

Plasma β -endorphin and cortisol profiles around periparturient period in Friesian Cattle suffering from reproductive disorders in Egypt

Abstract

The present study aimed to determine plasma β -endorphin and cortisol concentrations in cattle with reproductive disorders (dystocia and retained placenta) and weakness body condition score during periparturient period. Stress may cause an overproduction of beta-endorphins and free radicals. Twenty multiparous Friesian cows weighing 400–650 kg aged 6–10 years and between (3–8) parities at late pregnancy period was used for two months before parturition. From ten days before the expected birth date the birth canal of each animal was monitored by rectal and vaginal palpations, and the body temperature was checked daily to predict the probable time of parturition. Animals were divided into four groups according to parturition disorders: five animals in each one. First group delivered spontaneously at term with no obstetrical assistance (A), second group delivery was at term but was accompanied by dystocia (B), third group delivery was at term but was accompanied by retained placenta (C), and fourth group delivery was at term but animals showed weakness body condition score (D). Results illustrated that Plasma β -endorphin level in Friesian cattle during pregnancy state increased gradually through gestation period. It reaches its highest level during the third trimester due to stressful state. Also, it reaches its peak at zero time of parturition by appearing its analgesic action. Meanwhile, it decreases gradually towards re-establishment of ovarian cycle. Generally, cows suffering reproductive disorders had a clear impact on blood plasma β -endorphin concentration around parturition process.

Keywords: cattle, β -endorphin, cortisol, reproductive disorders

Volume 9 Issue 1 - 2020

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Received: December 22, 2019 | **Published:** January 20, 2020

Introduction

The term "periparturient" stems from the word "parturition" and the prefix "peri," which literally but vaguely means "around." This phase generally includes the dry period and the first 3 to 4 weeks after calving. Livestock animals were susceptible for many stressful conditions results from various factors which are responsible for changes in animal's body. Stress can occur in a variety of forms, physical or psychological, acute (lasts for a period of minutes to hours) or chronic (persists for at least several hours per day for weeks or months) and which cause unfavorable consequences Dhabhar & McEwen.¹ β -Endorphin is one of endogenous Opioid peptides hormones. It consists of 31 amino acids with molecular weight about 31000 Dalton.² It derived from Pro-opiomelanocortin (POMC) which is found in Hypothalamus, Pituitary and Placenta mRNA of many living organisms. Scientists suggest that beta-endorphin modulates activity of the hypothalamic-pituitary-adrenal axis. It also can be found in ovary and uterus of mice, monkeys, hamsters,³ rats⁴ and fertile women.⁵

In animals, β -endorphin is known as one of stress hormones. It has been analyzed to evaluate the effects of a stressful condition. Parturition is natural event that involves stress and pain for the mother. Plasma concentrations of β -endorphin have been reported to be elevated in response to a variety of forms of stress including hemorrhage, and hypoglycemia in sheep.⁶ Cortisol concentration

increased when stress has been implicated as one of the predisposing factors in the pathogenesis of infectious diseases in cattle Ettema & Santos.⁷ Furthermore, Akar & Gazioglu⁸ declared that dystocia which means difficult calving have been a big problem in animals industry. Difficult calving causes trauma, uterine infections, and reproductive disorders as retained placenta, metritis, longer calving intervals and reduced health of animals. Studies reveal that beta-Endorphin synthesis occurs in ovarian follicles, but the relatively low concentrations found in reproductive tissues indicate that it exerts autocrine or paracrine effects in the ovary.⁹ Various studies have shown that β -endorphin may affect ovarian function both directly and indirectly, by modulating LH secretion¹⁰ and follicle-stimulating hormone (FSH) secretion by exerting an inhibiting effect on GnRH secretion in humans and experimental animals.¹¹ The ovulation rate and the ovarian production of preovulatory prostaglandins were inhibited by β -endorphin and stimulated by naltrexone.¹² Moreover, Bruckmaier et al.¹³ found that dairy cows respond with elevated circulating levels of β -endorphin when placed in an unfamiliar room for milking.

