Differential gene expression analysis of TNFα, IL10, HSP70 and HSP90 in fetal dystocia and eutocia buffaloes

B.V.S. Bhavya Charitha[1*], B. Chandra Prasad[1], K. Sunny Praveen[3], G. Kamalakar[2], Ch. Srinivasa Prasad[4]

[1*]Department of Veterinary Gynaecology and Obstetrics, [2]Department of Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram – 521101; [3]Livestock Research Station, Lam farm, Guntur – 522034; [4]College of Veterinary Science, Prodattur – 516360
[*]Corresponding author email address: bhavyabaddula97@gmail.com,

Journal of Livestock Science (ISSN online 2277-6214) 16: 688-693
Received on 11/4/25; Accepted on 12/12/25; Published on 15/12/25
doi. 10.33259/JLivestSci.2025.688-693

Abstract

Fetal dystocia in buffaloes elicit changes in haemato-biochemical parameters and inflammatory molecules attributed primarily to stress. This study aimed to investigate the gene expression patterns of certain pro-inflammatory and anti-inflammatory cytokines as well as heat shock proteins (HSPs) in peripheral blood mononuclear cells of buffaloes affected by fetal dystocia. Buffaloes with fetal dystocia and eutocia were examined and RNA was isolated from peripheral blood mononuclear cells using the TRIzol method. First-strand cDNA synthesis was performed and qPCR was conducted to analyze the expression of TNF-α, IL-10, HSP70 and HSP90 genes. The results revealed significant ($P<0.05$) up-regulation of TNF-α, IL-10, HSP70 and HSP90 in buffaloes affected by fetal dystocia compared to eutocia. These findings suggest that dystocia induces cellular stress leading to tissue damage and inflammation as evidenced by the altered expression of inflammatory cytokines and HSPs. Further research is needed to elucidate the mechanisms underlying these molecular changes and their implications for the prognosis of dystocia in buffaloes.

Key words: Buffaloes, Differential gene expression, Dystocia, Eutocia, Heat shock proteins, Inflammatory cytokines.

Introduction

Fetal dystocia is associated with changes in hematobiochemical parameters, as well as alterations in certain inflammatory molecules which were mainly ascribed to stress factors (Prasad *et al.*, 2017; Charitha *et al.,* 2023). During parturition, the uterus becomes exposed to foreign materials and microorganisms, resulting in bacterial contamination post-calving (Mette *et al.,* 2010). Cytokines are regulatory proteins secreted mainly by immune cells in response to various stimuli and play a crucial role in modulating local and systemic inflammation (Stanilov *et al.,* 2024; Henderson and Wilson, 1996). Many cytokines are interleukins, indicating that they are secreted by leukocytes and act upon other leukocytes (Entrican, 2002). pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-8, IL-12, TNF-α and IFN-γ amplify the inflammatory responses, whereas anti-inflammatory cytokines including IL-1 Ra, IL4, IL-10, IL-13 and TGF-β suppress the genes for pro-inflammatory cytokine synthesis and aid in the resolution of inflammation (Stanilov *et al.,* 2024).

Pro-inflammatory cytokine mRNA expressions are elevated during the peripartum period to ward off infections, and together with anti-inflammatory cytokines, they regulate immune responses and determining reproductive outcomes (Wani *et al.,* 2018). Parturition and other calving-related disorders, including dystocia, alters the expression of Heat Shock Proteins (HSPs) expression and elevated HSP levels serve cytoprotective role and acts as markers of cellular injury under stressful conditions (Kregel, 2002; Catalani *et al.,* 2010; Biradar *et al*., 2024 and).

Few studies have investigated the gene expression in buffaloes during fetal dystocia and eutocia, highlighting the need for further research. With this backdrop, the present study was aimed to analyze pro- and anti-inflammatory cytokines expression in peripheral blood mononuclear cells of fetal dystocia and eutocia-affected buffaloes.

