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Investigation of Relationship Between Coagulation Parameters and Embryonic Loss in Embryo Transferred Cows and Heifers*

Öznur ASLAN[1,a], Kutlay GÜRBULAK[2,b], Uğur KARA[3,c], Erkan SAY[3,d], Esra CANOOĞLU[2,e], Murat ABAY[2,f]

[1]Erciyes University, Faculty of Veterinary Medicine, Department of Internal Medicine, Kayseri-TURKEY
[2]Erciyes University, Faculty of Veterinary Medicine Department of Obstetrics and Gynecology, Kayseri- TURKEY
[3]East Mediterranean Agriculture Research Institute, Adana-TURKEY

ORCID Numbers: [a]0000-0001-5479-3737; [b]0000-0002-1176-9881; [c]0000-0002-7977-6826; [d]0000-0003-0131-5912; [e]0000- 0002-7881-9484; [f]0000-0003-2457-1919

Corresponding author: Öznur Aslan; E-mail: oznuratalay@gmail.com
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Abstract: The aim of the study, the relationship between coagulation parameters and embryonic loss in embryo transferred (ET) cows and heifers wereevaluated. The animal material of this study consisted of 19 cows and 19 heifers on farms located in East Mediterranean Agriculture Research Institute in Adana. Blood samples were collected before the ET application from the recipient cows and heifers. Coagulation parameters measured included prothrombin time (PT), activated thromboplastin time (APTT), fibrinogen, thrombin time (TT), anti-thrombin-III (AT3) and D-dimer using by Sysmex® CA-7000 and activated protein C resistance, protein C and protein S using by Sysmex CS-5100. The pregnancy rate was found 5/19 (26.3%) and 5/19 (26.3%) in cows and in heifers, respectively (P>0.05). The embryonic mortality ratio in cows was 60% (3/5) and in heifers it was 40% (2/5). The differences of D-dimer levels between pregnant and non-pregnant animals were significant (P<0.05).The APTT levels between the groups with pregnancy and embryonic loss were significantly different (P<0.05). As a result, it was determined that there is a relationship between plasma D-Dimer levels and embryonic loss in cows that were transferred embryo.To the best of authors' knowledge, this is the first study reporting the relationship between coagulation parameters and embryonic loss in ET cows and heifers.
Keywords: Bovine, coagulation parameters, embryonic loss, embryo transfer

Embriyo Transferi Yapılan İnek ve Düvelerde Koagulasyon Parametreleri ile Embriyonik Kayıp Arasındaki İlişkinin Araştırılması

Öz: Çalışmanın amacı, embriyo transferi (ET) yapılan Holstein ırkı inek ve düvelerde, pıhtılaşma parametreleri ile embriyonik ölüm arasındaki ilişkinin araştırılmasıdır. Çalışmaya, Doğu Akdeniz Tarım Araştırmaları Enstitüsü (Adana) çiftliklerinde bulunan 19 inek ve 19 düve dahil edildi. Alıcı inek ve düvelerden ET yapıldığı gün transfer öncesinde kan örnekleri alındı. Protrombin zamanı (PZ), aktive edilmiş parsiyel tromboplastin zamanı (APTZ), fibrinogen, trombin zamanı (TZ), antitrombin III (AT3) ve D-dimer gibi koagulasyon parametreleri Sysmex® CA- 7000 ve aktive edilmiş protein C rezistansı, protein C ve protein S ise Sysmex CS-5100 kullanılarak ölçüldü. Çalışmada gebelik oranı ineklerde 5/19 (% 26.3) ve düvelerde 5/19 (%26.3) olarak belirlendi. Embriyonik ölüm oranı ineklerde %60 (3/5), ise düvelerde %40 (2/5) olarak belirlendi (P>0.05). Gebe olan ve olmayan hayvanlar arasında D-dimer seviyeleri arasındaki fark önemli bulundu (P<0.05). Gebelik devam eden ve embriyonik kayıp belirlenen gruplar arasında APTT seviyeleri arasındaki fark önemli bulundu (P<0.05). Sonuç olarak, embriyo transferi yapılan sığırlarda plazma D-dimer seviyeleri ile embriyonik kayıp arasında ilişki olduğu belirlendi. Yazarların bilgisine göre sunulan çalışma embriyo transferi yapılan inek ve düvelerde koagulasyon parametrelerinin değerlendirildiği ilk çalışma olması açısından önemlidir.
Anahtar kelimeler: Embriyonik ölüm, embriyo transferi, koagulasyon parametreleri, sığır

