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Hormonal Profile of Ovarian Follicular Fluid and Blood Plasma during Different Stages of Estrous Cycle in Holstein Cattle

Research Article

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ABSTRACT

The aim of this study was to evaluate the concentrations of reproductive hormones in blood plasma and in follicular fluids of different sized follicles during various stages of the estrous cycle in Holstein cattle. Jugular blood samples from 42 adult Holstein cattle were collected immediately after the slaughter. Antral fluids from small (4-5 mm), medium (6-9 mm) and large (10-20 mm) follicles were collected and the stages of estrous cycle were recorded. The gonadotropin and steroid concentrations in blood plasma and steroid levels in follicular fluid were measured. Estradiol-17-ß concentrations in antral fluids of small follicles in early diestrous were significantly ($P<0.05$) higher than the concentration of that in the follicular fluids from small antral follicles in other stages of the estrous cycle. Concentrations of estradiol-17-ß in follicular fluids from medium antral follicles in metestrous were significantly ($P<0.05$) higher than that of those in pro-estrous and estrous phase of the estrous cycle. There were no significant differences in the follicular fluid concentrations of estradiol-17-ß from the large follicles among the various stages of estrous cycle ($P>0.05$). The variation in progesterone concentration within the follicles of various size during different phases of estrous cycle was not significant ($P>0.05$). The plasma concentrations of FSH, LH and estradiol-17-ß in proestrous and estrous were higher than the other stages of the cycle ($P<0.05$). The plasma progesterone concentration in late diestrous was higher than in metestrous, proestrous and during estrous ($P<0.05$).

KEY WORDS bovine, estradiol-17-β, estrous cycle, gonadotropins, progesterone.

INTRODUCTION

Follicular dynamic is defined as the process of continual growth and regression of antral follicles. One to four waves of follicular growth and development occur during a single estrous cycle in cattle (Azawi *et al.* 2009). The dominant follicle of each wave of growth continues to grow at an accelerated rate and if its development coincides with corpus luteum lysis and the decrease of progesterone, it may ovulate (Rosales Torres *et al.* 2012). Ovarian follicles in cattle vary in number and in growth rate within size classes and stages of the estrous cycle (Maurasse *et al.* 1985). Due to day-to-day changes in the pattern of ovarian follicular growth, changes in hormone concentrations associated with the wave-like pattern of follicle growth has been investigated (Evans, 2003). Antral follicular development in cattle depends on pulsatile secretion of gonadotropins (FSH and LH) from the pituitary (Vizcarra *et al.* 1997). The hypothalamus controls the release of gonadotropins into the portal circulation of the pituitary gland by the pulsatile secretion of GnRH (Rodriguez and Wise, 1989). The follicular synthesis of estradiol requires the coordinated activities of

two ovarian cell types and the two gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Gordon, 2003). There was a highly significant positive correlation between the concentration of estradiol and follicular size in the healthy cattle follicles (Nishimoto *et al.* 2009). It is known that bovine granulose cells are capable of producing estradiol only when provided with an aromatizable substrate; thecal cells are the source of androgen in the follicles and androgen secretion is increased by LH, but not by FSH (Gordon, 2003). Anadrogen is produced by thecal cells under the influence of LH and aromatized to estradiol by P450 arom and 17-ß HSD in smooth endoplasmic reticulum of granulose cells, under the control of FSH (Gordon, 2003).

Each follicular growth wave in estrous cycle is associated with transient rise of FSH. In heifers, the wave stimulating FSH surge reaches peak concentrations, on average, when the largest follicle is about 5 mm. The mean concentration then decrease, with about a 3-day interval between peak concentrations and the beginning of deviation (Sunderland *et al*. 1994). The role of FSH after the peak of the surge involves the continued growth and development of follicles before deviation and the developing dominant follicle after deviation (Glister *et al*. 2001). Based on *in vitro* studies of Glister *et al.* (2001) and Ginther *et al.* (2003) with granulose cells, FSH stimulates the production of estradiol in cattle. Estradiol is one of the factors that has intrafollicular role in deviation (Glister *et al*. 2001; Ginther *et al*. 2003). LH stimulation of follicular theca cells is essential for androgen secretion which act as precursors for estradiol, the enhanced synthesis of which is always associated with success within the continued progress during follicular wave growth (Mihm and Bleach, 2003). The dominant follicle which grows to a much larger size than all other ovarian follicles is responsible for the high ovarian estradiol and inhibin secretion and maintains low FSH concentrations to prevent any other cohort of growing follicles (Ginther *et al.* 2000).

