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The effect of inflammatory cytokines on occurrence of retained placenta in cattle

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Abstract

Background: In simple terms, retained placenta is a common issue after parturition in cattle that can affect their reproductive ability. In pregnancies with fetal growth restriction (FGR), the placenta has low anti-inflammatory cytokines and high pro-inflammatory cytokines. By looking at variations in cytokine levels in the blood, we can diagnose the condition. The focus of a recent study was to examine the role of some interleukins (Interleukins -10, Interleukins -6, Interleukins -1β), C-reactive protein (CRP) and Tumor Necrosis Factor (TNF)-α, in retained placenta occurrence in cattle.

Methods: The study involved 40 cows, aged 3-8 years in the Salah-Din province. Each animal suffered from retained placenta also eleven healthy cows served as the control group in a period of January to August 2022. The blood serum was analyzed using enzyme immunoassay techniques (ELIZA) to measure the levels of IL-10, IL-6, IL-1β, CRP and TNF-α.

Results: Serum levels of IL-10, IL-6, IL-1β, CRP and TNF-α showed a significant increase in cows experiencing retained fetal membranes compared to healthy cows (2.31±0.11 vs 1.41 ±0.07) (10.48±0.24 vs 5.40±0.19), (13.6±2.1 vs 4±0.9), (0.9±0.02 vs 0.32±0.04) and (60.1 ± 12.79 vs 29.5 ± 16.58) respectively.

Conclusion: from the present study we conclude that IL10, IL6, IL1β and TNFα play an essential component in retained placenta incidence, and the estimation levels of these parameters in serum may be considered good indicator for occurrence in cattle. RT-PCR revealed increased expression of SDHA genes in the maternal compartment of the placenta.

Introduction

Retained placenta is described as the failure of cows to expel fetal membranes within approximately 24 hours following giving birth. This condition is a significant post-partum disorder in cattle and can result in reduced reproductive performance [1]. The closure of the cervix shortly after birth may contribute to the retention of fetal membranes [2]. In cows suffering from retained placenta the inflammation and oxidative stress increase [3]. Placenta of fetal growth restriction (FGR) pregnancies have an elevate in pro-inflammatory and decreases of anti-inflammatory cytokines [4]. The concentration difference in cytokines in serum, sweat, saliva and stool give good information for diagnosis many problems [5].

Soluble mediator interleukin 6 is generated quickly in response to tissue damage and infection, triggering both acute phase and immunological responses [6]. Type 2 helper cells (Th2), thymocytes, macrophages, B cells, and monocytes create interleukin-10, a vital pleiotropic immunomodulatory cytokine that is locally supplied from resistant tissues to aid alleviate irritation [7,8]. One of the primary cytokines that induce inflammation is interleukin-1 (IL1). There are two components to it. Because of its pro-inflammatory properties, immune cells are drawn to the area and secondary cytokines are produced, which in turn promote acute phase reactions. Type 2 phospholipase A, cyclooxygenase-2, and inducible IL-1R1 are all induced by nitric oxide synthase, which in turn causes inflammatory reactions (iNOS). The IL-1 precursor cannot attach to the receptor and is hence inactive. A cleavage is required for the compound to become its active form [9, 10].

This study aimed to quantify the contribution of IL10, IL6, IL1β, CRP and TNFα to the incidence of retained fetal membrane in cattle.

Methods

This study, which took place between January and August of 2022, 40 local Holstein cross-breed cows in the Salah-Din region, ranging in age from 3 to 8 years, suffered from retained placenta, while 11 animals had normal placenta expulsion served as the control group. When a cow fails to evacuate her fetal membranes more than 12 hours after giving birth, it is said that she has retained placenta [11]. Every animal underwent a clinical examination to determine whether it was suffering from a metabolic disease or a systemic illness [12,13].

Each experiment animal's jugular vein was used to draw five milliliters of blood, which was then separated into serum using a centrifuge and stored at -20 C until analysis. The following were measured using ELISA kits from United States Biological Company: interleukin-10, IL-6, IL-1β, CRP and TNF-α.two genes used (GAPDH and SDHA) were utilized to assess the integrity of RNA and normalize RT-PCR levels. For GAPDH and SDHA, in specific cow primers (Table 1).