Materials and methods

The present study was carried out in fetal dystocia affected Graded Murrah buffaloes, which were presented to the Obstetrical ward, Department of VGO, NTR CVSc, Gannavaram, Andhra Pradesh and from local dairy farms were selected. Fetal dystocia affected buffaloes categorized as Group-I (n=6), while normal calved buffaloes categorized as Group-II (n=6).

Sample collection

Whole blood samples were collected aseptically from six buffaloes affected with fetal dystocia and six eutocia buffaloes in EDTA coated sterile vacutainers for the isolation of Peripheral Blood Mononuclear Cells.

Gene expression analysis

PBMCs were isolated from whole blood using Histopaque- 1077 via density gradient centrifugation (Sigma-Aldrich, Cat. No. 10771) as per the manufacturer's protocol. The PBMC pellet was washed with PBS and total RNA was extracted using TRIzol reagent (Invitrogen, USA), according to manufacturer's instructions with minor modifications (Manjari *et al.,* 2015). Spectrophotometer (Nanodrop, ND-1000, Thermo-scientific, USA) was used to assess the quality and quantity of RNA and sample with acceptable absorbance ratios of 260/280 and 260/230 were used for cDNA synthesis.

The iScript^{TM} Select cDNA Synthesis Kit (Bio-Rad, USA) was employed to convert RNA into first-strand cDNA. Primers for the target genes (TNF-α, IL-10, HSP70 and HSP90) and the housekeeping gene (GAPDH) were designed using Primer3plus software from NCBI and validated using BLAST. The primer sequences of target genes were mentioned in Table 1. Assay optimization involved standard PCR amplification, melt-curve profiling and systematic evaluation of optimal primer and template concentration. Target gene expression levels were normalized against GAPDH and analysed using the $2^{-\Delta\Delta CT}$method, while amplicon specificity was confirmed by agarose gel electrophoresis with standard DNA ladders.

Statistical analysis

The statistical analysis of the data was done adopting computer software programme for Windows XP (version 17.0, SPSS Inc. Munich) and as per the procedures described by Snedecor and Cochran (1994). Relative gene expression i.e., fold change was calculated by $2^{-\Delta\Delta CT}$method.

Results

mRNA expression of pro-inflammatory cytokine (TNFα) and anti-inflammatory cytokine (IL-10) in buffaloes affected with fetal dystocia and eutocia:

The mean±SE values of ΔC_q, $\Delta\Delta C_q$ and fold change for TNFα and IL10 were depicted in Table 2. The mRNA expression of TNFα and IL-10 were significantly ($P<0.05$) up-regulated in fetal dystocia affected buffaloes compared to eutocia buffaloes (Fig.1 and 2, respectively). The amplicon size of qPCR product of TNFα and IL-10 visualized on 2% agarose gel was presented in Fig. 5.

mRNA expression of heat shock proteins (HSP70 and HSP90) in buffaloes affected with fetal dystocia and eutocia:

The mean±SE values of ΔC_q, $\Delta\Delta C_q$ and fold change for HSP70 and HSP90 were depicted in Table 2. The mRNA expression of HSP70 and HSP90 were significantly (P<0.05) up-regulated in fetal dystocia affected buffaloes compared to eutocia buffaloes (Fig.3 and 4, respectively). The amplicon size of qPCR product of HSP70 and HSP90 were visualized on 2% agarose gel was presented in Fig. 5.

Discussion

In the present study, the mRNA expression of pro-inflammatory cytokine (TNFα) was significantly (P<0.05) up-regulated in fetal dystocia affected buffaloes compared to control animals (Wani *et al.,* 2018). On the contrary, non-significant increase in TNFα expression at 35 days postpartum in dystocia affected buffaloes was recorded (Wakayo, 2014), while significant increase in TNFα level at the time of parturition in buffaloes during the transition period was observed (Gomaa *et al.,* 2021). On the other hand, a non-significant difference in TNFα levels during transition period in dairy cows was also on record (Sabzikar *et al.,* 2023). The higher mRNA expression of TNFα in fetal dystocia affected might be due to manipulative procedures at delivery that caused increased tissue injury and bacterial contamination of the uterus, which favoured creation of persistent inflammatory state with up-regulation on inflammatory mediators (Goto *et al.,* 2024).