Introduction

Embryonic death refers to embryonic losses occurring on the 45th day post breeding, covering the period from fertilization to the completion of differentiation (McNeill et al., 2006). The attributes of embryonic death are in two parts; genetic and environmental (King, 1991). Ergene (2009) ascribed (or listed or mentioned or described) early embryonic mortalities to genetic and chromosomal anomalies, hormonal factors, dietary disorders and heat stress. Embryonic mortalities have recently been identified through ultrasonographic examination (Diskin and Morris,

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2008), and analyses of serum and milk progesterone levels (McNeill et al., 2006; Ergene, 2009).

It has proven burdensome to prevent embryonic losses even in fertile cows within optimum conditions. The losses may occur during the embryonic or fetal period, or sometimes even during- or post-delivery (Alaçam, 1994). Peters (1996) reported that the pregnancy rate may reduce by 50% due to embryonic losses (25-30%), fetal losses (10%) and abortion (5%). Annual calving rate is associated with productivity, and the fertilization rate can be as high as 90% in heifers and dairy cows with high and average milk yields. However, calving rate of cows with high milk yield may be lower (40%) than the cows with average milk yield (55%) (Diskin et al., 2011). The data above indicate that mortality rate in embryonic and fetal period ranges between 35% and 50%. Silke et al. (2002) stated that while some losses occur in the first 8 days post fertilization, total embryonic loss is observed at 70-80% about 8-16 days after insemination; at 10% on days 16-42; and at 5-8% during subsequent days. Research shows that embryonic mortality has a significant impact on production and economics in beef and dairy production systems (Diskin and Morris, 2008; Diskin et al., 2011).

Haemostasis process possesses a dynamic equilibrium between coagulation and fibrinolytic system. Although there are not many studies on hemostasis during pregnancy in cattle, there are many studies on hemostasis in women during pregnancy.During normal pregnancy, it is common to observe that procoagulant effect dominates in haemostasis. The changes in hemostasis during pregnancy are considered being part of the complex physiological harmony, which allow circulation of fetus and mother on uteroplacental surface, and control the placental bleeding in a fast and effective way during the placental dispersion. It is believed that the activation of the coagulation system in uteroplacental circulation prepares this circulation beforehand against abnormal fibrin accumulation. Excessive uteroplacental thrombosis is a characteristic of important clinical complications in pregnant women, and it is best defined in preeclampsia (O'Riordan and Higgins, 2003).

In women, hemorrhagic defects possibly leading to inadequate fibrin formation, associated with fetal wasting syndrome have been reported to prevent adequate implantation of the fertilized ovum into the uterus, and thrombotic defects causing early thrombosis of placental vessels, resulting in fetal waste.The earlier the pregnancy, the smaller the placental and uterine vessels and therefore the greater the tendency for partial or complete occlusion with thrombus formation. Thrombotic occlusion of both venous and arterial placental vessels prevents adequate nutrition and therefore fetal viability (Bick and Hoppensteadt, 2005). In women, to assists in the diagnosis and the identification of patients at risk for the development of DIC, coagulation assays including PT, PTT, fibrinogen and D-dimer or fibrin split products are used (Erez et al., 2014).

Although literature exists in evaluating some of the coagulation parameters in different stages of gestation in pregnant cows, there is a lack of research presenting the coagulation parameters of cows with embryonic losses (Gentry et al., 1991; Heuwieser et al., 1990a; Heuwieser et al., 1990b). Therefore, this study was conducted to investigate the relationship between coagulation parameters and embryonic loss in embryo transferred (ET) Holstein breed cows and heifers.

Materials and Methods

Animal material of this study consisted of 19 ET cows and heifers (totally 38), of which body condition scores were measured. Recipient animals were administrated two intramuscular injection of 500 µg PGF2α cloprostenol (Lutelen®, Topkapı İlaç Premiks San ve Tic AŞ, Türkiye) 11 days apart. Embryos were transferred on the 7th day of the oestrus cycle in the upper 1/3 part of the cornu uterine (ipsilateral) where corpus luteum was present. Before the ET, blood samples collected from all animals into tubes containing sodium citrate were centrifuged for 15 minutes at 1500 x g at room temperature to separate the serum and were then stored at -20°C until the tests were performed.