Materials and methods

This study was conducted at the experimental station of Animal Production Research Institute, EGYPT. Mahalet Mosa farm is located in the North Center of Nile Delta, Kafr El-Sheikh Governorate. Twenty multiparous Friesian cattle weighing 400–620 kg aged from 5–11

years and, between 3-8 parities at late pregnancy period were used for two months before parturition. From 10 days before the expected birth date the birth canal of each animal was monitored by rectal and vaginal palpations, and the body temperature was checked daily to predict the probable time of parturition. All animals were housed in semi open pens then they were transferred to the maternity unit before 1 or 2 days of the expected birth date. Water was offered freely in water troughs. after delivery all cattle were allowed to nurse the newborn calves for only a week as a period of colostrums intake. Then dams transferred to the milking unit they were milked twice daily at 7a.m and 5 p.m. and they were subjected to the regular managerial practices of the breeding stock. Follow-up using ultrasound sonar for ovarian activity after birth to study link functions, the growth of ovarian follicles and first ovulation occurs after birth and its association with occurrence of heat or not. The animals did not receive any hormonal treatment during pregnancy, before as well as after parturition.

Animals were divided into four groups according to parturition disorders' five animals in each one. First group delivered spontaneously at term with no obstetrical assistance(A), second group delivery was at term but was accompanied by dystocia(B), third group delivery was at term but was accompanied by retained placenta(C), and fourth group delivery was at term but animals showed weakness body condition score (D). Blood samples (10 ml) were collected from the jugular vein into clean dried and heparinized evacuated tubes, kept on ice, centrifuged at 3000 revolution per minute for 15-30 minutes to obtain the plasma immediately and stored at -20°C until extraction of β -endorphin and measured by RIA.¹⁴ Also, Cortisol concentrations were quantified by enzyme immunoassay (EIA).¹⁵

Blood samples were collected from pregnant animals by a permanent catheter was inserted under local anesthesia provided with an extension tube that was fixed to the neck with adhesive tape. It was therefore possible to withdraw blood without having to restrain the animal. Because of the large variations in the length of parturition and its occurrence at any time of day or night as follow:

1: a weekly sample started from 8 months till parturition. 2: a sample at 1-2 days before the expected date of parturition. 3: a sample at 0 times of parturition and 3 h after delivery. 4: samples at days 1, 3, 5 of parturition and every 10 days until corpus luteum formation that following first ovulation. The day of first ovulation was estimated by determination of plasma progesterone conc., (≥ 1 ng/ml) and rectal palpation.¹⁶ Statistical analysis was carried out using the general linear model (SAS 2003). Data was analyzed using the following model.

Results and discussion

I: Concentration of β -endorphin and Cortisol in blood plasma in late pregnancy period:

Results in table (1a &b) represented that there was significant difference at ($P \leq 0.05$) between concentrations of β -endorphin in plasma during pregnancy; this difference started from the beginning of two months before parturition. Plasma β -endorphin level in cattle during pregnancy state increased gradually through gestation period. It reaches its highest level during the third trimester due to stressful state. It reached its highest concentration in the case of Retained Placenta (176.16 ± 9.57) and its lowest concentration in the case of weakness in body score condition (143.60 ± 2.99) at the last week of parturition. Meanwhile, there was significant difference at ($P \leq 0.05$) between concentrations of Cortisol in plasma during pregnancy beginning from the seven week before parturition in groups C, B, D and A (26.60 ± 1.89 , 22.60 ± 0.98 , 21.00 ± 1.22 and 22.40 ± 0.60 , respectively) till the beginning of the last week of parturition in groups C, B, D and A (176.16 ± 9.57 , 168.70 ± 4.17 , 148.16 ± 4.91 and 143.60 ± 2.99 , respectively). Cortisol concentrations tended to increase significantly at ($P \leq 0.01$) till the beginning of the last two weeks before parturition then it tended to increase significantly at ($P \leq 0.01$) day zero (the moment of delivery fetus). It reached its highest concentration in the case of Retained Placenta group C (39.80 ± 2.44) and its lowest concentration (20.00 ± 0.71) in the case of group delivered spontaneously at term with no obstetrical assistance group A or control group (Table 1a&1b).