In the current study, the mRNA expression of anti-inflammatory cytokine (IL10) was significantly (P<0.05) up-regulated in fetal dystocia affected buffaloes compared to control animals. These findings were in agreement with the previous studies (Wani *et al.,* 2018, Van Engelen *et al.,* 2009). Van Engelen *et al*. (2009) also reported that IL10 expression increased 10 folds at parturition when compared with 185th day of pregnancy, while Sheikh *et al*. (2016) recorded higher values of IL10 on the day of parturition. The higher mRNA expression of IL 10 in fetal dystocia affected buffaloes might be due to dystocia induced stress and tissue damage during assisted calving which lead to inflammation and the inflamed cells would respond by producing anti-inflammatory cytokines.

Table 1: Primer sequences of TNF-α, IL10, HSP70, HSP90 and GAPDH genes

Genes	Accession number	Forward primer (5---->3)	Reverse primer (3--->5)	Product size (bp)
TNF-α	XM_006041930.4	5'GAAGTTGCTTGTGCCTCAGC3'	5'AACCAGAGGGCTGTTGATGG3'	143
IL-10	NM_001290845.1	5'TCAGCACTACTCTGTTGCCTG'	5'AGTAAGCTGTCCAGTTGGTCC3	185
HSP 70	XM_006041891.2	5'TCCCCATCCGAACCGTATCA3'	5'ATCGGTGAAGGCCACATAGC3'	205
HSP 90	XM_025271500.3	5'CACAAGCACATACGGCTGGAC'	5'GGCAGCCATGTAACCCATCG3'	91
GAPDH	XM_006065800.4	5'AAAGTGGACATCGTCGCCAT3'	5'CCGTTCTCTGCCTTGACTGT3'	116

Table 2: Mean±SE of ΔC_q, $\Delta\Delta C_q$ and fold change of pro-inflammatory cytokine (TNFα), anti-inflammatory cytokine (IL-10), HSP70 and HSP90 in buffaloes affected with fetal dystocia and eutocia

Cytokines and HSPs	Group	ΔC_q	$\Delta\Delta C_q$	Fold change
Pro-inflammatory cytokine (TNFα)	Eutocia (control)	3.94±0.17	0.00±0.00	1.00±0.00^{a}
	Fetal dystocia	3.38±0.14	-0.57±0.15	1.50±0.16^{b}
Anti-inflammatory cytokine (IL-10)	Eutocia (control)	4.92±0.15	0.00±0.00	1.00±0.00^{a}
	Fetal dystocia	4.54±0.21	-0.37±0.12	1.31±0.10^{b}
HSP70	Eutocia (control)	3.37±0.12	0.00±0.00	1.00±0.00^{a}
	Fetal dystocia	2.73±0.24	-0.64±0.18	1.60±0.18^{b}
HSP90	Eutocia (control)	1.13±0.07	0.00±0.00	1.00±0.00^{a}
	Fetal dystocia	0.72±0.06	-0.41±0.07	1.33±0.06^{b}

Means with different superscripts (a,b) in a column for fold change differ significantly (P<0.05).
ΔΔCq = ΔCq(Target) – ΔCq(Reference); ΔΔCq = ΔCq(Treatment) – ΔCq(control); Fold change = 2^- ΔΔCq,