The blood samples were analysed for Prothrombin time (PT), Activated thromboplastin time (APTT), thrombin time (TT), fibrinogen, D-dimer on the Sysmex® CA-7000 (Siemens Healthcare Diagnostics) and activated protein C resistance (APC), protein C and protein S using by Sysmex® CS-5100 (Siemens Healthcare Diagnostics) at the Central Laboratory of Erciyes University.

The proportional distributions of pregnancy rates were calculated and shown as %. Fisher's Exact test was used to compare the pregnancy rates in heifers and cows.Descriptive statistics of coagulation parameters according to groups were shown with mean and standard error. Coagulation parameters in relation to pregnancy and embryonic losses in pregnant cows were compared using Student T test and Mann Whitney U test, respectively.Shapiro-Wilk-W test to determine the compliance of the data to normal distribution; Levene's test to determine the homogeneous distribution of variances were used.Statistical significance level was accepted as $P<0.05$.Statistical analyses were performed using "Minitab 17 (Minitab, UK)" software package.

The experimental protocol was approved by the Erciyes University Local Ethics Committee for Animal Experiments (meeting number: 02, decision number:

15/34).

Results

The pregnancy rate for ET cows and also in heifers was found 26.3%, reflecting 10 animals (5 cows and 5 heifers) being pregnant. An ultrasound scan of all pregnant animals at day 50 showed 50% embryonic death (3 cows and 2 heifers).

The average body condition score values of animals were found 3.75±0.52, which was not significant in relation to pregnancy (P>0.05). D-dimer levels were relatively higher in pregnant animals than their non-pregnant counterparts (P<0.05) (Table 1). Coagulation parameters except for D-dimer did not differ statistically in pregnant and non-pregnant cows (P>0.05) (Table 2). The differences in APTT parameters between animals with continuing pregnancy and embryonic loss were significant (P<0.05), and low in animals with embryonic mortality (Table 2).

Table 1. Pregnancy rates of ET cows and heifers

		Pregnancy		Total
		Not pregnant	Pregnant	
Cow	Head	14	5	19
	%	73.7	26.3	
Heifer	Head	14	5	19
	%	73.7	26.3	
Total	Head	28	10	38
	%	73.7	26.3	

The difference in pregnancy rate between cows and heifers was not found statistically significant (P>0.05).

Discussion

Results of the study showed that procoagulant effect becomes dominant during normal pregnancy. While these changes in hemostasis regulate the circulation of fetus and mother on maternal-placental surface, they are also considered to control the placental bleeding during placental dispersion in the fastest and most effective way by being part of the complex

Table 2. Coagulation parameters of animals with pregnancy and embryonic loss

	Pregnancy	N	$\bar{X} \pm S_{\bar{X}}$	Statistical significance (Student T test)	Embryonic loss	N	$\bar{X} \pm S_{\bar{X}}$	Statistical significance (Mann Whitney U Test)
PT	negative	28	44.80 ±2.60	T=1.909 P=0.065	negative	5	40.54 ±2.14	P=0.548
	positive	10	38.50 ±2.03		positive	5	36.46 ± 3.46	
APTT	negative	28	40.28 ±0.78	T=0.255 P=0.808	negative	5	43.00 ±1.43	**P=0.016**
	positive	10	39.88 ±1.39		positive	5	36.76 ±1.34	
FIB	negative	28	143.22 ±8.39	T=0.232 P=0.818	negative	5	131.18 ±7.08	P=0.151
	positive	10	139.93 ±4.73		positive	5	148.69 ±3.50	
TT	negative	28	22.08 ±0.57	T=0.643 P=0.524	negative	5	21.76 ±1.72	P=0.841
	positive	10	21.32 ±1.14		positive	5	20.88 ±1.68	
AT3	negative	28	84.60 ±1.65	T=-0.184 P=0.855	negative	5	83.86 ±3.50	P=1.000
	positive	10	85.15 ±1.77		positive	5	86.44 ±1.00	
D-DIMER	negative	28	0.20 ±0.06	T=-2.356 **P=0.024**	negative	5	3.12 ±2.15	P=0.095
	positive	10	1.75 ±1.11		positive	5	0.37 ±0.21	
APC	negative	28	0.48 ±0.01	T=-1.615 P=0.115	negative	5	0.51 ±0.02	P=0.310
	positive	10	0.50 ±0.01		positive	5	0.49 ±0.02	
PC	negative	28	48.48 ±1.53	T=0.440 P=0.663	negative	5	47.42 ±1.94	P=0.690
	positive	10	47.26 ±1.74		positive	5	47.10 ±3.13	
PS	negative	28	50.18 ±2.43	T=0.399 P=0.692	negative	5	47.56 ±6.40	P=0.548
	positive	10	48.31 ±3.85		positive	5	49.06 ±5.05	

