

The effect of short term progesterone-releasing intravaginal device treatment on acute inflammation markers for Holstein heifers

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ABSTRACT

The purpose of this study was to determine the effect of using a progesterone-releasing intravaginal device (PRID) for treatment using the Cosynch-72 protocol combined with short term progesterone, which causes the concentration of acute phase markers in heifers. The material for this study consisted of 200 Holstein heifers which were 14-16 months of age and weighed 360-380 kg. In this protocol, blood was taken from the animals 10 days prior to synchronization, on day 0 (the day the PRID was inserted), on day 5 (the day PRID was removed) and on day 8 (the day of artificial insemination). The values of haptoglobin (Hp), ceruloplasmin (CP), albumin, Fe, total iron-binding capacity (TIBC) and transferrin saturation (TS) were measured colorimetrically from the serum specimens which were taken. It was determined that the concentration of albumin, CP, Fe, TIBC and TS were similar on day -10 and day 0 ($P>0.05$) but different on the other days (5 and 8), and that this change was statistically significant ($P=0.000$). Consequently, it was determined that short term PRID treatment for the aim of synchronization in heifers increased the values of Hp, CP and TIBC, but decreased the concentration of albumin, Fe and TS.

Keywords: Intravaginal device, haptoglobin, ceruloplasmin, albumin, Fe, transferrin saturation

RÉSUMÉ

Effet d'un dispositif intravaginal libérant de la progestérone durant une courte durée sur différents marqueurs d'inflammation aiguë chez les génisses Holstein

Le but de cette étude était de déterminer l'effet de l'utilisation d'un dispositif intravaginal libérant de la progestérone (PRID) dans le cadre d'un protocole combiné de synchronisation (Cosynch-72) sur des marqueurs de la phase aiguë de l'inflammation chez les génisses. Deux cents génisses Holstein de 14-16 mois pesant 360-380 kg ont subi un prélèvement sanguin 10 jours avant la synchronisation, le jour 0 (jour où le PRID a été inséré), le jour 5 (jour où le PRID a été retiré) et le jour 8 (jour de insémination artificielle). Les valeurs de l'haptoglobine (Hp), céruloplasmine (CP), albumine, Fe, la capacité de fixation du fer totale (TIBC) et saturation de la transferrine (TS) ont été mesurées par colorimétrie à partir des échantillons de sérum. Les concentrations d'albumine, CP, Fe, TIBC et TS étaient similaires au jour -10 et au jour 0 ($P>0,05$), mais différentes aux jours 5 et 8 ($P=0,000$), les valeurs de HP, CP et TIBC, étaient augmentées alors que les valeurs albumine, Fe et TS étaient diminuées.

Mots-clés: dispositif intravaginal, progestatif, éponge, haptoglobine, céruloplasmine, transferrine

Introduction

Progesterone can be administered with an ear implant or an intravaginal device (PRID: Progesterone-releasing intravaginal device or CIDR: Controlled internal drug release) for the purpose of estrous synchronization in cows or heifers [14, 24, 30]. Within three days after the end of this treatment, synchronized estruses are prevalent [4]. Studies have reported that the use of progesterone (which can be short term or long term) in synchronization protocols did not change pregnancy rates [12]. In recent years, Cosynch (GnRH/PGF_{2α}/GnRH) protocols combined with short term progesterone treatment have been developed for heifers [6, 21, 24, 26, 27]. It has been observed that PRID or CIDR administered in these kinds of synchronization protocols causes tissue damage, irritation and vaginitis [5, 36, 38]. Also, studies have determined that there were increases in oxidative stress markers after such treatment [1, 23].

It is known that acute phase proteins increase or decrease in animals that are exposed to infection, inflammation, tissue damage or irritation, neoplasia and stress [9, 10]. In ruminants, haptoglobin (Hp), serum amyloid A, C-reactive

protein, fibrinogen, ceruloplasmin (CP), protease inhibitors, albumin and transferrin are often used for diagnostic purposes [11, 34]. Furthermore, it has been ascertained that increases in serum Hp occur with genital tract inflammations (metritis, endometritis, subclinical endometritis and vaginitis) [15, 25, 39]. Hirvonen et al. [18] determined that Hp increased in metritis with systemic clinical symptoms. In that regard, some researchers indicated that cows diagnosed with toxic puerperal metritis had high Hp concentrations and decreased with treatment [33]. In one study, it was determined that the concentration of Fe decreased when inflammation was present [16].

The purpose of this study was to evaluate the use of short term PRID treatment for heifers and determine its effect on serum Hp, CP, albumin, Fe, total iron-binding capacity (TIBC) and transferrin saturation (TS).

Material and methods

This study was conducted after obtaining approval from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK). The material for this study

consisted of 200 Holstein heifers that were clinically healthy, intensively fed, 14-16 months of age and weighed 360-380 kg. This study was conducted from June to September 2014.