Table 1a Plasma β -endorphin during pregnancy period (3rd trimester) in Friesian cattle

Groups	β endorphin concentration (pg/ml) Number of weeks before parturition (Mean \pm SE) Two months before parturition				One month before parturition Ave. in the pregnancy period			
	8w	7w	6w	5w	4w	3w	2w	1w
Control	121.62 \pm 6.01	121.22 \pm 6.16	124.28 \pm 5.56	124.32 \pm 5.48	128.22 \pm 5.20	132.28 ^a \pm 4.55	141.14 ^b \pm 3.47	148.16 ^b \pm 4.91
Dystocia	138.92 \pm 4.85	139.88 \pm 4.77	142.74 \pm 4.85	145.22 \pm 5.10	147.12 \pm 4.78	150.04 ^a \pm 4.86	161.14 ^a \pm 4.30	168.70 ^a \pm 4.17
Retained placenta	128.62 \pm 4.17	131.54 \pm 5.43	130.04 \pm 6.25	136.68 \pm 7.06	144.02 \pm 8.37	151.50 ^a \pm 6.94	159.90 ^a \pm 6.77	176.16 ^a \pm 9.57
Weakens	127.06 \pm 3.60	127.42 \pm 3.74	129.28 \pm 3.13	131.68 \pm 3.44	133.48 \pm 2.42	136.44 ^{ab} \pm 3.08	138.14 ^b \pm 2.85	143.60 ^b \pm 2.99
P -value	0.114	0.111	0.106	0.086	0.097	0.035	0.003	0.003

S, Significant ($P \leq 0.05$); HS, Highly significant ($P \leq 0.01$); NS, Not significantly differ

^{a,b}Means bearing different superscripts in the same column are significantly different ($P \leq 0.05$)

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Table 1b Plasma Cortisol during pregnancy period (3rd trimester) in Friesian cattle

Groups	Cortisol concentration (ng/ml) Number of weeks before parturition(Mean \pm SE)							
	Two months before parturition				one month before parturition			
	8w	7w	6w	5w	4w	3w	2w	1w
Control	20.80 \pm 1.46	21.00 \pm 1.22	19.00 \pm 1.14	20.80 \pm 1.24	22.20 \pm 1.43	21.20 \pm 0.73	19.80 \pm 1.11	20.00 \pm 0.71
Dystocia	24.40 \pm 0.68	22.60 \pm 0.98	24.80 \pm 0.86	23.40 \pm 0.93	30.40 \pm 0.93	32.80 \pm 0.80	29.40 \pm 1.17	33.00 \pm 0.95
Retained placenta	21.20 \pm 1.85	26.60 \pm 1.89	26.40 \pm 1.91	24.80 \pm 2.44	30.40 \pm 1.96	31.20 \pm 3.15	36.20 \pm 3.06	39.80 \pm 2.44
Weakens	22.60 \pm 2.06	22.40 \pm 0.60	26.80 \pm 1.85	28.00 \pm 2.19	31.20 \pm 1.28	29.00 \pm 1.67	29.20 \pm 1.80	27.80 \pm 2.35
P –value	.406	.036	.008	.038	.097	.035	.003	.003

S, Significant ($P \leq 0.05$); HS, Highly significant ($P \leq 0.01$); NS, Not significantly differ.

^{a,b}Means bearing different superscripts in the same column are significantly different ($P \leq 0.05$)

Olsson & Dahlborn¹⁷ declared that there were no differences in cortisol concentrations between heifers that needed assistance and those that did not, the high cortisol values during expulsion may have been partly due to the stress and pain that accompany all parturitions. On the day before parturition, McMillen et al.¹⁸ found that cortisol concentration in the goats tended to increase above the value that could be explained by diurnal variation, and, when labour started, cortisol levels increased stepwise. Plasma cortisol concentrations increase several-fold in the ovine fetus late in pregnancy and decrease threefold within 48 h following birth.¹⁹ In farming animals, Sathya et al.²⁰ declared that many factors such as pregnancy, parturition processes and periparturient disorders can be considered a natural event that causes various physiological stressors for dams.