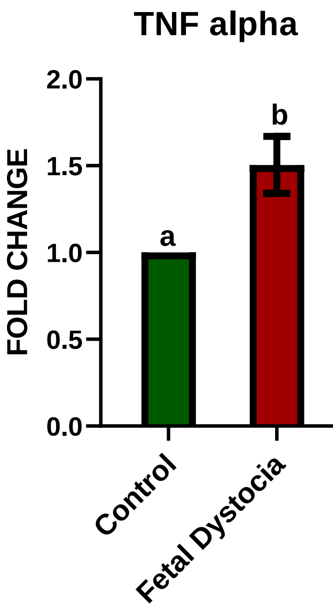

Figure 1: mRNA expression of TNFα in PBMC of fetal dystocia affected buffaloes

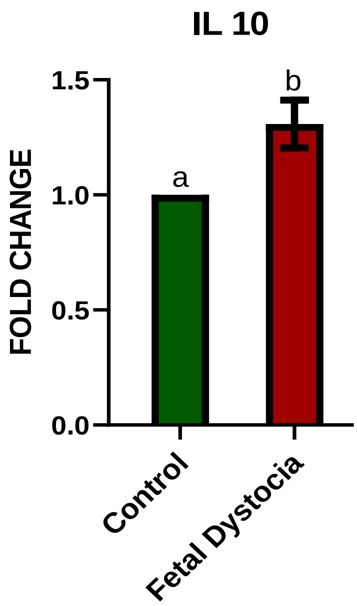

Figure 2: mRNA expression of IL10 in PBMC of fetal dystocia affected buffaloes

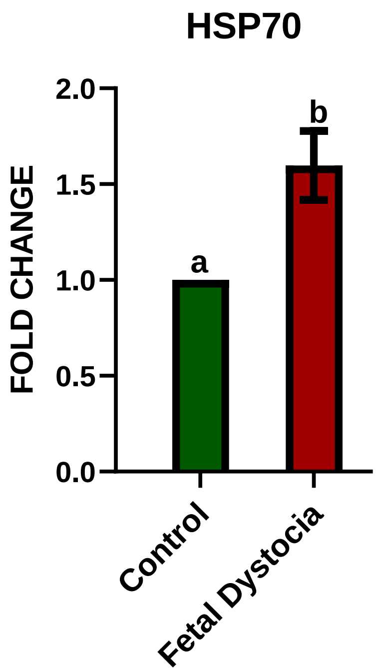

Figure 3: mRNA expression of HSP70 in PBMC of fetal dystocia affected buffaloes

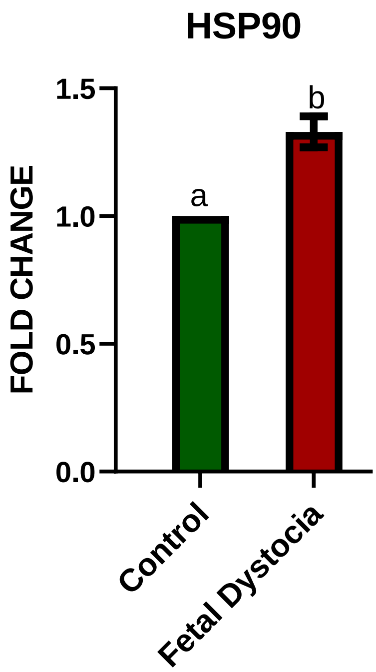

Figure 4: mRNA expression of HSP90 in PBMC of fetal dystocia affected buffaloes

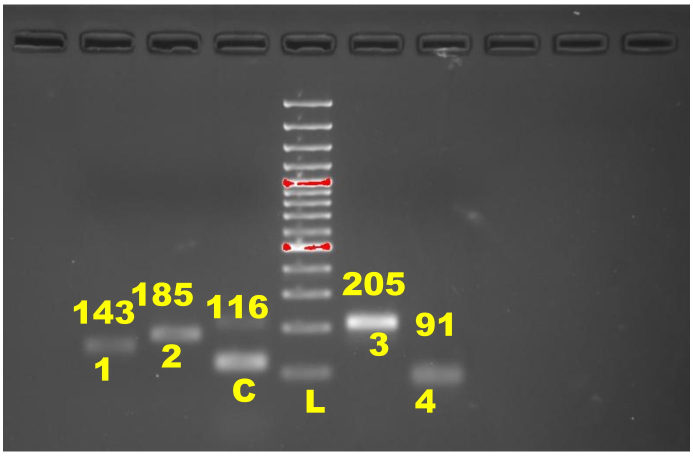

Fig 5: Amplicon sizes: L= ladder (100bp), C= GAPDH (116bp), 1=TNFα (143bp), 2= IL10 (185bp), 3= HSP70 (205bp), 4= HSP90 (91bp)

In the present investigation, the RNA expression of HSP70 and HSP90 were significantly ($P<0.05$) up-regulated in fetal dystocia affected buffaloes when compared with normal parturition controls. Sheikh *et al*. (2016) reported a higher expression of HSP70 and 90 on the day of parturition in cows and Aggarwal *et al*. (2012) recorded higher plasma levels of HSP70 on the day of parturition in cows. Whereas, Catalani *et al.* (2010) found peak levels of HSP72 on day14 post calving in periparturient Holstein cows. Yaniz *et al*. (2009) reported that concentration of HSP70 increased as the gestation progresses in Holstein-Friesian cows. Relatively higher mRNA expression of HSP70 and HSP90 might be due to higher degree of stress during assisted calving. Generally, activation of heat shock proteins would occur during cellular stress and would provide cytoprotective effect (Swelum *et al.,* 2020). Nevertheless, the current study's findings showed that during the process of abnormal delivery there was an increased stress levels at the cellular level which leads to tissue damage and further aggravated by bacterial contamination which favorably influenced the development of a persistent inflammatory state and the upregulation of pro-inflammatory mediators and heat shock proteins. The cells that are inflamed may react by producing anti-inflammatory mediators in an effort to reduce inflammation. Therefore, heat shock proteins, pro-inflammatory mediators and anti-inflammatory mediators were used to evaluate the prognosis of the condition.