PT: Prothrombin time, APTT: Activated thromboplastin time, FIB: Fibrinogen, TT: thrombin time, AT3: Antithrombin III APC: Activated protein C resistance, PC: Protein C, PS: Protein S

physiological harmony. There is a scientific consensus that the activation of coagulation system in the uteroplacental circulation prepares this circulation against excessive fibrin accumulation (O'Riordan and Higgins, 2003). Fibrinogen levels have been reported to increase in cows and dogs during mid-pregnancy and postpartum (Gentry et al., 1991). D-dimer is formed as a consequence of the degradation of cross -linked fibrin clot by plasmin through activation of coagulation system for no apparent reason. It is the sensitive and trustworthy indicator of fibrin accumulation and stability (Shalhub et al., 2014). The elevated levels of D-dimer in the present study in pregnant animals in relation to non-pregnant animals were attributed to the commencement of fibrin accumulation during the ET.Treatment of patients due to insufficient fibrin formation is usually recommended plasma substitution therapy or 1-deamino-8-d-arginine-vasopressin (DDAVP) therapy in appropriate disorders (Bick and Hoppensteadt, 2005), while the treatment of common procoagulant defects has been reported to be a viable alternative, 81 mg / day low-dose aspirin followed by low-dose unfractionated porcine heparin or dalteparin and low molecular weight heparin immediately after conception (Bick and Hoppensteadt, 2005; Simon and Laufer, 2012).

Increased coagulation in systemic circulation clinically indicates venous thromboembolism (O'Riordan and Higgins, 2003). Thrombophilia is described as a genetic disorder during fibrinolytic process, coagulation factors, anticoagulants, and excessive formation of coagulation for several reasons. Thrombophilic defects are known to increase not only the venous thrombus, but also the risks of fetal loss and pregnancy complications (Ivanov et al., 2012).

Venous thromboembolism is a disease caused by genetic, non-congenital and seconder conditions (Kujovich, 2011). Intrinsic (factor XII, XI, IX and VIII) and common (factor V, X, prothrombin, fibrinogen) hemostasis pathways are evaluated using activated partial thromboplastin time test (Radostits et al., 2007). On the other hand, PT (tissue factor, factor VII, and common system; factor V, X, prothrombin, fibrinogen) is used for evaluating the extrinsic system; fibrinogen, TT, the degredation product D-dimer are used for evaluating the clot formation and degradation rate (Noyan, 2012); and finally the APC resistance is used for assessing the natural anticoagulant system (Arnljots and Dahlbäck, 1995), all of which are known as the hemostatic profile tests (Kujovich, 2011; Harvey, 2006; Herring and McMichael 2012; Gökçe and Irmak, 2007). Previous studies found a correlation between the intrinsic factor Factor XI and embryonic loss in cattle, reflecting that a repeat breeder may develop due to a mutation in Factor XI gene (Mukhopadhyaya et al., 2006; Akyüz et al., 2012). The change in the APTT in this study in animals with continuing pregnancy and embryonic mortality may be due to an alteration of factors forming intrinsic hemostasis in animals with embryonic loss.

To the best of the authors' knowledge, this is the first study that presented and evaluated the coagulation parameters of ET cows and heifers. Further studies are needed with higher numbers of animals to a detailed evaluation. Since the D-dimer test used in the determination of fibrinolytic activity was found to be high in pregnancy, plasma substitution treatment or DDAVP treatment may be recommended to low-level animals by following the D-Dimer levels during embryo transfer in cattle.

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