Jacob et al.²¹ found that there is a trend towards high levels of cortisol. Furthermore, Hoyer et al.,²² suggesting that cows at the time of parturition exposed to severe stress which resulted in hyperadrenocortical activity with higher levels of circulating steroids like cortisol. They also found that biochemical blood parameters as cortisol levels resulted in hyperadrenocortical activity especially during periparturient period. On the other hand, in cows at parturition, the stress of labour and delivery does not seem to influence plasma β -endorphin levels in cows and no correlation was found between β -endorphin concentration and type of calving. Whereas, β -endorphin values in dystocia group tended to be higher than that normal group. It is possible that cows with abnormal calving have more stress than that normal one, leading to an increase of peripheral β -endorphin level. Add to that, there was a concomitantly trend between both of plasma β -endorphin and cortisol profile.²³

II: Concentration of β -endorphin and Cortisol in blood plasma around parturition process (before, at and after parturition):

Results of this study in Table 2ab represented that there was highly significant difference at ($P \leq 0.01$) between concentrations of plasma β -endorphin and Cortisol in plasma around parturition period (periparturient). Plasma β -endorphin elevated two days before parturition till the day of parturition (day zero) in groups C, B, D and A as follow: (194.06 \pm 10.43, 181.40 \pm 5.10, 160.56 \pm 5.10 and 157.60 \pm 4.40, respectively) then it tended to decrease in level after

that. Plasma cortisol elevated two days before parturition till the day of parturition (day zero) in groups C, B, D and A as follow: (49.60 \pm 2.42, 38.40 \pm 1.54, 28.00 \pm 0.89 and 36.00 \pm 2.55, respectively) then it tended to decrease in level after that for about five days.

Elevated plasma cortisol concentrations, measured at different times around parturition, have previously been reported in cattle.²⁴ Meanwhile, through frequent blood sampling, Kitts²⁵ demonstrated that the cortisol increases during parturition in heifers and goats correlate strongly with the phases of parturition. The elevation of cortisol levels around parturition may be due to the increased need for glucocorticoids, to accelerate mammary growth and initiate lactation, and the fact that oestrogens reduce the metabolic clearance rate of cortisol. In sheep there were different responses of plasma β -EP and cortisol to a change of environment.²⁶ It was reported that in response to acute stress, plasma β -EP, ACTH and cortisol levels in sheep increased and then β -EP declined while ACTH and cortisol levels remained elevated (Table 2a&b).²⁷

In according to the present results, Osawa et al.²⁸ found that plasma cortisol level increased in both of cows showed or don't showed higher levels of β -endorphin nearest and/ or at calving. Furthermore, in normal parturition cases distinguish of β -endorphin excretion was noticed when the uterine constriction and labour pain increased as a result to amniorrhexis. Adding to that, during periparturient period β -endorphin and cortisol concentrations were higher in dystocia group comparison to normal parturition one and there are no significant differences between groups. Moreover, at parturition they found that concentrations of β -endorphin were higher in cows suffering from dystocia and retained placenta (79%) in compare with normal one (42%). Healthy cow exhibited a single elevated cortisol levels (>8 nmol/l) at the onset of parturition. Cortisol levels then fell below 4 nmol/l, 8 h later in each case, except for a mild increase to 8.8 nmol/l for cow on day 1 after calving. But, the cows with mastitis showed prolonged increases in cortisol values or 2nd maximal values of 12–32 nmol/l during the first 2 d post-partum. By day 4 cortisol values had decreased to below 4 nmol/l in all these cows. Cortisol values in the cows with mastitis were very similar to those in the healthy cows at each time interval examined in 2 d post-partum and the values of cortisol ranged from, 0.6 to 3.99nmol/l.²⁹

Table 2a Plasma β endorphin around parturition period (*periparturient*) in Friesian cattle

Groups	β endorphin concentration (pg/ml)						
	Times around parturition (Mean \pm SE)						
	Before parturition		during parturition	After parturition			
	-2 days	-1 day	Zero time	3h.	1 day	3days	5days
Control	151.68 ^a \pm 4.56	155.10 ^{bc} \pm 4.46	160.56 ^b \pm 5.10	153.06 ^b \pm 4.47	145.44 ^c \pm 3.52	140.12 ^c \pm 4.50	132.10 ^c \pm 3.71
Dystocia	171.98 ^a \pm 4.25	172.04 ^{ab} \pm 4.24	181.40 ^a \pm 5.10	177.64 ^a \pm 3.93	173.00 ^b \pm 3.14	163.08 ^b \pm 2.95	150.30 ^b \pm 1.69
Retained placenta	178.76 ^a \pm 11.01	185.42 ^a \pm 9.84	194. ^{06a} \pm 10.43	192.22 ^a \pm 9.53	191.84 ^a \pm 8.45	190.54 ^a \pm 7.86	189.80 ^a \pm 7.51
Weakens	145.02 ^b \pm 3.14	147.34 ^c \pm 2.65	157.60 ^b \pm 4.40	155.90 ^b \pm 4.32	154.08 ^c \pm 4.63	142.80 ^c \pm 4.99	141.28 ^{bc} \pm 5.43
P –value	.006	.002	.004	.001	.000	.000	.000

