

RELATIONSHIP BETWEEN SERUM PROGESTERONE AND ESTRADIOL-17_β AND EMBRYONIC LOSS IN FRISIAN COWS UNDER EGYPTIAN CONDITION

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ABSTRACT

Association between circulating progesterone and oestradiol-17_β concentrations and embryonic loss in Frisian cows (n=105) under Egyptian condition was investigated. Total embryonic losses in present study were 26.4%. On d25, progesterone (P₄) concentrations and sonography revealed 79.3% cows were continuing gestation and 20.7% lost their pregnancies (early embryonic loss, EEL). Furthermore, 7.4% cows with late embryonic loss (LEL) were detected between d25-42. An increase in P₄ levels started from day 4 to 7 in cows diagnosed pregnant compared to low P₄ in cows that lost pregnancy (p<0.05). Cows with LEL had decrease in P₄ from d28. Estradiol (E₂) concentrations increased (p<0.05) in cows with EEL starting from d4 to 21 after AI compared to cows diagnosed pregnant. Starting from d4 to 40 after AI, P₄/E₂ ratio was higher (p<0.05) in cows diagnosed pregnant compared to cows exhibiting EEL loss, while cows with LEL had highest P₄/E₂ ratio till d25 post-AI and declined from d28 to 40.

Keywords: Embryonic loss, Friesian cows, Estradiol-17_β, Pregnancy, Progesterone

INTRODUCTION

There is no practical way to reduce the early pregnancy loss in dairy cattle, however the management strategies may be helpful in increasing the calving rate. Prior to the development of ultrasonography for pregnancy diagnosis, it was difficult to determine the viability of fetuses at an early stage of gestation (Chaudhary and Purohit, 2012). Moreover, an estimation of early pregnancy loss by progesterone (milk or blood) can not be detected with certainty. The aim of present study was to assess the embryonic loss with the help of transrectal ultrasonography and serum progesterone (P₄) and estradiol (E₂) determination in Frisian cows under Egyptian condition.

MATERIALS AND METHODS

Friesian cows (n=105; age, 30-96 mo.; b. wt., 440-660 kg) were used in this study which was carried out between Oct, 2012 to July, 2013. The tagged experimental animals were fed on concentrate feed mixture, maize silage and rice straw in summer. While, in winter, animals were fed concentrate feed mixture,

fresh berseem and rice straw according to their body weight and milk production. All heifers were free from any disease with healthy appearance and were housed in separated two groups under semi-open sheds, partially roofed by asbestos.

Estrus was visually detected two times daily between 6 a.m. and 7 p.m. using a teaser bull. Cattle detected in estrus in morning were recorded and artificially inseminated (AI) in the afternoon of same day and cattle detected in estrus in the afternoon were inseminated next morning. Pregnancy was diagnosed by serum progesterone on d4, 7, 11, 13 and 22 post AI, as well as using ultrasonography starting at d22 and d45 post AI.

Blood samples were collected for the determination of progesterone (P₄) and Estradiol-17_β (E₂) in blood serum. Within an hour after collection, samples were centrifuged for 15 min at 3000 rpm for serum separation. Serum samples were stored at -20 °C till the hormonal assay.

A direct radioimmunoassay technique was performed for determination of serum P₄ using

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ready antibody coated tubes kit (Diagnosis Systems Laboratories, Texas, USA). The standard curve of P_4 ranged from 0.0 to 40.0 ng/ml. The sensitivity value was reported as 0.12 ng/ml. The intra and inter-assay coefficients of variation were 8 and 13.1%. Serum E_2 was determined by radioimmunoassay procedure (Sirois and Fortune, 1990). Intra-assay coefficient of variation for E_2 was 4%. Inter-assay coefficient of variation was 10.9%. The detection limit of assay was 2.9 pmol/l.

For diagnosing pregnancy and embryo loss of mated animals, ultrasonographic examinations were performed using a B-mode ultrasound scanner (Falco, Easote/Piomedical, Maastricht, 6-8 MHZ Linear array transducers, Alliance Medical Int.) on day 25 and 42 post AI. The animals were considered pregnant on the basis of presence of anechoic fluid with visualization of embryo and heart beat in either of uterine horns. Early pregnancy loss was confirmed by non-visibility of embryo/fetal heart beat or non-visibility of embryo or the absence of positive signs of pregnancy in an animal previously diagnosed pregnant or the presence of signs of embryo/ fetal degeneration.

The results were statistically analyzed using completely randomized design for the data of diagnostic and therapeutic studies. The significant differences among treatment groups were tested using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

From 105 Friesian cows, 70.8% cows were pregnant on day 25 by both serum P_4 and sonography.

Out of these, 79.3% continued their gestation and 20.7% lost their pregnancies by d25 (Table 1). Late embryonic loss was detected in 7.4% from d25-42, thus the total embryonic losses were 26.4% (Table 1). This estimate of early embryonic loss was in close agreement with an earlier study exhibiting 22.8% (Chaudhary and Purohit, 2012).