Continued growth and estradiol synthesis by the first dominant follicle of the cycle does not usually occur for more than 3-4 days as the developing corpus luteum with its progesterone secretion negatively regulate the LH pulse pattern resulting in the LH dependent dominant follicle becoming atretic (Ginther *et al*. 2000; Azawi *et al*. 2009). The relative follicular fluid concentrations of steroids and gonadotrophins vary widely between follicles, and that the intrafollicular environment of steroids and gonadotrophins may be an important regulator of follicular developmet in animals (Henderson *et al*. 1982).

The study on hormonal profile of follicular fluid in different stages of estrous cycle provide a useful indication of the requirements for oocyte and follicular cell growth *in vitro* and may be used as a guide for the formulation of cell culture conditions according to follicular size in various stages of estrous cycle. Therefore, the aim of this study was to evaluate the steroid and gonadotropin concentrations of blood plasma and follicles of various sizes during different stages of the estrous cycle in Holstein cattle.

MATERIALS AND METHODS

Collection of blood sample and ovaries

Jugular blood samples and ovaries of 42 adult Holstein cattle were collected from Ahwaz Iran slaughterhouse, immediately after the slaughter. The blood samples were placed in a vacutainer containing ethylene diamine tetra acid (EDTA) as an anticoagulant. Ovaries and blood samples from pregnant cattle and those that have any pathological lesions such as cystic follicles (>20 mm in diameter) were not included in the study. The selected ovaries, as well as blood sample of each animal, were placed in plastic bags and transported to the laboratory in an ice box. In the laboratory, blood tubes were centrifuged at 3,000 rpm for 15 minutes then the plasma was separated and stored at -20 ˚C for further analysis.

Estrous cycle phase determination and processing of follicles

In the laboratory, each ovary was cleaned from the extragonadal tissues and stages of estrous cycle were determined and classified as metestrous (days 1-4) (n=10), early diestrous (days 5-10) (n=10), late diestrous (days 11-17) (n=10) and proestrous and estrous (days 18-21) (n=12) phases from the appearance of corpus luteum as previously described by Ali *et al*. (2003) in cattle. In each stage of the estrous cycle, diameter of various follicles present in ovaries was measured by using vernier calipers. These follicles were placed in three groups according to their diameter, i.e. small (4-5 mm), medium (6-9 mm) and large (10-20 mm). Then, the fluid from the antral cavity of each follicle category was aspirated by using a disposable sterilized insulin syringe. The fluid collected from the same sized follicle in paired ovaries was pooled. The pooled follicular fluid from each group was centrifuged for sedimentation of cell debris. The upper portion of the fluid was collected. Follicular fluid samples were stored at -20 ˚C for further analysis. The follicular fluids from three different sizes of follicles, in different stages of estrous cycle, were subjected to steroid (estrogen and progesterone) concentration analysis.

Hormone analysis

Follicular fluid and blood plasma concentrations of steroids and gonadotropins were measured by radioimmunoassay (RIA) method by using commercial kits (Immunotech, France).

Statistical analysis

The mean values ± SEM for concentrations of various hormones in follicular fluid of small, medium and large follicles and blood plasma were computed.

In order to determine the magnitude of variation in concentrations of various hormonal constituents of follicular fluid and plasma in different stages of estrous cycle, the data were subjected to one-way analysis of variance. Significance between means was tested using Duncan multiple range test.

RESULTS AND DISCUSSION

The follicular fluid concentrations of estradiol-17-ß and progesterone in follicles of different size during various stages of the estrous cycle are presented in Table 1. The estradiol-17-ß concentrations of follicular fluids form small follicles was significantly (P<0.05) higher in early diestrous compared with other stages of estrous cycle. The estradiol-17-ß level of antral fluid from medium follicles in metestrous was significantly higher (P<0.05) than that in proestrous and the estrous phase of the cycle. There were no significant differences in estradiol-17-ß concentrations of antral fluids from the large follicles among different stages of the estrous cycle (P>0.05). The follicular fluid concentrations of progesterone in small, medium and large follicles were not different among various phases of the estrous cycle. The concentrations of blood plasma estradiol-17-ß, progesterone, FSH and LH during different stages of estrous cycle are presented in Table 2 and Figure 1. In proestrous and estrous, the blood plasma levels of FSH, LH and estradiol-17-ß were significantly higher than other phases of estrous cycle (P<0.05). The blood plasma progesterone concentration in late diestrous was significantly (P<0.05) higher than during metestrous and proestrous and estrous phases of the estrous cycle.

The concentration of plasma estradiol-17-ß remained constant during metestrous and diestrous and then increased during proestrous and estrous phases of estrous cycle. Contrary to this study, estrogen level in Punganur cattle showed a significant decrease from estrous to day 10 with a significant rise on day 15 of estrous cycle (Naik *et al.* 2013). Also, in opposite to results of Wise (1987) in cattle, blood serum estradiol was significantly higher on days 7-10 (early diestrous) of estrous cycle. In goat, the blood plasma estradiol-17-ß profile was characterized by a gradual increase during metestrous and then decreased to the basal level during the luteal phase (Medan *et al.* 2003), which was similar to the present study.