S, Significant ($P \leq 0.05$); HS, Highly significant ($P \leq 0.01$); ^{NS}, Not significantly differ

^{a,b}Means bearing different superscripts in the same column are significantly different ($P \leq 0.05$)

Table 2b Plasma cortisol around parturition period (*periparturient*) in Friesian cattle

Groups	Cortisol concentration (ng/ml) Times around parturition (Mean \pm SE)						
	Before parturition		during parturition	After parturition			
	-2 days	-1 day	Zero time	3h.	1 day	3days	5days
Control	21.60 ^c \pm 0.68	21.60 ^c \pm 0.60	28.00 ^c \pm 0.89	25.80 ^c \pm 0.73	23.60 ^c \pm 1.54	19.40 ^c \pm 1.08	19.00 ^c \pm 0.89
Dystocia	32.60 ^b \pm 0.75	32.80 ^b \pm 0.58	38.40 ^b \pm 1.54	36.00 ^b \pm 1.30	32.60 ^b \pm 0.81	27.80 ^b \pm 0.97	27.20 ^b \pm 2.08
Retained placenta	40.20 ^a \pm 1.43	45.40 ^a \pm 1.86	49.60 ^a \pm 2.42	48.60 ^a \pm 2.32	48.40 ^a \pm 2.40	46.20 ^a \pm 2.48	46.20 ^a \pm 1.98
Weakens	28.60 ^b \pm 2.84	29.00 ^b \pm 2.55	36.00 ^b \pm 2.55	34.80 ^b \pm 2.35	30.20 ^b \pm 3.12	25.00 ^b \pm 3.08	21.20 ^c \pm 1.71
P –value	.000	.000	.000	.000	.000	.000	.000

S, Significant ($P \leq 0.05$); HS, Highly significant ($P \leq 0.01$); ^{NS}, Not significantly differ

^{a,b}Means bearing different superscripts in the same column are significantly different ($P \leq 0.05$)

III: Concentration of β -endorphin and cortisol in blood plasma during the postpartum period:

Results in Table 3a&b represented that there was highly significant difference at ($P \leq 0.01$) between concentrations of plasma β -endorphin and Cortisol in plasma in postpartum period. Plasma β -endorphin tended to decrease among ten days after delivery till two months postpartum. It ranged in groups C, B, D and A as follow: (186.80 \pm 6.22 to 151.62 \pm 2.04, 144.94 \pm 3.02 to 119.04 \pm 2.23, 141.28 \pm 5.43 to 131.00 \pm 5.30 and 124.00 \pm 2.06 to 119.90 \pm 2.78, respectively). Furthermore, plasma cortisol levels tended to decrease among ten days after delivery till two months postpartum. It ranged in groups C, B, D and A as follow: (46.40 \pm 1.60 to 44.40 \pm 3.06, 28.80 \pm 1.71 to 32.80 \pm 0.80, 21.60 \pm 1.21 to 23.20 \pm 1.28 and 23.60 \pm 0.93 to 30.00 \pm 1.58, respectively).

Meanwhile, Osawa et al.¹⁴ conducted that there was no effect of dystocia, retained placenta or fall in BCS on the plasma β -EP level during the period between calving and first ovulation was found. We previously reported that there is a positive correlation between time to first ovulation and plasma β -EP level in dairy cow. Osawa et al.²⁸ reported that time to first ovulation was not different between cows with abnormal calving and those with normal calving, and between cows with decreased BCS and those with maintained BCS. Mohammad and Abdel-Rahman³⁰ summarized that the cortisol level during the first stage of parturition was significantly higher in the serum of parturient animals suffering from dystocia. Also, Nakao & Grunert³¹ demonstrated that dystocia significantly elevated cortisol level as compared to normal parturition. Adding that, the rises in the cortisol concentration in dystocia heifers appear to be induced during calving stress (Table 3a&b).