An increase in serum P_4 started from d4 to d7 in cows diagnosed pregnant ($p < 0.05$) compared to low P_4 in cows that lost pregnancy (Table 2). In addition, serum P_4 was higher ($p < 0.05$) from d4 to d40 post-AI in pregnant cows compared to cows that lost their embryos. A reduced capacity P_4 secretion can explain around 5% embryonic mortalities in buffalo synchronized and mated by AI during a period of low reproductive activity (Campanile *et al.*, 2005). Cows with late embryonic loss exhibited increasing serum P_4 till d25, thereafter, P_4 started to decrease from d28 onward (Table 2). A significant negative correlation was reported between serum P_4 and late embryonic loss as P_4 is responsible for maintaining the pregnancy (Gabor *et al.*, 2008).

Serum E_2 increased ($p < 0.05$) in cows with early embryonic loss starting from d4 to 21 post-AI compared to cows diagnosed pregnant (Table 3). Estrogen secretion from a large follicle from d14 to 17 of pregnancy may negatively affect embryo survival (Inskeep, 2004). In addition, cows pregnant at day 28 had lower E_2 ($p < 0.05$) compared to cows with late embryonic loss (Table 2).

The present results showed that P_4/E_2 ratio was

Table 1: Early (EEL) and late (LEL) embryonic losses in Friesian cows

Parameter	Total	Pregnant		Pregnancy loss	
	N	N	%	N	%
Conception rate, %	105	87	82.8	-	-
EEL, till d 25	87	69	79.3	18	20.7 ^a
LEL, d 25-42	69	64	92.7	5	7.4 ^b
Total	87	64	73.6	23	26.4

^{a,b} $p < 0.05$

Table 2: Serum progesterone (P₄) and Estradiol-17_β (E₂) concentrations (ng/ml) during post-mating period in Friesian cows. EEL, Early embryonic loss; LEL, Late embryonic loss

Day	Pregnant		EEL		LEL	
	P ₄	E ₂	P ₄	E ₂	P ₄	E ₂
0	0.43±0.05 ^b	16.36±1.0	0.60±0.04 ^a	15.79±0.69	0.49±0.06 ^{ab}	15.65±1.02
4	8.03±0.75 ^a	4.28±1.57 ^b	4.40±1.17 ^b	7.67±1.10 ^a	7.57±0.65 ^a	3.66±1.62 ^b
7	8.98±0.99 ^a	3.68±0.68 ^b	4.91±1.17 ^b	8.23±1.52 ^a	8.37±0.87 ^a	3.57±0.84 ^b
11	9.23±1.23 ^a	3.17±0.58 ^b	4.56±1.53 ^b	11.12±1.81 ^a	9.06±1.11 ^a	2.87±1.06 ^b
12	10.11±0.79 ^a	3.16±0.70 ^b	4.61±1.33 ^b	11.36±1.96 ^a	9.27±0.86 ^a	2.96±0.95 ^b
13	11.20±0.87 ^a	3.15±0.53 ^b	6.85±1.55 ^b	8.67±1.35 ^a	9.96±0.92 ^a	3.06±1.27 ^b
21	9.61±0.76 ^a	2.81±0.47 ^b	3.77±0.80 ^b	13.17±2.80 ^a	8.57±0.78 ^a	4.56±1.35 ^b
28	9.09±0.61 ^a	2.83±0.54 ^b	-	-	5.15±1.12 ^b	8.14±1.21 ^a
40	9.56±0.84 ^a	2.14±0.43 ^b	-	-	2.95±0.46 ^b	5.91±0.96 ^a

^{a,b}p<0.05, within a column**Table 3: P₄/E₂ ratio during post-mating period in Friesian cows. EEL, Early embryonic loss; LEL, Late embryonic loss**

Day	Pregnant	EEL	LEL
0	0.03±0.02	0.04±0.003	0.03±0.03
4	1.87±0.15 ^a	0.57±0.23 ^b	2.07±0.20 ^a
7	2.44±0.32 ^a	0.59±0.22 ^b	2.34±0.51 ^a
11	2.92±1.35 ^a	0.41±0.24 ^b	3.17±0.52 ^a
12	3.19±1.21 ^a	0.51±0.34 ^b	3.12±0.45 ^a
13	3.55±0.98 ^a	0.79±0.28 ^b	3.26±0.36 ^a
21	3.42±0.57 ^a	0.28±0.11 ^b	1.88±0.28 ^a
28	3.13±0.57 ^a	-	0.63±0.54 ^b
40	4.46±2.02 ^a	-	0.49±0.31 ^b

^{a,b}p<0.05, within a column

higher ($p < 0.05$) in cows diagnosed pregnant starting from d4 to 40 post-AI compared to cows that lost their embryos between d4 to 25 post-AI (Table 3). The cows with late embryonic loss had highest P₄/E₂ ratio till d25 post-AI and declined on d28 to 40 (Table 3). The high ratio occurs when P₄ is high relative to E₂ and this describes the classic situation of P₄ dominance. An embryo must secrete sufficient amounts of interferon-tau by d16 to prevent the regression of corpus luteum (Vasconcelos *et al.*, 1997). It was also noticed that P₄/E₂ ratio was high on d0 (day of estrus and AI) in cows with early embryonic loss than cows diagnosed

pregnant or cows with late embryonic loss (d25-40, Table 3).

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