Uterine luminal fluid proteins during different reproductive stages (follicular and luteal stages and early pregnancy) in goat

Surya Prakash Pannu and Govind Narayan Purohit

Abstract

This study was carried out on abattoir derived goat genitalia to evaluate the changes in uterine luminal fluid proteins during different reproductive stages. Genital tracts were classified into follicular phase (n=26), luteal phase (n=21) and early pregnancy (n=13). The genitalia were dissected and uterine fluid samples were collected in eppendorf tubes. SDS-PAGE analysis was performed on the uterine fluid samples and molecular weights (MWs) of protein bands were estimated by comparing their migration rates on the gel with those of protein markers with known MWs. By comparing these fractions with those of the standards, MWs of 198.15, 168.27, 149.64, 118.83, 75.26, 50.33, 46.09, 37.69, 32.96, 21.05 and 15.76 kilodaltons (kDa) were calculated. Protein with molecular weights 46.09 kilodalton were observed only in follicular phase. Proteins with MW 50.33, 37.69 and 32.96 kDa were observed in all the phases but the higher proportions were recorded in early pregnancy. Proteins of 15.76 kDa were observed in early pregnancy. Proteins with MW 168.27, 149.64, 118.83 and 75.26 kDa were observed in follicular phase and luteal phase but the higher proportions were recorded in follicular phase. Protein with molecular weights 198.15 kilodalton was observed in all the phases but was specific during follicular phase. It was concluded that there is a preponderance of high molecular weight proteins during follicular phase and higher proportion of low molecular weight proteins appear during early pregnancy in goat uterus.

Keywords: Goat, uterine luminal fluid proteins, follicular phase, pregnancy, luteal phase

Introduction

During reproductive cycles and gestation of many mammalian species the presence of proteins in the uterine luminal fluid are altered. Such changes in the uterine luminal fluid proteins (ULFP) appear to be necessary as different proteins act as protease inhibitors (Fazleabas et al., 1982) [10] enzymes (Hansen et al., 1985) [21] carrier molecules for hormones, vitamins and minerals (Zavy et al., 1979; Buchi et al., 1982; Pentacost and Tang, 1987) [39, 7, 34] signalling molecules for pregnancy recognition (Godkin et al., 1984; Bartol et al., 1985; Godkin et al., 1988) [17, 34, 18] immunoregulatory molecules (Murray et al., 1978) [31] and many other probably unidentified functions.

Most ULFP’s are sequestrated from the blood into the uterine lumen however some are synthesized from the uterine endometrium (Fischer and Beier, 1986) [11]. Appreciable changes in the ULFP’s have been recorded during the estrous cycle in mares (Zavy et al., 1978; Zavy et al., 1979) [43-44], cows (Alavi-Shoushtari et al., 2006; Alavi-Shoushtari et al., 2014) [1-2], pigs (Kayser et al., 2006) [25] and buffaloes (Kumar and Purohit, 2018) [26]. The altered circulating steroids are probably responsible for these changes.

The influence of circulating progesterone on uterine protein secretion is well established (Chen et al., 1975; Adams et al., 1981; Simmen et al., 1991; Trout et al., 1992) [8, 1, 38, 40]. Progesterone receptors are known to be up or down regulated during the follicular and luteal phase of the estrous cycle (Geisert et al., 1994) [14]. The changing steroid concentrations are probably also responsible for the rapid release of histotroph from the endometrial epithelium that occurs at around Day 10-11 of conception (Geisert et al., 1982) [13]. The morphology and secretory activity of the endometrium is probably altered by angiogenesis (Kaczmarek et al., 2010) [24], apoptosis (Ziecik et al., 2011) [45] and extracellular matrix (ECM) remodelling (Diao et al., 2011) [9]. The uterine glandular epithelium is highly proliferated during the luteal phase compared to follicular phase (Gray et al., 2001; Hettinger et al., 2001) [19, 22].
Screening criteria for selection of genital organs

The collected genitalia were examined in the laboratory and classified as follicular/luteal stage or early pregnancy. Luteal stage were further classified as early, mid and late luteal phase. Follicular phase included genitalia in proestrus and estrus phase. For determination of the stage of estrous cycle (follicular/luteal) the presence/absence of follicles on the surface of the ovary was utilized as described previously (Roy et al., 2006; Miranda-Moura et al., 2010) [18, 29].

Briefly the tracts were classified into early-luteal (days 1–4, day1: ovulation), mid-luteal (days 5–10), late-luteal (days 11–16) and follicular phase (day 17–20) based on the corpus luteum morphology.

Follicular phase was characterized not only by the presence of regressed CL with no vasculature, cream color and hard texture in cut surface but also with at least one 5 mm or above diameter follicle. Genitalia with disorganized red color and lobular structure of the corpus luteum (CL) tissues and ovulation point not covered by surface epithelium were considered as early luteal stage. Genitalia in mid-luteal phase had CL tissues that were soft in texture, full of blood vasculature, had covered ovulation point, incomplete folding pattern in cut surface with reddish brown colour. Late-luteal CL were characterized by presence of surface vascularization, complete folding in cut surface with moderately hard texture and brown CL colour. All luteal stages were considered under the luteal phase.

Early pregnancy was considered when a conceptus was visible in the uterus at late luteal phase as mentioned for cattle. (Forde et al., 2014) [13].

