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Short Paper

Changes of acute-phase proteins during different phases of the estrous cycle in Ovsynch-synchronized Holstein cows

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Abstract

Background: Acute-phase proteins (APPs) may be increased due to different stresses during estrus phase in farm animals. **Aims:** Determining changes of APPs at different phases of non-synchronized estrous cycle group (NSEG), and Ovsynch-synchronized estrous cycle group (SEG) in Holstein cows. **Methods:** Twelve non-pregnant clinically and paraclinicaly healthy Holstein cows with a body condition score (BCS) of 2.75 and 70 days in milk were chosen. Two groups including NSEG and SEG were performed. Blood sampling was carried out from NSEG at the time of diestrus, proestrus, and estrus. In SEG, blood sampling was performed on day 7 (at the time of prostaglandin injection, equivalent diestrus), day 9 (at the time of gonadotropin-releasing hormone (GnRH) injection, equivalent proestrus), and day 10 (at the time of insemination, equivalent estrus) of synchronization protocol. Concentrations of haptoglobin (Hp), serum amyloid A (SAA), ceruloplasmin (Cp), and fibrinogen (Fib) were measured. **Results:** Concentration of Hp at estrus phase was significantly higher compared with diestrus (P=0.001) and proestrus (P=0.019) in NSEG. Moreover, Hp level in the NSEG was significantly higher than SEG at estrus phase (P=0.002). Concentrations of SAA, Cp, and Fib had no significant differences during various phases of estrous cycle in each group or between equivalent phases of both groups. **Conclusion:** It seems that unlike SAA, Fib, and Cp, concentrations of Hp may be affected by different phases of estrous cycle. Although APPs are not specific indicators, their changes besides other clinical and paraclinical indices may be helpful for more accurate heat detection in dairy cows.

Key words: Ceruloplasmin, Dairy cows, Fibrinogen, Haptoglobin, Serum amyloid A

Introduction

Due to genetic selection, improvement of nutrition and milk production of dairy cows, physiological behaviors are changed. Estrus detection is one of the most important problems in the dairy cow industry (Noakes *et al*., 2019). In order to eliminate this problem, timed artificial insemination protocols such as Ovsynch (Pursley *et al*., 1995) have been introduced.

Assessment of acute-phase proteins (APPs) can be considered for detection of stress (environmental, physiological and pathological) (Murata *et al*., 2004). Study of changes in APPs in different stages of the estrous cycle (proestrus, estrus, and diestrus) might be helpful to diagnose estrus phase (Krakowski and Zdzisinska, 2007; Ceciliani *et al*., 2012). According to our knowledge, no published articles were found about the evaluation of APPs in non-synchronized and synchronized dairy cows. So, this study aimed to determine changes of APPs at different phases of non-synchronized estrous cycle group (NSEG) and Ovsynch-synchronized estrous cycle group (SEG) in Holstein cows.

Materials and Methods

The trial was approved by members of the State Commission on Animal Ethics, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran (approval ID number: IR.UK.VETMED. REC.1398.034). The authors confirm that they have followed the recommendations of the European Council Directive (2010/63/EU) of September 22, 2010 for animals welfare during scientific purposes.

Twelve non-pregnant Holstein cows with body condition scores (BCS) higher than 2.75 were enrolled in the study. All cows were at the same lactation status (after 70 days in milk) to minimize negative energy balance (NEB) effects. The cows were kept in the same management conditions. All animals were fed with a similar total mixed ration (TMR) diet according to maintenance requirements including: alfalfa, wheat straw, corn silage, barley and corn grains, soybean meal, wheat bran, salt, and mineral supplements. The animals were evaluated by clinical examination as well as paraclinical (including hematobiochemical and faecal parasitologic evaluation) tests before each sampling time and healthy cows were included during this study.