In mare, serum estradiol concentration increased concurrently with LH and coincided with the onset of estrous (Pattison *et al.* 1974). A peak of estradiol on the day before ovulation and additional peaks of this hormone on days 3-4, 9-10 and 12-13 of the estrous cycle associated with wave-like pattern of follicular development during stages of ovine estrous cycle have been shown (Cox *et al.* 1971; Zieba *et al.* 2001). However, no differences in concentrations of estrogens were observed during different stages of the bovine estrous cycle (Ireland *et al.* 1979).

In disagreement with our study, in buffalo, large follicles present during the early luteal stage contained as much oestradiol-17-ß in the follicular fluid as large follicles during the follicular stage (Kruip and Dieleman, 1985). In goat, blood plasma estradiol concentration decreased on about day 6 of the estrus cycle and remained low from days 7-15 compared with the pre-luteal and follicular phases (Pang *et al.* 2010). In contrast to our results, peak level of estradiol in follicular fluid of buffalo ovaries was recorded during estrous phase (Eissa, 1996). Simillar to our results, the mean concentrations of plasma progesterone in beef cattle increased from days 1 through 17 and then declined (Ireland *et al.* 1979).

Table 1 Follicular fluid concentration of steroids in different sized follicles at various stages of estrous cycle in Holstein cattle

Stages of estrous cycle	Small follicle (≤5 mm)		Medium follicle (6-9 mm)		Large follicle (10-20 mm)	
	Estradiol-17-β*	Progestrone**	Estradiol-17-β	Progestrone	Estradiol-17-β	Progestrone
Metestrus (1-4 days)	2212.8±425.35^{a}	58.23±8.46	11596±893.65^{b}	41.68±13.94	-	-
Early diestrous (5-10 days)	11776±777.79^{b}	42.82±5.12	6243.8±2607^{ab}	34.68±8.13	13208±671.14	62.83±5.35
Late diestrous (11-17 days)	1680.5±312.30^{a}	64.45±11.97	6703±3407.5^{ab}	64±10.82	11968±803.63	61.53±10.06
Proestrous and estrous (18-21 days)	2891±699.78^{a}	57.18±9.38	1350.7±386.91^{a}	32.20±7.67	13003±68.35	69.00±11.01

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* pg/mL and ** ng/mL.

Table 2 Blood plasma steroid concentrations in different stages of estrous cycle in Holstein cattle

Stages of estrous cycle	Metestrous (1-4 days)	Early diestrous (5-10 days)	Late diestrous (11-17 days)	Proestrous and Estrous (18-21 days)
Estradiol-17-β*	44.20±7.62^{a}	56.15±6.57^{a}	62.73±4.54^{a}	105.30±22.62^{b}
Progesterone**	0.28±0.07^{a}	0.97±0.14^{ab}	1.59±0.51^{b}	0.29±0.07^{a}

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* pg/mL and ** ng/mL.

Also, blood progesterone raised to peak levels about 12 to 14 days after estrous in Thai native cattle (*Bos indicus*) with 3 ovarian follicular waves (Sakhong *et al.* 2011) and days 13 to 15 of the estrous cycle in buffaloes (Mondal *et al.* 2007). In Punganur cattle, the peripheral blood concentration of progesterone increased significantly from estrous to day 15 and thereafter it decreased (Naik *et al.* 2013). Similar to present study, no significant changes in follicular progesterone occurred during the estrous cycle of cattle (Wise, 1987). In contrast, Eissa (1996) reported the significantly maximum concentration of progesterone in follicular fluid of buffalo during estrous stage.

Progestin levels rose and fell coincident with the growth and regression of the corpus luteum (Christensen *et al.* 1974). Plasma progesterone levels in Huanghuai goats remained low during the follicular phase (Pang *et al.* 2010). Serum progestin level in beef cow was lowest on day 0 and remained low until day 4, then levels began to increase, reaching a peak on day 15 (Christensen *et al.* 1974).