Heifers were synchronized with the Cosynch-72 protocol using treatment with short term (5 days) progesterone (PRID, PRID Delta[®], CEVA-DIF, Turkey) like the method employed by Colazo and Ambrose [6]. To synchronize the animals, the vulva was first washed with warm water and dried before the PRID was inserted. The treatments were administered intravaginally after the PRID applicator was washed with a diluted antiseptic [39]. Blood was taken 4 times: 10 days prior to the synchronization protocol, on day 0 (the day the PRID was inserted), on day 5 (the day PRID was removed) and on day 8 (the day of artificial insemination). Blood was taken from the *vena coccygea* into 8.5 ml vacuumed gel tubes (BD Vakutainer[®], Tiplkimsan, Turkey) that did not contain anticoagulant. Blood specimens were taken to the laboratory at most 2 hours after they were taken. After the blood specimens were centrifuged (Hettich Universal 320[®], Hettich, Germany) at 3000 rpm for 10 minutes, the serum specimens were stored at -20°C until the analyses were made.

Concentration of CP measured spectrophotometrically (Epoch[®], Biotek, USA) based on p-phenylenediamine oxidase activity at pH 5.6 and 546 nm as described Richterich and Colombo [7]. The obtained results was calculated by the formula [Ceruloplasmin (mg / dl) = 237 x (A test- A blank)]. Serum Hp concentration was measured by determining the binding capacity for hemoglobin. In this context, between the serum free hemoglobin and Hp-dependent hemoglobin peroxidase activity gives the serum Hp concentration differences. The reagent of methemoglobin used for measurement of hemoglobin binding capacity was prepared in University of Kafkas Faculty of Veterinary Biochemistry Laboratory as described Skinner et al [32]. Measurement of the serum albumin (Albumin[®], DDS, Turkey), Fe (Total Iron[®], DDS, Turkey) and unsaturated iron binding capacity (UIBC, UIBC[®], DDS, Turkey) concentration was done spectrophotometrically (Epoch[®], Biotek, USA) by using the commercial test kit. Total iron binding capacity (TIBC) is obtained by adding the concentration of serum Fe and UIBC. TS concentration was calculated according to the formula (TS (%) = Fe / TIBC x 100). [3, 20].

Statistical evaluation of the results was done using SPSS[®] (SPSS 20, IL, USA) software. The distribution of the groups was assessed by the Shapiro-Wilk test. Groups were compared with the nonparametric tests because of the data is not normal distribution. The statistical differences between groups were evaluated with the Kruskal Wallis-H and Mann Whitney-U tests. In addition, correlations between variables were determined using the Pearson correlation test. Results were reported as mean±SD (standard deviation), min-max and median. In the statistical evaluation, P<0.05 was considered to be statistically significant.

Result

On the day (5th day) the PRID was removed, vaginoscopy revealed that vaginitis had developed in all heifers. Also, a purulent discharge was found on the PRID and between the vulva lips.

Hp concentration did not changed before PRID implantation; it was highest on day of removal and decreased after but remained higher than days 0. The study determined that Hp concentrations were similar (P=0.099) on days -10 and 0, but there was a statistically significant difference for these days in comparison to days 5 and 8 (P=0.000).

CP concentration changed on different days. In the study, CP concentrations were similar on days -10 and 0 (P=0.14), but these days was statistically significant for days 5 and 8 (P=0.000).

In measurements which were done on different days, it was determined that albumin concentrations on days -10 and 0 (P=0.1) were similar to those on days 5 and 8 (P=0.241). Furthermore, the difference in serum albumin concentration was statistically significant on days 5 and 8 when compared to days -10 and 0 (P=0.000).

It was determined from serum specimens taken on the days of the synchronization protocol that Fe concentrations changed with PRID treatment. The measurements revealed that serum Fe concentrations were statistically similar on days -10 and 0 (P=0.08) and on days 5 and 8 (P=0.074). However, there was a statistically significant difference in serum Fe concentrations on days -10 and 0 when compared to days 5 and 8 (P=0.000).

The measurements revealed that serum TIBC changed with PRID treatment (P=0.000). It was found that the TIBC concentration increased on the PRID removal (the 5th day), and the difference in this value was statistically significant when compared to the other days (days -10 and 5).

Based on the serum specimens taken in this study, it was determined that TS changed with PRID treatment. It was found that the TS concentration of the serum specimens taken before starting the synchronization protocols (on day -10 and 0) decreased, and there was a statistically significant difference in TS concentration on day 5 and 8 when compared to the other days (P=0.000).

Table I shows the average values of Hp, CP, albumin, Fe, TIBC and TS concentration, which were obtained from measurements of serum specimens taken on days -10, 0, 5 and 8.

Discussion

Haptoglobin is a positive acute phase protein which can be used for diagnostic purposes in cattle [34]. Skinner et al. [32] determined that Hp increases with cases of acute septic metritis, retention secundinarum and chronic endometritis that are caused by genital tract inflammations. Walsh et al. [39] report that PRID treatment does not statistically affect the Hp of cows.