The cows were categorized into two groups (n=6): NSEG and SEG. In the SEG, the estrus was synchronized by the Ovsynch protocol (Pursley *et al.*, 1995). During this study, diestrus phase was confirmed by detection of a corpus luteum (CL) on ovaries by B-mode ultrasonographic (V8, EMP, China) examination. Small regressing CL or absence of simultaneous large follicles was ovarian evidence of proestrus and estrus. In NSEG, sampling was carried out at diestrus (8 to 10 days after the previous estrus phase), proestrus and estrus (simultaneously by standing heat) phases. In SEG, sampling was performed at day 7 (at the time of prostaglandin $F_{2\alpha}$ injection, equivalent diestrus), day 9 (at the time of gonadotropin-releasing hormone (GnRH) injection, equivalent proestrus) and day 10 (at the time of insemination, equivalent estrus) of synchronization protocol. Blood samples were collected from coccygeal vein by venipuncture syringes after aseptic skin preparation.

Serums were separated by centrifuging blood samples at 3000 g for 10 min. Measurement of haptoglobin (Hp) and serum amyloid A (SAA) was conducted by quantitative sandwich enzyme immunoassay method according to manufacturer's instructions (Bioassay Technology Laboratory, China). The analytical sensitivity of this test in serum is determined as 1.06 μg/ml for Hp and 0.054 μg/ml for SAA by the manufacturer. Also, the assay range of this test in serum was defined as 2-600 μg/ml for Hp and 0.1-40 μg/ml for SAA according to manufacturer instructions. The intra- and inter-assay precision of both kits were coefficient of variation (CV) < 8% and CV < 10%, respectively. Ceruloplasmin (Cp) activity was measured according to its p-phenylenediamine oxidase activity (Sunderman and Nomoto, 1970). Whole blood samples, containing anticoagulant (EDTA), were utilized to determine plasma fibrinogen (Fib) value by heat precipitation-refractory method (Thrall, 2012).

Statistical analysis

Statistical analysis was calculated by SPSS software version 23 (SPSS for Windows, SPSS Inc., Chicago, Illinois). The normality of data was evaluated by Kolmogorov-Smirnov test before statistical assessment. Evaluation of APPs means in equivalent phases of estrous cycle between two groups was determined using two independent t-tests. Repeated measure ANOVA was applied to compare quantitative data at each group's specific sampling times. P-value under 0.05 was considered as significant.

Results

Changes in APPs are shown in Table 1. Serum concentration of Hp at estrus phase was significantly higher compared with diestrus (P=0.001) and proestrus (P=0.019) in NSEG. Also, Hp level in the NSEG was significantly higher than SEG at estrus phase (P=0.002). Concentrations of SAA, Cp and Fib had no significant differences during various phases of estrous cycle in each group or between equivalent phases of both groups (Table 1).

Discussion

Levels of APPs are affected by a wide range of pathological (such as infectious and noninfectious diseases), physiological (such as nutritional status, age, pregnancy, and lactation) and environmental conditions (Nazifi *et al.*, 2012). During estrus phase, by increasing LH pulses, ovarian follicles are developed and produce estrogens which leads to estrus behaviors in animals (Noakes *et al.*, 2019). In estrus phase, cows have more walking and mounting resulting in the occurrence of physical damages, which may increase APPs.

The normal range of Hp was less than 0.1 g/L (Ceciliani *et al.*, 2012). Serum concentrations of Hp at estrus phase of NSEG were higher than other sampling times (Table 1). Although no certain causes were found for the elevation of Hp (unlike other APPs) at estrus phase in NSEG, it may be related to the following reasons. Measurement of haptoglobin is mostly produced by the liver (Murata *et al.*, 2004). Furthermore, uterine tubes and endometrium could produce Hp (unlike SAA)

Table 1: Concentrations (mean±SD) of APPs at various phases of NSEG and SEG in Holstein cows

Acute phase proteins	Groups	Sampling times		
		Luteal phase	Follicular phase	
		Diestrus	Proestrus	Estrus
Haptoglobin (g/L)	NSEG	0.05 ± 0.01^{a}	0.09 ± 0.04^{a}	$0.17 \pm 0.03^{b*}$
	SEG	0.07 ± 0.01	0.06 ± 0.02	$0.07 \pm 0.04^{*}$
Serum amyloid A (g/L)	NSEG	1.54 ± 0.25	1.52 ± 0.18	1.45 ± 0.24
	SEG	1.25 ± 0.23	1.30 ± 0.23	1.44 ± 0.2
Ceruloplasmin (g/L)	NSEG	0.30 ± 0.11	0.26 ± 0.04	0.27 ± 0.09
	SEG	0.29 ± 0.08	0.27 ± 0.07	0.28 ± 0.08
Fibrinogen (g/L)	NSEG	6.58 ± 1.55	6.3 ± 0.92	6.66 ± 1.21
	SEG	6.80 ± 1.00	6.9 ± 0.92	7.19 ± 1.23