In agreement with this study, the peak blood concentration of LH in cattle was observed at the onset of estrous (Christensen *et al.* 1974). LH stimulate the theca cells for androgen synthesis which act as precursors for estradiol, the enhanced synthesis of which is always associated with success within the cohort and continued progress during follicle wave growth (Mihm and Bleach, 2003). High progesterone levels during luteal phase, suppress the pituitary LH pulse frequency (Menchaca and Rubianes, 2002). Secretion of estradiol by the dominant follicle in the follicular phase is acutely responsive to LH pulses that are infrequent during the late luteal phase (Souza *et al.* 1997). In the proestrous plus estrous phase, the blood plasma level of FSH was significantly higher than other phases of estrous cycle. In opposite to present study, maximum plasma FSH achieved between days 0-4 of estrous cycle for Angus, Brahman and Senepol cows (Alvarez *et al.* 2000). FSH secretion was not affected directly by progesterone but was regulated by estradiol and inhibin, which was produced mainly by the large follicles (Menchaca and Rubianes, 2002). The importance of FSH in ovarian folliculogenesis in ewes has been demonstrated (Zieba *et al.* 2001). There is a relationship between elevations in the mean daily serum concentrations of FSH and the emergence of follicular waves in ewes (Medan *et al.* 2005). In contrast to our results, Souza *et al.* (1997) reported a significant decline in blood plasma FSH concentration during the ovine follicular phase (proestrous and estrous) of estrous cycle. Sakhong *et al.* (2011) reported the maximum FSH level on day 12 after ovulation in Thai native cattle with 4 follicular waves. Follicular fluid steroid concentrations (progesterone and estrogens) are the primary indicators that are utilized for the classification of follicular status (Wise, 1987).

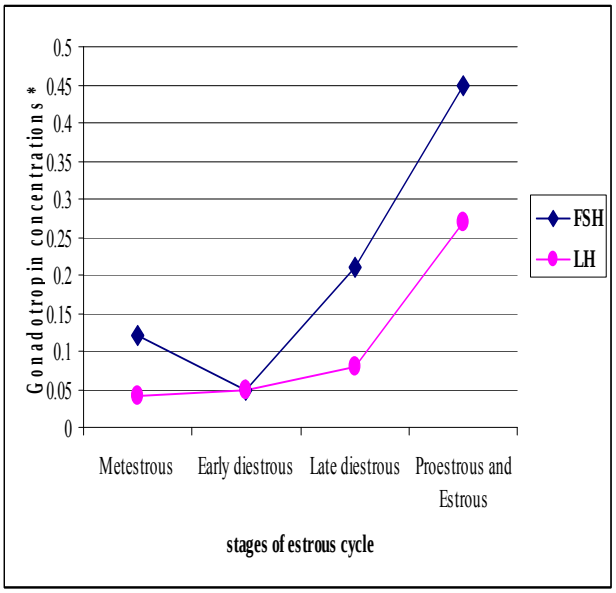

Figure 1 Mean blood plasma concentrations of gonadotropins in different stages of estrous cycle in Holstein cattle
* pg/mL

The evidence from this study and the retrospective data analyses in ewe provides strong support for implicating progesterone as the key regulator of circulating FSH concentrations in ewes and in determining the number of antral follicular waves per estrous cycle in ewes (Baby and Bartlewski, 2011).

The mechanism whereby progesterone regulates periodic increases in serum FSH concentrations remains to be elucidated. The above estradiol-17-ß value of medium follicles in metestrous was in agreement with findings of Souza *et al.* (1998) in ewe, where the concentration of estradiol in ovarian venous plasma increased progressively from day 1 until day 3.5 (metestrous) and then decreased by day 7 to low values similar to the onset of the luteal phase. Frequent GnRH and LH pulses are known to lead to prolonged growth of follicles and increased follicular estrogen synthesis (Opara *et al.* 2006). The decline in steroid secretion after day 3 of the luteal phase could be due to the decrease in LH pulse frequency (Souza *et al.* 1997).

In the present study, significant differences in estradiol-17-ß content of large follicles between different phases of estrous cycle were not found. In a study by Bridges *et al.* (2002) in mare, large follicular phase follicles contained 3-72 times greater levels of estradiol than large follicles in the luteal phase, small follicles in the follicular or luteal phase and medium follicles in either the follicular or luteal phase. FSH stimulates granulosa cell aromatase activity and in this way stimulates follicular estradiol-17-ß production (Henderson *et al.* 1982).

The concentration of progesterone in small, medium and large follicles in the present study was not different among various phases of estrous cycle. This is in agreement with

another study (Wise, 1987) that found no significant changes in bovine follicular progesterone concentrations during the estrous cycle in large and small follicles. Contrary to this study, Ireland *et al*. (1979) reported that the amounts of follicular progestins were the highest in days 5-10 of estrous cycle within the follicles in the small size and which declined steadily through days 18-20 in cattle. Also, Ireland *et al*. (1979) found the increased pattern for progestins of medium size follicles during days 1-10 of estrous cycle and remained high during the remainder of the estrous cycle.

CONCLUSION

In conclusion, ovarian steroid (estradiol-17-ß and progesterone) and gonadotropin (FSH and LH) concentrations of blood plasma and in follicles of different sizes (small, medium and large) may be affected by stages of the estrous cycle in Holstein cattle.

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