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Maternal plasma Interleukin-6 and early conception failure in dairy cows

Maryam Hemmatzadeh-Dastgerdi[1], Farid Barati[2*], Azar Baradaran[3], Abdonaser Mohebbi[2] and Mohammad Barati[4]

[1] DVSc Student of Theriogenology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
[2] Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
[3] Professor, Department of Pathology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[4] Assistant Professor Department of Applied Chemistry, Faculty of Chemistry, University of Kashan, Kashan, Iran

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Abstract

Pro-inflammatory cytokines may contribute to physiological, e.g., tissue reconstruction and vascularization, or pathological, e.g., inflammation or some metabolic disturbances, processes during pregnancy establishment. The current study aimed to investigate IL-6 plasma levels at 30 and 45 days post-insemination (dpi) in pregnant and non-pregnant dairy cows. The cows were examined for pregnancy diagnosis at 30 and 45 dpi. Cows (n=71) were assigned into four groups: Non-pregnant at 30 dpi (n=16), pregnant at 30 dpi (n=36), non-pregnant at 45 dpi (n=11), and pregnant at 45 dpi (n=8). Blood samples were collected at 30 and 45 dpi for IL-6 assay. Results showed high levels of plasma IL-6 at 30 dpi either cow-conceived or not. Cows with healthy embryos at 45 dpi had lower concentrations of IL-6 than cows with late embryonic death. The plasma IL-6 concentrations showed different patterns of changes in dairy cows during the first two months of pregnancy.

Key words: Pregnancy loss, Embryonic death, IL-6, Cows

Introduction

Interleukin-6 (IL-6) is a cytokine with multitude functions of pro-inflammatory and anti-inflammatory. It is an indicator of systemic inflammatory response syndrome. Endotoxins, viral and bacterial infections, double-strand DNAs, epidermal growth factor, platelet-derived growth factor, and tumor necrosis factor can stimulate different types of cells, e.g., fibroblasts, immune cells, endothelial and some neural cells to produce IL-6 (Srinivasan et al., 2017). Some human reproductive pathologies such as preterm birth, chorioamnionitis, and fetal and neonatal inflammations elevate the levels of IL-6 (Markert et al., 2011; Prins et al., 2012). General infections such as mastitis (Campos et al., 2018) and some specific bovine abortificients e.g., bovine viral diarrhea virus (Atluru et al., 1990) can increase the bovine serum IL-6.

IL-6 plays a role in mediating early embryonic development (Jauniaux et al., 1996). The expression of the IL-6 gene in the endometrium was lower in heifers with more retarded embryos compared to heifers

* **Corresponding Author**: Farid Barati, Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
E-mail: fabrtir@yahoo.com

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with healthy embryos (Beltman et al., 2013). Endometrium and embryonic trophoblast also produce IL-6 (Blitek et al., 2012; Ding et al., 2021). The circulatory level of IL-6 in the mother is a biomarker of Th1/Th2 immune status (Aris et al., 2008) and may contribute to fetal brain development (Smith et al., 2007). Implantation is a highly neo-organization process of tissues with extensive angiogenic events (Balakrishnan et al., 2018; Sakurai et al., 2012). Human chorionic villi express IL-6 mRNA during the first trimester of gestation (Bennett et al., 1999) which normally may increase at term in the human placenta (Agarwal, Loganath, Roy, Wong, & Ng, 2000). The aim of the present study was to evaluate the maternal plasma IL-6 at 30 and 45 days post-insemination (dpi) in cows.

Materials and methods

The experiment was carried out at the Zagros Milk and Meat Complex located around Shahrekord city (32°19′32″N 50°51′52″E), Iran, from February to May 2022. Animals were fed a balanced ration based on their physiological state to receive adequate energy, protein, and minerals (NRC). Water was *ad libitum*. The farm had a total of 2537 lactating cows with an average of 39.8 kg daily milk yield, 45.7% of pregnancy rate, and 191 average days in milk. The experimental procedures followed the ethical committee of the university guidelines on animals in research.

All cows were examined for pregnancy diagnosis at 30 and 45 dpi using an ultrasound B-mode transducer (7.5 MHz; V9, Shenzhen empower electronic technology Co., LTD, China). The blood samples were collected from the tail vein at the pregnancy diagnosis test (30-45 dpi) to measure plasma IL-6. Cows were assigned to one of four groups; G1: Non-pregnant at 30 (n=16); G2: pregnant at 30 dpi (n=36); G3: conception loss at 45 dpi (n=11) and G4: established pregnancy at 45 dpi (n=8). Plasma IL-6 was assayed using a bovine-specific ELISA kit (ZellBio GmbH, Germany). The kit was based on biotin double antibody sandwich technology. Intra- and inter-assay coefficient of variation for 10 and 12 replicates were less than 10% and 12%, respectively.