Table 3a Plasma β -endorphin at the postpartum period in Friesian cattle

Groups	β endorphin concentration (pg/ml) Times at the postpartum period per day (Mean \pm SE)					
	10 d	20 d	30 d	40 d	50 d	60 d
Control	124.00 \pm 2.06	119.62 \pm 2.50	119.06 \pm 2.48	113.30 \pm 2.11	111.88 \pm 3.86	119.90 \pm 2.78
Dystocia	144.94 \pm 3.02	137.04 \pm 3.74	123.30 \pm 2.12	120.24 \pm 2.77	121.00 \pm 2.20	119.04 \pm 2.23
Retained placenta	186.80 \pm 6.22	172.58 \pm 6.12	172.88 \pm 5.38	157.10 \pm 3.61	153.98 \pm 3.41	151.62 \pm 2.04
Weakens	141.28 \pm 5.43	129.66 \pm 6.96	127.24 \pm 6.49	127.44 \pm 5.28	126.76 \pm 6.65	131.00 \pm 5.30
P –value	.000	.000	.000	.000	.000	.000

S, Significant ($P \leq 0.05$); HS, Highly significant ($P \leq 0.01$); NS, Not significantly differ

^{a, b} Means bearing different superscripts in the same column are significantly different ($P \leq 0.05$)

Table 3b Plasma Cortisol at the postpartum period in Friesian cattle

Groups	Cortisol concentration (ng/ml) Times at the postpartum period per day (Mean \pm SE)					
	10 d	20 d	30 d	40 d	50 d	60 d
Control	21.60 \pm 1.21	21.00 \pm 1.52	21.80 \pm 1.02	22.00 \pm 0.77	23.20 \pm 0.37	23.20 \pm 1.28
Dystocia	28.80 \pm 1.71	28.60 \pm 2.14	30.80 \pm 1.46	32.20 \pm 0.58	33.00 \pm 1.45	32.80 \pm 0.80
Retained placenta	46.40 \pm 1.60	45.60 \pm 1.60	45.80 \pm 1.80	45.40 \pm 2.09	44.80 \pm 1.83	44.40 \pm 3.06
Weakens	23.60 \pm 0.93	23.00 \pm 1.05	25.40 \pm 0.60	26.60 \pm 0.81	30.00 \pm 1.52	30.00 \pm 1.58
P –value	.000	.000	.000	.000	.000	.000

S, Significant ($P \leq 0.05$); HS, Highly significant ($P \leq 0.01$); NS, Not significantly differ

^{a, b} Means bearing different superscripts in the same column are significantly different ($P \leq 0.05$)

IV: Concentration of β -endorphin and cortisol in blood plasma during the lactation period:

Table 4a&b represented that there was highly significant difference at ($P \leq 0.01$) between concentrations of plasma β -endorphin and cortisol in plasma during lactation periods in dairy cattle. Plasma β -endorphin decreased gradually from the first month of lactation till the fourth month of lactation in groups C, B, D and A as follow: (185.90 \pm 6.22 to 151.32 \pm 2.24, 143.32 \pm 3.89 to 129.46 \pm 2.15, 124.36 \pm 2.43 to 116.58 \pm 1.79 and 139.20 \pm 4.17 to 128.74 \pm 2.41, respectively). Meanwhile, plasma cortisol increased gradually from the first month of lactation till the fourth month of lactation in groups C, B, D and A as follow: (45.80 \pm 2.13 to 50.00 \pm 1.48, 27.60 \pm 2.09 to 33.60 \pm 2.29, 20.80 \pm 1.16 to 21.80 \pm 0.86 and 23.00 \pm 0.55 to 33.60 \pm 2.09, respectively).

The relationship between cortisol and function of the mammary gland was evidenced, and cortisol seems to be important in the reduction of mammary epithelial cell tight junction leakiness in the udder of dairy cows.^{32,33} Since milking can represent psychophysical and emotional stimulations, with or without effects on the behavior,

welfare^{34,35} and metabolic status,³⁶ aim of study was to obtain insight into the dynamics of the release of β -endorphin, ACTH and cortisol in response to machine milking in dairy cows (Table 4a&b).