* Indicate the significant difference (P<0.05) between natural and induced estrus groups, and [a, b] Show the significant differences (P<0.05) in every natural or induced estrus phases. APPs: Acute phase proteins, NSEG: Non-synchronized estrous cycle group, and SEG: Synchronized estrous cycle group

at the preovulatory phase in cattle (Lavery *et al*., 2003; Krakowski and Zdzisinska, 2007). Also, the secretion of Hp (unlike other APPs) is regulated by the ovarian steroid hormones (Lavery *et al*., 2003; Fischer *et al*., 2010). In the present study, elevation of Hp at the estrus phase may be due to higher production from the reproductive tract around the times of ovulation in the cattle. Increased behavioral stresses around ovulation (Krakowski and Zdzisinska, 2007), as well as increased oxidative activity and released free radicals during estrus (Al-Gubory *et al*., 2010; Sciorsci *et al*., 2020), could be other reasons for increased Hp. Haptoglobin level of the NSEG was higher than SEG at estrus phase. Although no certain causes were found, it may be related to the lack of enough time for production and secretion of Hp from the reproductive tract in SEG. The minimum time for production and increased serum of Hp is about 6-12 h (Nazifi *et al*., 2008). During induced estrus by hormones or drugs, as well as in SEG, this time may be insufficient. Moreover, physical activities and subsequent traumatic damages in Ovsynch-synchronized estrus cows may not be sufficient to increase Hp. There were no significant differences in Hp during various stages of the estrous cycle and pregnancy in heifers (Krakowski and Zdzisinska, 2007) and dogs (Ulutas *et al*., 2009). But in Kuru *et al*.'s (2015) study, short term progesterone-releasing intravaginal device (PRID) treatment increased Hp in Holstein heifers due to local inflammation of the vagina (Kuru *et al*., 2015). However, it should be noted that elevation in Hp occurs under various physiological and pathological conditions (El-Deeb and El-Bahr, 2017) including transportation, weaning, dystocia, clinical and subclinical infections (Murata *et al*., 2004; Nazifi *et al*., 2008; Ceciliani *et al*., 2012); further, it is not a specific biomarker related to estrus but may assist heat detection in cows.

In the present study, there were no significant differences in Cp, SAA, and Fib at various phases of the estrous cycle in both groups (Table 1). An increase in Cp levels has been reported on the first day after transportation (Kim *et al*., 2011). Also, PRID treatment accelerated Cp in Holstein heifers (Kuru *et al*., 2015). Moreover, decreased BCS and glucose led to acceleration in Cp concentrations in dairy cows (Trevisi *et al*., 2009) and pregnancy reduces it (Cooke *et al*., 2009; Ceciliani *et al*., 2012). The influence of different phases of the estrous cycle and pregnancy on SAA in dogs has not been reported (Ulutas *et al*., 2009). However, an increase in SAA levels in the induced follicular phase of heifers was shown (Krakowski and Zdzisinska, 2007). Controversial findings may be due to differences in age and animal species, estrus synchronization protocol and lactation status. In addition, factors such as farm management, ration, season and species differences were effective (Ceciliani *et al*., 2012). Fibrinogen values during different phases of the estrous cycle were within normal range (between 2-7 g/L) for this species at this age. This is in accordance with the finding of Constable *et al*. (2017). However, values of Fib in this study were higher than in Ceciliani *et al*.'s (2012) study. Fibrinogen is a moderate APP, and its concentration in livestock is accelerated due to moderate or severe inflammatory process, weaning and transportation (Ceciliani *et al*., 2012).

It seems that, unlike SAA, Fib, and Cp concentrations of Hp may be affected by different phases of estrous cycle. Although APPs are not specific indicators, their changes beside other clinical and paraclinical indices may be helpful for more accurate heat detection in dairy cows. Further studies with more biochemical investigations and larger number of animals are required.

Conflict of interest

The authors declare that they have no conflict of interest.

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