All data were not normally distributed ($p<0.0001$), so the nonparametric one-way Wilcoxon was applied. Comparing the groups was done using Kruskal-Wallis multi-comparison test. Data were expressed as the mean and standard error of means, median, and mode values. Data were analyzed, and the graphs were produced in the statistical analysis system (SAS) 9.1.

Results

Plasma IL-6 concentration (pg/ml) was 63.1±7.82 (median: 51.5 and mode: 16) which was dependent on both the day of examination and the pregnancy outcome ($p=0.0419$). Figure 1 shows lower ($p=0.0144$) scores for IL-6 levels in cows of G1 (38.8±14.82; median: 48; mode: 16) than in G2 (79.9±10.15; median: 64; mode: 32). Cows in G4 (14.4±23.58; median: 8; mode: 8) had lower ($p=0.0138$) score of IL-6 level than cows in G3 (78.3±19.92; median: 72; mode: -). The respective scores of plasma IL-6 were not different between cows in G2 and G3 (Figure 2; $p=0.1275$). However, the respective scores of plasma IL-6 level declined in G4 compared to G1 ($p=0.0019$).

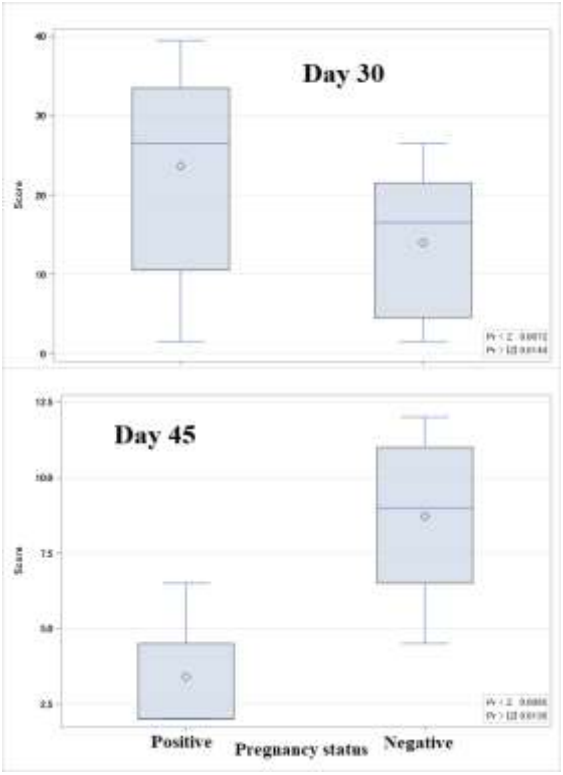

Figure 1. The distribution of Wilcoxon rank score of bovine plasma IL-6 levels in positive and negative cows for pregnancy at 30 and 45 dpi.

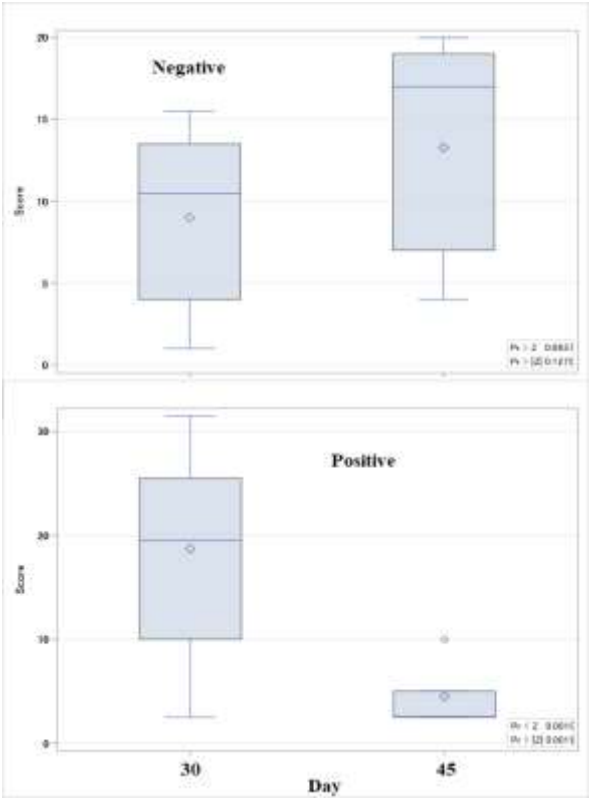

Figure 2. The distribution of Wilcoxon rank score of bovine plasma IL-6 levels at 30 and 45 dpi between positive and negative cows for pregnancy.

Discussion

This study showed different patterns of IL-6 fluctuation among cows with different pregnancy outcomes during the first trimester of pregnancy.