Moreover, a decrease of plasma β -endorphin concentrations during machine milking in cows with disturbed milk ejection and in control animals with normal milk removal were shown.³⁷ In addition, a recent experiment of Lacasse et al.³⁸ showed that β -endorphin releases was not affected by milking frequency and not correlated with the magnitude of prolactin release. Moreover, they summarized the highest cortisol concentrations observed in dairy cows milked during the morning, both at the 1st and the 2nd day could indicate that these cows were very sensitive to milking stimuli, even if all animals appeared to be calm and relaxed during milking. Overall, the magnitude of cortisol increases after morning machine milking may suggest a stimulus-response relationship and a pulsatile mode of releases cortisol that was probably superimposed on baseline circadian variations. A comparison of the results obtained in dairy cows after machine milking with published data by Sutherland et al.³⁹ showed a similar pattern of cortisol concentrations, with a cortisol increases after milking than baseline values.

Table 4a Plasma β -endorphin during lactation periods in Friesian cattle

Groups	β endorphin concentration (pg/ml) Times during lactation period (Mean \pm SE)							
	1 month		2 month		3 month		4 month	
	4w	2w	4w	2w	4w	2w	4w	2w
Control	124.36 ^c \pm 2.43	117.56 ^b \pm 2.88	113.30 ^c \pm 2.11	120.84 ^c \pm 2.65	120.84 ^c \pm 2.31	118.40 ^c \pm 1.59	116.24 ^c \pm 1.86	116.58 ^c \pm 1.79
Dystocia	143.32 ^b \pm 3.89	123.92 ^b \pm 2.24	119.32 ^{bc} \pm 2.22	118.76 ^c \pm 2.90	123.00 ^c \pm 0.95	128.76 ^b \pm 2.40	130.00 ^b \pm 3.02	129.46 ^b \pm 2.15
Retained placenta	185.90 ^a \pm 6.22	174.34 ^a \pm 5.34	156.22 ^a \pm 3.82	150.84 ^a \pm 1.50	149.36 ^a \pm 1.59	145.44 ^a \pm 0.85	151.66 ^a \pm 3.62	151.32 ^a \pm 2.24
Weakens	139.20 ^b \pm 4.17	127.84 ^b \pm 5.42	127.44 ^b \pm 5.28	133.92 ^b \pm 4.21	134.56 ^b \pm 3.71	134.16 ^b \pm 3.21	128.78 ^b \pm 3.18	128.74 ^b \pm 2.41
P –value	.000	.000	.000	.000	.000	.000	.000	.000

S, Significant (P \leq 0.05); HS, Highly significant (P \leq 0.01); ^{NS}, Not significantly differ

^{a,b}Means bearing different superscripts in the same column are significantly different (P \leq 0.05)

Table 4b Plasma Cortisol during lactation periods in Friesian cattle

Groups	Cortisol concentration (ng/ml) Times during lactation period (Mean \pm SE)							
	1 month		2 month		3 month		4 month	
	4w	2w	4w	2w	4w	2w	4w	2w
Control	20.80 ^c \pm 1.16	20.80 ^c \pm 1.02	22.00 ^c \pm 0.77	23.20 ^c \pm 0.37	22.80 ^c \pm 0.58	22.00 ^c \pm 0.71	22.40 ^c \pm 0.51	21.80 ^c \pm 0.86
Dystocia	27.60 ^b \pm 2.09	28.80 ^b \pm 1.53	29.40 ^b \pm 1.36	30.40 ^b \pm 0.68	32.60 ^b \pm 0.93	33.80 ^b \pm 1.66	35.00 ^b \pm 2.17	33.60 ^b \pm 2.29
Retained placenta	45.80 ^a \pm 2.13	45.80 ^a \pm 2.15	46.40 ^a \pm 2.29	44.60 ^a \pm 1.75	46.40 ^a \pm 2.54	46.60 ^a \pm 2.64	46.60 ^a \pm 1.72	50.00 ^a \pm 1.48
Weakens	23.00 ^{bc} \pm 0.55	24.40 ^{bc} \pm 1.08	26.60 ^b \pm 0.81	29.40 ^b \pm 0.81	32.40 ^b \pm 1.47	32.60 ^b \pm 1.08	32.60 ^b \pm 0.93	33.60 ^b \pm 2.09
P –value	.000	.000	.000	.000	.000	.000	.000	.000

S, Significant (P \leq 0.05); HS, Highly significant (P \leq 0.01); ^{NS}, Not significantly differ