Conclusion

The present study provides significant insights in to the molecular alterations associated with fetal dystocia in buffaloes, with upregulation of TNFα, IL-10, HSP70 and HSP90 mRNA expression highlights a pronounced cellular response to stress and inflammation during assisted parturition, thereby underlining the complexity of physiological and immunological responses during dystocia and emphasizing the importance of these markers in evaluating stress and inflammation.

Acknowledgements: The authors are highly grateful to Sri Venkateswara Veterinary University, Andhra Pradesh for providing facilities for research.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1) Aggarwal A, Chandra G, Singh AK, 2012. Heat shock protein 70, oxidative stress, and antioxidant status in periparturient crossbred cows supplemented with α-tocopherol acetate. Tropical Animal Health and Production 45: 239-245.

2) Biradar S.R., Komatwar S.J., Dongre V.B., Jadhav P.V., Bhosale A.V., Vaidya M.M. 2024. Molecular characterization of heat shock protein 70 gene in Deoni cattle (Bos indicus). Journal of Livestock Science 15: 203-207 doi. 10.33259/JLivestSci.2024.203-207

3) Catalani E, Amadori M, Vitali A, Bernabucci U, Nardone A, Lacetera N, 2010. The Hsp72 response in peri-parturient dairy cows: relationships with metabolic and immunological parameters. Cell Stress and Chaperones 15: 781-790.

4) Charitha BVSB, Prasad BC, Praveen KS, Kamalakar G, Prasad CS, 2023. Haemato-biochemical profile in graded Murrah buffaloes with fetal dystocia and eutocia. International Journal of Veterinary Sciences and Animal Husbandry 8: 481-484.

