the endometrial transcriptome: how soon does the cow know she is pregnant? Biol. Reprod. 85, 144–156.
Galligan, D., et al., 2009. Economic concepts regarding early pregnancy testing. Proceedings of the Annual Conference.
Gifford, C.A., et al., 2007. Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. J. Dairy Sci. 90, 274–280.
Gough, D.J., et al., 2012. Constitutive type I interferon modulates homeostatic balance through tonic signaling. Immunity 36, 166–174.
Graves, P.R., Haystead, T.A., 2002. Molecular biologist's guide to proteomics. Microbiol. Mol. Biol. Rev. 66, 39–63.
Green, J.C., et al., 2010. Measurement of interferon-tau (IFN-τ) stimulated gene expression in blood leukocytes for pregnancy diagnosis within 18–20 d after insemination in dairy cattle. Anim. Reprod. Sci. 121, 24–33.
Haller, O., et al., 2015. Mx GTPases: dynamin-like antiviral machines of innate immunity. Trends Microbiol. 23, 154–163.
Hansen, T.R., Pru, J.K., 2014. ISGylation: a conserved pathway in mammalian pregnancy. Posttranslational Protein Modifications in the Reproductive System. pp. 13–31.
Hicks, B.A., et al., 2003. Expression of the uterine Mx protein in cyclic and pregnant cows, gilts, and mares. J. Anim. Sci. 81, 1552–1561.
Hoffmann, A.R., et al., 2013. Ovine fetal immune response to Cache Valley virus infection. J. Virol. 87, 5586–5592.
Kizaki, K., et al., 2013. Differential neutrophil gene expression in early bovine pregnancy. Reprod. Biol. Endocrinol. 11, 6.
Kunii, H., et al., 2018. Hot topic: pregnancy-induced expression of interferon-stimulated genes in the cervical and vaginal mucosal membranes. J. Dairy Sci. 101, 8396–8400.
Lewis, G.S., 2003. Role of ovarian progesterone and potential role of prostaglandin F2α and prostaglandin E2 in modulating the uterine response to infectious bacteria in postpartum ewes. J. Anim. Sci. 81, 285–293.
Lonergan, P., et al., 2013. Role of diestrus progesterone on endometrial function and conceptus development in cattle. Anim. Reprod. 10, 223–227.
Lucy, M., et al., 2011. Pregnancy determination in cattle: a review of available alternatives. Proceedings of the Applied Reproductive Strategies in Beef Cattle.
Manjari, P., et al., 2016. Neutrophil gene dynamics and plasma cytokine levels in dairy cattle during peri-implantation period. Vet. Immunol. Immunopathol. 173, 44–49.
Manjari, P., et al., 2018. Exploring the concentration-dependent actions of interferon-τ on bovine neutrophils to understand the process of implantation. J. Cell. Biochem. 119, 10087–10094.
Matsuyama, S., et al., 2012. Relationship between quantity of IFNT estimated by IFN-stimulated gene expression in peripheral blood mononuclear cells and bovine embryonic mortality after AI or ET. Reprod. Biol. Endocrinol. 10, 21.
Mauffré, V., et al., 2016. Interferon stimulated genes as peripheral diagnostic markers of

early pregnancy in sheep: a critical assessment. Animal 10, 1856–1863.
Mohammed, S., et al., 2017. Fluctuation in the number, type and activity of blood neutrophils in cows exhibiting successful and unsuccessful completion of gestation cycle. Biol. Rhythm Res. 48, 855–865.
Mor, A., et al., 2015. Genes regulating maternal recognition of pregnancy in domestic animals: an update. Braz. Arch. Biol. Technol. 58, 854–863.
Owhashi, M., et al., 2003. Identification of a ubiquitin family protein as a novel neutrophil chemotactic factor. Biochem. Biophys. Res. Commun. 309, 533–539.
Pantarelli, C., Welch, H.C., 2018. Rac-GTP ases and Rac-GEF s in neutrophil adhesion, migration and recruitment. Eur. J. Clin. Invest. 48, 12939.
Pohler, K.G., et al., 2015. Predicting embryo presence and viability. Adv. Anat. Embryol. Cell Biol. 216, 253–270.
Posthuma-Trumpie, G.A., et al., 2009. Perspectives for on-site monitoring of progesterone. Trends Biotechnol. 27, 652–660.
Pugliesi, G., et al., 2014. Conceptus-induced changes in the gene expression of blood immune cells and the ultrasound-accessed luteal function in beef cattle: how early can we detect pregnancy? Biol. Reprod. 91, 95–101.
Purohit, G., 2010. Methods of pregnancy diagnosis in domestic animals: the current status. Webmed Central Reprod. 1.
Ruhmann, B., et al., 2017. Interferon-τ induced gene expression in bovine hepatocytes during early pregnancy. Theriogenology 104, 198–204.
Schmitt, R.A., et al., 1993. Uterine cellular changes in 2′, 5′-oligoadenylate synthetase during the bovine estrous cycle and early pregnancy. Biol. Reprod. 48, 460–466.
Schneider, W.M., et al., 2014. Interferon-stimulated genes: a complex web of host defenses. Annu. Rev. Immunol. 32, 513–545.
Sheikh, A.A., et al., 2018a. JAK3 and PI3K mediate bovine Interferon-tau stimulated gene expression in the blood neutrophils. J. Cell. Physiol. 233, 4885–4894.
Sheikh, A.A., et al., 2018b. Development of enzyme-linked immunosorbent assay for early pregnancy diagnosis in cattle. Anim. Reprod. Sci. 197, 126–133.
Sheikh, A.A., et al., 2019. Interferon tau stimulated gene expression and proinflammatory cytokine profile relative to insemination in dairy cows. Biol. Rhythm Res. 50, 335–345.
Shirasuna, K., et al., 2011. Upregulation of interferon-stimulated genes and interleukin-10 in peripheral blood immune cells during early pregnancy in dairy cows. Malays. J. Reprod. Health 1111010406-1111010406.
Shirasuna, K., et al., 2015. Possible role of interferon tau on the bovine corpus luteum and neutrophils during the early pregnancy. Reproduction 150, 217–225.
Soloff, M.S., et al., 2011. Effects of progesterone treatment on expression of genes involved in uterine quiescence. Reprod. Sci. 18, 781–797.
Stevenson, J.L., et al., 2007. Correlation between reproductive status and steady-state messenger ribonucleic acid levels of the Myxovirus resistance gene, MX2, in peripheral blood leukocytes of dairy heifers. J. Anim. Sci. 85, 2163–2172.
Toji, N., et al., 2017. Evaluation of interferon-stimulated genes in peripheral blood granulocytes as sensitive responders to bovine early conceptus signals. Vet. J. 229, 37–44.
Weiner, C.M., et al., 2012. Interferon stimulated genes, CXCR4 and immune cell responses in peripheral blood mononuclear cells infected with bovine viral diarrhea virus. Res. Vet. Sci. 93, 1081–1088.
Yoshino, H., et al., 2018. A predictive threshold value for the diagnosis of early pregnancy in cows using interferon-stimulated genes in granulocytes. Theriogenology 107, 188–193.
Zhang, D., Zhang, D., 2011. Interferon-stimulated gene 15 and the protein ISGylation system. J. Interferon Cytokine Res. 31 (1), 119–130.