Conception failure either before 30 or between 30 and 45 dpi was associated with high levels of maternal plasma IL-6. High levels of plasma IL-6 were detected in 30 dpi pregnant animals and significantly reduced in 45 dpi pregnant cows. High levels of plasma IL-6 in non-pregnant cows can be due to pathologic factors that can increase maternal plasma levels of IL-6. Bacterial lipopolysaccharides (LPS) are the common source of IL-6 gene stimulation in mothers (Campos et al., 2018). Almeria et al. showed higher expression of IL-6 in the caruncle of *Neospora caninum*-infected cows (Almeria et al., 2011). Endometrial biopsies from cows with endometritis expressed higher levels of IL-6 compared to cows without endometritis (Fagundes et al., 2019). β-endorphins (Cui et al., 2021), cortisol (Cui et al., 2020), and progesterone (Cui et al., 2022) reduce IL-6 expression in LPS-exposed bovine endometrial cells. A role for trophoblast source of IL-6 after exposure to bacterial peptidoglycan through the high expression of TLR-6 was described (Abrahams et al., 2008).

Higher levels of IL-6 can be related to the structural changes in endometrium and trophoblast during implantation and producing fetal membranes (Bednarska-Czerwińska et al., 2015; Goryszewska et al., 2021). The IL-6 gene is expressed in uterine villi during human pregnancy with higher levels in the third trimester (Agarwal, Loganath, Roy, Wong, Lindoff, et al., 2000). High levels of plasma IL-6 in pregnant cows at 30 dpi that were lower in pregnant cows at 45 dpi indicate that the source of maternal plasma IL-6 differs from the non-pregnant. Seekford et al. (2021) showed that supplemented medium with bovine recombinant IL-6 improves post-transfer pregnancy outcomes in the IVP-transferred bovine embryos. The essential

role of IL-6 in the implantation process during porcine early gestation (Blitek et al., 2012) and its stimulatory effect on human chorionic gonadotropin production were confirmed previously (Neki et al., 1993). In vitro exposure of trophoblast to IL-6 activates endothelial cells (Chen et al., 2010), which may have contributed to extracellular matrix remodeling and early placental angiogenesis (Chang & Vivian Yang, 2013; Singh et al., 2012), and invasion and migration of trophoblast (Ding et al., 2021). The results of the present study showed different plasma levels of IL-6 in cows at 30 and 45 dpi either cow was pregnant or not.

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Conflict of interest

The authors declare no conflict of interest.

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اینترلوکین-۶ پلاسمای مادری و شکست زودرس آبستنی در گاوهای شیری

مریم همت‌زاده‌دستگردی[۱]، فرید براتی[۲*]، آذر برادران[۳]، عبدالناصر محبی[۲] و محمد براتی[۴]

[۱] دانشجوی دکترای تخصصی مامایی و تولیدمثل دام، دانشکده دامپزشکی، دانشگاه شهرکرد، شهرکرد، ایران
[۲] دانشیار گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهرکرد، شهرکرد، ایران
[۳] استاد، گروه آسیب شناسی، دانشکده پزشکی، دانشگاه علوم پزشکی اصفهان، اصفهان، ایران
[۴] استادیار گروه شیمی کاربردی، دانشکده شیمی، دانشگاه کاشان، کاشان، ایران

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چکیده

سیتوکین‌های پیش التهابی ممکن است در فرآیندهای فیزیولوژیکی، مانند ترمیم بافت و رگ‌زایی، یا پاتولوژیک، مانند التهاب و برخی اختلالات متابولیک، در دوران بارداری نقش داشته باشند. مطالعه حاضر با هدف بررسی سطوح پلاسمایی IL-6 در ۳۰ و ۴۵ روز پس از تلقیح در گاوهای شیری آبستن و غیر آبستن انجام شد. تشخیص آبستنی در روزهای ۳۰ و ۴۵ پس از تلقیح با استفاده از سونوگرافی صورت پذیرفت. گاوها (۷۱ رأس) به چهار گروه تقسیم شدند: غیرآبستن در روز ۳۰ (۱۶ رأس)، آبستن در روز ۳۰ (۳۶ رأس)، غیر آبستن در روز ۴۵ (۱۱ رأس) و آبستن در روز ۴۵ (۸ رأس). نمونه‌های خون در روزهای ۳۰ و ۴۵ پس از تلقیح برای سنجش IL-6 جمع‌آوری شدند. نتایج سطوح بالایی از IL-6 پلاسما را در تمامی گاوهای مورد آزمایش در روز ۳۰ نشان داد. گاوهای با جنین سالم در روز ۴۵، غلظت پلاسمایی کمتری از IL-6 نسبت به گاوهای با مرگ جنینی دیررس داشتند. غلظت IL-6 پلاسمای گاوهای با وضعیت آبستنی مختلف در .

کلمات کلیدی: شکست آبستنی، مرگ و میر اولیه رویان، اینترلوکین-۶

* نویسنده مسئول: فرید براتی، دانشیار، گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهرکرد، شهرکرد، ایران
E-mail: fabrtir@yahoo.com

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