Gene	Sequence	Primer Sequence	NCBI reference sequence
GAPDH	Forward	5′-GCGATACTCACTCTTCTACCTTCG A-3′	NM_001034034
	Reverse	5′-TCGTACCAGGAAATGAGCTTGAC-3′	
SDHA	Forward	5′-ATGGAAGGTCTCTGCGCT AT-3′	NM_174178
	Reverse	5′-ATGGACCCGTTCTTCTATGC-3′	

Table 1: Primers used in real-time-PCR.

Statistical analysis

The data was presented as mean ± SD. Data evaluated using a one-way ANOVA were deemed significant using the Student's –test if the A value was ⩽ 0.01.

Results

Figure 1 shows that in RP cows, the mean values of IL10, IL6 and IL1β were considerably (P<0.01) higher compared to healthy cows (2.31±0.11 versus 1.41±0.07 pg per ml), (13.6±2.1 versus 4±0.9 pg per ml) and (10.48±0.24 versus 5.40±0.19 pg per ml), respectively.

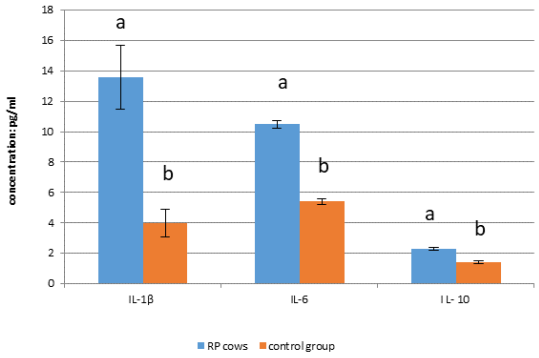

Figure 1: Serum IL10, IL6 and IL1β Level (pg per ml) in Retained fetal membranes group and the control group (mean ± SE). Letter variations between groups indicate significant variations (P ≤ 0. 01).

Figures 2 and 3 show that when RP cows were compared to healthy cows, the mean value of CRP was lower (P ⩽ 0. 01) in RP cows (0.9±0.02 vs. 0.32±0.04 mg/L, respectively), whereas the mean value of TNF-α was higher (P ⩽ 0. 01) in RP cows (60.1 ± 12.79 vs. 29.5 ± 16.58 pg/ml, respectively).

Gene Expression in Retained Placenta in cattle

Semi quantitative RT-PCR could detect mRNA expression of SDHA in both paternal and fetal tissue samples , to assess the expression of SDHA polarization markers in mother and fetal cells in the cattle term placenta the level of expression was determined separately in fetal and mother tissues. The results revealed that SDHA expression was considerably higher in both tissues mother than in all other genes (Figure 4).

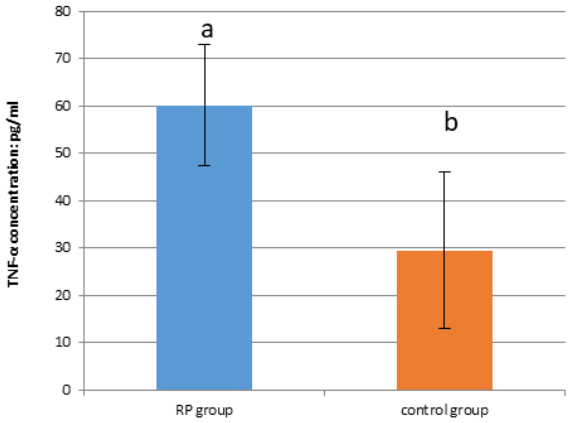

Figure 2: Serum TNFα Level (pg per ml) in Retained fetal membranes group and the control group (mean±SE). Letter variations between groups indicate significant variations (P ≤ 0.01).

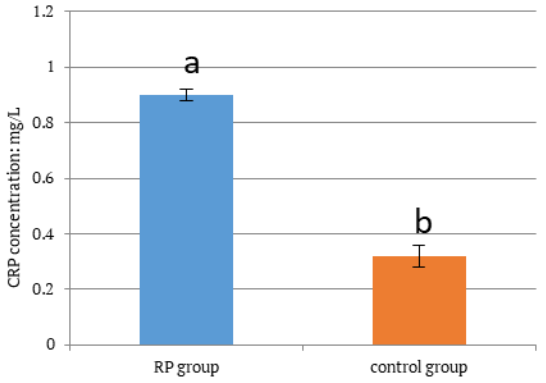

Figure 3: Serum CRP levels (pg per ml) in Retained fetal membranes group and the control group (mean±SE). Letter variations between groups indicate significant variations (P ≤ 0.01).