5) Entrican G, 2002. New technologies for studying immune regulation in ruminants. Veterinary Immunology and Immunopathology 87: 485-490.
6) Gomaa NA, Darwish SA, Aly MA, 2021.Immuno-metabolic response in Egyptian water buffalo cows during the transition period. Veterinary World 14: 2678-2685.
7) Goto, Shinya, Osamu Mikami, Yuya Nagasawa Atsushi Watanabe, 2024. Bovine neutrophils stimulated with Streptococcus uberis induce neutrophil extracellular traps, and cause cytotoxicity and transcriptional upregulation of inflammatory cytokine genes in bovine mammary epithelial cells. Journal of Veterinary Medical Science 82:2: 141-149.
8) Henderson B, Wilson M, 1996. Cytokine induction by bacteria: beyond lipopolysaccharide. Cytokine 8: 269-282.
9) Kregel KC, 2002. Invited review: heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. Journal of Applied Physiology 92: 2177-2186
10) Manjari R, Yadav M, Ramesh K, Uniyal S, Rastogi SK, Seijan V, Hyder I, 2015. HSP70 as a marker of heat and humidity stress in Tarai buffalo. Tropical Animal Health and Production 47: 111-116.
11) Mette C, Dooleweerdt BC, Stine J, Miki BA, Roenn PM, Henrik LJ, 2010. Evaluation of the systemic acute phase response and endometrial gene expression of serum amyloid A and pro-and anti-inflammatory cytokines in mares with experimentally induced endometritis. Veterinary Immunology and Immunopathology 138: 95-105.
12) Prasad B.C., Srinivas M., Phanikumar L., Rao K.S. 2017. Dystocia due to fetal lipomatous in a murrah buffalo. Journal of Livestock Science 8: 137-138
13) Sabzikar ZN, Mohri M, Seifi HA, 2023. Variations of some adipokines, pro-inflammatory cytokines, oxidative stress biomarkers, and energy characteristics during the transition period in dairy cows. Veterinary Research Forum 14: 87-95.
14) Sheikh AA, Aggarwal A, Aarif O, 2016. Effect of in vitro zinc supplementation on HSPs expression and Interleukin 10 production in heat treated peripheral blood mononuclear cells of transition Sahiwal and Karan Fries cows. Journal of Thermal Biology 56: 68-76.
15) Snedecor G M and Cochran W C 1994 Statistical Methods. 9th edition Oxford and IBM Publishing Company. Mumbai, India. pp: 124 165.
16) Stanilov N, Velikova T, Stanilova S, 2024. Navigating the Cytokine Seas: Targeting Cytokine Signaling Pathways in Cancer Therapy. International Journal of Molecular Sciences 25:1009.
17) Swelum AAA, Hashem NM, Abo-Ahmed AI,Abd El-Hack ME, Abdo M, 2020.The role of heat shock proteins in reproductive functions. In Heat shock proteins in inflammatory diseases. Cham: Springer International Publishing 407-427.
18) Van Engelen E, De Groot MW, Breeveld-Dwarkasing VNA, Everts ME, Van Der Weyden GC, Taverne MAM, Rutten VPMG, 2009. Cervical ripening and parturition in cows are driven by a cascade of pro-inflammatory cytokines. Reproduction in Domestic Animals 44: 834-841.
19) Wakayo BU, 2014. Study on endometrial cytokine expression, uterine health and reproductive performance in postpartum dairy cows and buffalo. Ph.D thesis submitted to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.www.http://gadvasu.in
20) Wani AA, Mavi PS, Rafee MA, Brar PS, Gandotra VK, 2018. Haemato-biochemical and inflammatory markers of fetotomy operated buffaloes. Exploratory Animal and Medical Research 8: 85-89
21) Yaniz JL, Lopez-Gatius F, Almeria S, Carretero T, García-Ispierto I, Serrano B, Smith RF, Dobson H, Santolaria P, 2009. Dynamics of heat shock protein 70 concentrations in peripheral blood lymphocyte lysates during pregnancy in lactating Holstein-Friesian cows. Theriogenology 72: 1041-1046.