Ceruloplasmin is a ferroxidase which contains copper and oxidizes toxic Fe ions in blood to non-toxic Fe ions [19]. One study determined that CP decreases with involution after birth, that total bacterial load increases and that it tends to increase in cases of inflammation in cows [31]. Regassa and Noakes [9] found that in sheep, postnatal CP concentration does not significantly change due to bacterial contamination of the uterus. However, they stated that bacterial contamination or an inflammation of the genital tract can cause an increase in CP. In our study, it was observed that a purulent discharge was released at the time of PRID removal and that the vagina was inflammatory during the vaginoscopic examination. Biochemical measurements revealed that the CP concentration increased with PRID treatment ($P=0.000$). It is thought that the increase in CP concentration may be caused by inflammation (vaginitis) and stress in the vagina due to PRID treatment.

Albumin is a negative acute phase protein which can be measured in the serum and urine [34]. Research on

cattle has shown that albumin concentrations reported in inflammatory cases or diseases have been contradictory [15, 17, 35]. Studies have found no statistically significant change in serum albumin for subclinical or clinical endometritis [15] and the peripartum period [35]. However, albumin concentrations have been observed above the reference interval after cesarean section or experimental laparotomy operations [17]. Conner et al. [8] reported that in their study, serum albumin decreased after inflammation that happened as a result of a turbentin injection. In our study, we found that after PRID treatment, there was a statistically significant decrease ($P=0.000$) in albumin values which had been around the reference interval. It is thought that this was due to PRID treatment causing tissue damage in the vagina and because catabolism increases due to inflammation.

Many studies have determined that Fe concentrations can be used to detect cases of inflammation. In cases of local or systemic inflammation, it was determined that serum Fe concentrations tends to decrease [2, 4, 22]. Borges et al. [4] determined that in cases of local inflammation, Fe values decrease even though they are within the reference interval. In our study, we found that Fe concentrations decreased with PRID treatment ($P=0.000$). It is thought that when the values are within the reference interval [28], this decrease may be caused by local inflammation which occurs because of PRID treatment. TIBC can vary between 110 and 350 $\mu\text{g}/\text{dl}$ in cattle [20, 28]. In this study, TIBC that was within the reference interval increased during PRID treatment. We concluded that this was related to the decrease in Fe ($P<0.05$). TS is a

		Day -10	Day 0	Day 5	Day 8	P value
Hp (g/L)	Mean \pm SD	0.09 \pm 0.02 ^a	0.09 \pm 0.03 ^a	0.21 \pm 0.07 ^b	0.17 \pm 0.06 ^c	0.000*
	Min-Max	0.05-0.15	0.04-0.14	0.07-0.37	0.06-0.37	
	Median	0.09	0.09	0.20	0.17	
CP (mg/L)	Mean \pm SD	1.44 \pm 0.37 ^a	1.52 \pm 0.40 ^a	2.91 \pm 0.75 ^b	2.39 \pm 0.71 ^c	0.000*
	Min-Max	0.76-2.48	0.83-2.73	1.42-4.42	1.24-4.26	
	Median	1.43	1.44	2.82	2.25	
Albumin (g/L)	Mean \pm SD	0.35 \pm 0.06 ^a	0.33 \pm 0.06 ^a	0.28 \pm 0.07 ^b	0.30 \pm 0.06 ^b	0.000*
	Min-Max	0.19-0.48	0.21-0.48	0.12-0.42	0.18-0.48	
	Median	0.34	0.33	0.29	0.29	
Fe ($\mu\text{g}/\text{L}$)	Mean \pm SD	12.65 \pm 2.63 ^a	12.15 \pm 2.46 ^a	10.74 \pm 2.40 ^b	11.22 \pm 2.72 ^b	0.000*
	Min-Max	4.73-18.93	7.60-18.93	5.47-16.77	5.48-17.83	
	Median	12.77	12.49	10.60	11.75	
TIBC ($\mu\text{g}/\text{L}$)	Mean \pm SD	29.41 \pm 3.07 ^a	30.17 \pm 3.29 ^a	31.19 \pm 4.16 ^b	30.74 \pm 3.15 ^b	0.000*
	Min-Max	22.72-36.55	20.79-36.55	22.07-38.41	22.76-37.86	
	Median	28.98	29.77	31.22	31.41	
TS (%)	Mean \pm SD	4.61 \pm 0.78 ^a	4.52 \pm 0.87 ^a	3.78 \pm 0.92 ^b	3.91 \pm 0.79 ^b	0.000*
	Min-Max	2.11-6.07	2.68-6.34	1.97-6.41	1.97-6.41	
	Median	4.66	4.46	3.76	3.84	

a:b; $P=0.000$ a:b:c; $P=0.000$ *Between groups

TABLE I: The change in serum Hp, CP, albumin, Fe, TIBC and TS concentration according to the day the blood was taken in 200 heifers in which a PRID was inserted on day 0 and removed on day 5.

ratio of serum Fe and total TIBC, measured as a percentage (%), and it is known that it correlates with Fe [20, 37]. After the measurements were made, we determined that TS values decrease and that they correlate to changes in Fe ($P < 0.01$).

In conclusion, short term PRID treatment for synchronization in heifers causes local inflammation in the vagina, and it raises the values of Hp, CP and TIBC while reducing albumin, Fe and TS concentrations, even though they are all within their reference interval. The relationship between acute phase response (Hp, CP and albumin) and fertility parameters in comprehensive studies will be better understood in heifers.

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