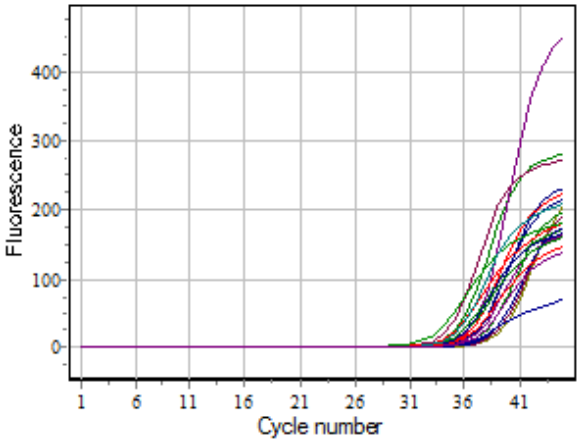

Figure 4: Dependence of FAM channel fluorescence on cycle number.

Discussion

Our investigation found that cows with RP had noticeably higher levels of IL-10, IL-6 and IL-1β than did healthy cows. These differences could be the result of an inflammatory insult that occurred in the mammary gland eight weeks prior to birth. Comparing the results to those of other studies, Trevisi et al. [14] and Sheldon et al. [15] found that elevated levels of inflammatory markers or cytokines in cows during the dry period were linked to inflammatory events or a high incidence of mastitis in the vicinity of parturition. Islam et al. [16] revealed that the IL-10 concentration can be used to identify cows that are prone to reproductive illnesses that may arise after calving, and they proposed that the IL-1 level decline during the two weeks prior to or at parturition can be utilized to do so. According to Ishikawa et al. [17], some retained fetal membranes were linked to higher levels of interleukin 6 prior to calving as opposed to later. C-reactive protein is a pentraxin family plasma protein that is produced by hepatocytes. Its expression is controlled by inflammatory cytokines produced by adipocytes, such as interleukin 6 (IL-6), and it serves primarily as an anti-inflammatory and pro-inflammatory molecule [18, 19]. By attaching phospholipids, phosphocholine, chromatin, fibronectin, and histone, it plays a crucial role in the identification and elimination of infections and damaged cells [20, 21].

In the present study, retained placental cows' CRP levels were considerably lower than those of normal cows. Acute phase proteins, primarily CRP, are more concentrated in retained fetal membranes when mastitis and endometritis are present. This is because lymphocytes and macrophages in retained fetal membranes secrete more IL-6, which causes CRP to bind to the surface of dying cells and make it easier for macrophages to phagocytose these cells, reducing the risk of infection and inflammation in the placental tissue [22, 23]. Activated macrophages, natural killer cells, and T lymphocytes create tumor necrosis factor-alpha (TNF-α), a pleiotropic cytokine that regulates inflammation and is elevated in serum and placental tissue [24]. Several cancers are susceptible to the growth-inhibitory and cytotoxic actions of tumor necrosis factor-alpha. An essential factor in initiating the host's immune response is the rising levels of tumor necrosis factor-alpha in the serum of cows with retained fetal membranes, which triggers the release of interleukins 1 and 6 [25, 26].

The high level of SDHA expression in fetal and Mother tissues shows that the majority of macrophages are M1 type. In cow, it has been shown that during the second half of pregnancy, the proportion of M2 macrophages increases, contributing to the anti-inflammatory milieu that is essential to prevent fetal rejection [27].

Determining the blood concentrations of interleukins IL-10, IL-6, IL-1β, and TNF-α is a useful method for predicting placental retention in cows since these molecules have a significant role in the development of

placental retention. The high expression of SDHA suggests a role for this transcription factor in the overexpression of cytokines.

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Author Contributions

Nawaf Nooraldeen and Manar Sabah conceived the project. Hayder Al-Mutar and Maythem Abdulealah supervised the project and provided guidance. All authors wrote, edited, and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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