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

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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-B 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. FSH (Glister et al. 2001; Ginther et al. 2003). LH stimulation of follicular theca cells is essential for anadrogen 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 development 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 extraglandular tissues and stages of estrous cycle were determined and classified as metestrus (days 1-4) (n=10), early diestrus (days 5-10) (n=10), late diestrus (days 11-17) (n=10) and proestrus and estrous (days 18-21) (n=12) phases from the appearance of corpus luteum according to 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 gonadotrophins were measured by radioimmunoassay (RIA) method by using commercial kits (Immunotech, France).
The stages of the estrous cycle are presented in Table 1. The progesterone in follicles of different size during various estrous phases of the estrous cycle was significantly higher (P<0.05) than that in proestrous and the estrous phase of the cycle. There were no significant differences in estradiol-17β 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-17β, progesterone, FSH and LH during different stages of estrous cycle were computed.

The mean values ± SEM for 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-17β and progesterone in follicles of different size during various stages of the estrous cycle are presented in Table 1. The estradiol-17β concentrations of follicular fluids form small follicles was significantly higher (P<0.05) than that in proestrous and the estrous phase of the cycle. The estradiol-17β level of antral fluid from medium follicles in metestrus was significantly higher (P<0.05) than that in proestrous and the estrous phase of the cycle. There were no significant differences in estradiol-17β 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-17β, 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-17β 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 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-17β 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 estrus (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-17β in the follicular fluid as large follicles during the follicular stage (Kruij 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). Similar 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

<table>
<thead>
<tr>
<th>Stages of estrous cycle</th>
<th>Small follicle (≤5 mm)</th>
<th>Medium follicle (6-9 mm)</th>
<th>Large follicle (10-20 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estradiol-17β*</td>
<td>Progestrone**</td>
<td>Estradiol-17β*</td>
</tr>
<tr>
<td>Metestrus (1-4 days)</td>
<td>2212.8±425.35a</td>
<td>58.23±8.46</td>
<td>11596±393.6a</td>
</tr>
<tr>
<td>Early diestrous (5-10 days)</td>
<td>11776±777.79b</td>
<td>42.82±5.12</td>
<td>6243±8±2607b</td>
</tr>
<tr>
<td>Late diestrous (11-17 days)</td>
<td>1680±5312.30a</td>
<td>64.45±11.97</td>
<td>670±3±1407.5a</td>
</tr>
<tr>
<td>Proestrous and estrous (18-21 days)</td>
<td>2891±699.78a</td>
<td>57.18±9.38</td>
<td>1350±7±386.91a</td>
</tr>
</tbody>
</table>

*pg/mL and ** ng/mL.

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

<table>
<thead>
<tr>
<th>Stages of estrous cycle</th>
<th>Metestrus (1-4 days)</th>
<th>Early diestrous (5-10 days)</th>
<th>Late diestrous (11-17 days)</th>
<th>Proestrous and Estrous (18-21 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estradiol-17β*</td>
<td>Progestrone**</td>
<td>Estradiol-17β*</td>
<td>Progestrone**</td>
</tr>
<tr>
<td></td>
<td>44.20±7.62a</td>
<td>56.15±6.57b</td>
<td>62.73±4.54a</td>
<td>105.30±22.62a</td>
</tr>
<tr>
<td>Progestrone**</td>
<td>0.28±0.07*</td>
<td>0.97±0.14*</td>
<td>1.59±0.51*</td>
<td>0.29±0.07*</td>
</tr>
</tbody>
</table>

*pg/mL and ** ng/mL.

The concentration of plasma estradiol-17β remained constant during metestrous and diestrous and then increased during proestrous and estrous phases of estrous cycle.
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 progestrone 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 (proestrus and estrus) 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).
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.

ACKNOWLEDGEMENT

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