^{a,b}Means bearing different superscripts in the same column are significantly different (P \leq 0.05)

V: Concentration of β -endorphin and cortisol in blood plasma during the estrus:

Table 5a&b represented that there was highly significant difference at (P \leq 0.01) between concentrations of plasma β -endorphin and cortisol in plasma during the estrus cycle stages in dairy cattle. Plasma β -endorphin and cortisol levels showed an elevated increase during stages of estrus cycle. Plasma β -endorphin levels reached its highest values at the follicular stage in groups C, B, D and A as follow: (150.80 \pm 1.16, 127.76 \pm 2.11, 131.84 \pm 4.24 and 121.36 \pm 1.72, respectively). Moreover, Plasma cortisol levels reached its highest

values at the follicular stage in groups C, B, D and A as follow : (53.8 \pm 0.58, 36.20 \pm 1.32, 32.40 \pm 0.40 and 23.80 \pm 0.58, respectively).

This result is comparable to the findings in sheep in which there were the different responses of plasma β -endorphin and cortisol to a change of environment. On the other hand, it was reported that in response to acute stress, plasma β -endorphin, ACTH and cortisol levels in sheep increased and then β -endorphin declined while ACTH and cortisol levels remained elevated. Also, the different profiles of β -endorphin and cortisol may be related to the half-life of plasma degradation of these two hormones (Table 5a&b).²⁷

Table 5a Plasma β -endorphin at stages of estrus cycle in Friesian cattle

Groups	β -endorphin concentration (pg/ml) Stages of estrus cycle (Mean \pm SE)			
	Metestrus	Early diestrus	Late diestrus	Follicular
Control	116.62 \pm 2.27	117.50 \pm 2.20	119.30 \pm 1.76	121.36 \pm 1.72
Dystocia	122.10 \pm 1.68	123.64 \pm 1.65	127.30 \pm 1.81	127.76 \pm 2.11
Retained placenta	143.88 \pm 1.47	146.58 \pm 1.24	149.60 \pm 1.24	150.80 \pm 1.16
Weakens	127.48 \pm 3.73	129.02 \pm 3.93	129.76 \pm 3.96	131.84 \pm 4.24
P –value	.001	.000	.000	.000

S, Significant ($P \leq 0.05$); HS, Highly significant ($P \leq 0.01$); NS, Not significantly differ

^{a,b}Means bearing different superscripts in the same column are significantly different ($P \leq 0.05$)

Table 5b Plasma Cortisol at stages of estrus cycle in Friesian cattle

Groups	Cortisol concentration (ng/ml) Stages of estrus cycle (Mean \pm SE)			
	Metestrus	Early diestrus	Late diestrus	Follicular
Control	22.00 \pm 0.71	22.60 \pm 0.68	23.40 \pm 0.51	23.80 \pm 0.58
Dystocia	33.40 \pm 1.57	34.00 \pm 1.34	34.80 \pm 1.69	36.20 \pm 1.32
Retained placenta	46.00 \pm 1.34	49.40 \pm 1.21	49.40 \pm 1.47	53.80 \pm 0.58
Weakens	29.60 \pm 1.33	31.80 \pm 0.73	31.60 \pm 0.60	32.40 \pm 0.40
P –value	.000	.000	.000	.000

S, Significant ($P \leq 0.05$); HS, Highly significant ($P \leq 0.01$); NS, Not significantly differ

^{a,b}Means bearing different superscripts in the same column are significantly different ($P \leq 0.05$)

Conclusion

In conclusion, β -endorphin secretion tends to increase together with cortisol at the time of calving. Plasma β -endorphin level in Friesian cattle during pregnancy state increased gradually through gestation period. It reaches its highest level during the third trimester due to stressful state. Also, it reaches its peak at zero time of parturition meanwhile; it decreases gradually towards re-establishment of ovarian cycle. It is concluded that β -endorphin, like oxytocin, is released in an episodic manner during parturition in cows and that both hormones are released in conjunction with uterine and abdominal contractions and distension of the uterine cervix. There is little known concerning β -endorphin as a promising diagnostic/prognostic indicator is and not all research results published so far allow the drawing of definite conclusions. For these reasons we propose that the questions raised above require further scientific attention.

Acknowledgements

None.

Conflicts of interest

No conflicts of interest exist.

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