Collection of uterine luminal fluid

The uterine fluid was collected by excision of the uterine horns followed by gentle scraping of the endometrium by a curette and collecting the fluid in a 2 mL Ependorf tube as mentioned previously (Alavi-Shoustari et al., 2006; Kumar and Purohit, 2018) [3, 26]. A total 2 ml uterine fluid was collected.

Experimental procedure

The organs were divided into 3 groups (follicular, luteal and early pregnant animal organs). The uterine luminal fluid was collected in Eppendorf tubes and immediately processed for SDS-PAGE analysis of uterine lumen proteins.

SDS-PAGE analysis

For qualitative estimation of proteins the uterine luminal fluid was processed as per method described previously (Nandi and Lewis, 1970; Kumar and Purohit, 2018) [12, 26] with some modification using commercially available kits (Hi-Media, India).

Analysis of results

We plotted the scatter curve between the log value and the migration distance of the known molecular weight marker to find out the m and b value (m and b are the absolute cells). Absolute cells means we calculate the scatter curve slope value in the excel sheet that is y = -0.182x + 5.570 In this value m = -0.182, b = 5.570.

The migration distance(cm), log value and molecular weight (kDa) of protein bands obtained in SDS-PAGE during different reproductive stages were calculated with the help of Excel sheet and formula. To calculate-

Log value = absolute cell (m) × migration distance in different reproductive stages + absolute cell (b)

Molecular weight (kDa) = 10^log value in different reproductive stages

Results

The uterine luminal proteins were classified (26 in follicular phase, 21 in luteal phase and 13 in early pregnancy) on the basis of molecular weights estimated by comparison with standard ladder on a SDS-PAGE (Figure 1). Proteins with molecular weight between 11 kDa to 245 kDa were evaluated in the present study. The migration distance of the known molecular weight markers (11kDa -245kDa) varied from 1.02 cm to 8.04 cm on the ladder. (Table I)
Table 1: Migration distance of the known molecular weight markers

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Known molecular weight (kDa)</th>
<th>Migration distance of known molecular weight (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>245</td>
<td>1.02</td>
</tr>
<tr>
<td>2.</td>
<td>180</td>
<td>1.70</td>
</tr>
<tr>
<td>3.</td>
<td>135</td>
<td>2.20</td>
</tr>
<tr>
<td>4.</td>
<td>100</td>
<td>2.90</td>
</tr>
<tr>
<td>5.</td>
<td>75</td>
<td>3.90</td>
</tr>
<tr>
<td>6.</td>
<td>63</td>
<td>4.60</td>
</tr>
<tr>
<td>7.</td>
<td>48</td>
<td>5.10</td>
</tr>
<tr>
<td>8.</td>
<td>35</td>
<td>5.70</td>
</tr>
<tr>
<td>9.</td>
<td>25</td>
<td>6.30</td>
</tr>
<tr>
<td>10.</td>
<td>20</td>
<td>6.70</td>
</tr>
<tr>
<td>11.</td>
<td>17</td>
<td>7.60</td>
</tr>
<tr>
<td>12.</td>
<td>11</td>
<td>8.04</td>
</tr>
</tbody>
</table>

The log value of known molecular weight markers was calculated as per formula described previously. The log value of the markers on ladder varied from 4.041 - 5.389. (Table 2)

Table 2: Log value of known molecular weight markers

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Known molecular weight (kDa)</th>
<th>Log value of known molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>245</td>
<td>5.389166084</td>
</tr>
<tr>
<td>2.</td>
<td>180</td>
<td>5.255272505</td>
</tr>
<tr>
<td>3.</td>
<td>135</td>
<td>5.130333768</td>
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<tr>
<td>4.</td>
<td>100</td>
<td>5.0</td>
</tr>
<tr>
<td>5.</td>
<td>75</td>
<td>4.875061263</td>
</tr>
<tr>
<td>6.</td>
<td>63</td>
<td>4.799340549</td>
</tr>
<tr>
<td>7.</td>
<td>48</td>
<td>4.681241237</td>
</tr>
<tr>
<td>8.</td>
<td>35</td>
<td>4.544068044</td>
</tr>
<tr>
<td>9.</td>
<td>25</td>
<td>4.397940009</td>
</tr>
<tr>
<td>10.</td>
<td>20</td>
<td>4.301029996</td>
</tr>
<tr>
<td>11.</td>
<td>17</td>
<td>4.230448921</td>
</tr>
<tr>
<td>12.</td>
<td>11</td>
<td>4.041392685</td>
</tr>
</tbody>
</table>

The scatter curve between the log value and the migration distance of the known molecular weight markers was prepared. (Figure 2)

We plotted the scatter curve between the log value and the migration distance of the known molecular weight marker to find out the m and b value (m and b are the absolute cells). Absolute cells means we calculate the scatter curve slope value in the excel sheet that is \( y = -0.182x + 5.570 \) In this value \( m = -0.182 \), \( b = 5.570 \)

The migration distance(cm), log value and molecular weight (kDa) of protein bands obtained in SDS-PAGE during different reproductive stages were calculated with the help of excel sheet and formula. To calculate-

Log value = absolute cell (m) \times\ migration distance in different reproductive stages + absolute cell (b)

Molecular weight (kDa) = 10^log value in different reproductive stages

Uterine fluid proteins during follicular phase of estrus cycle
Both proestrus and estrus phase sample are described as follicular phase. The proportion of proteins bands with their molecular weight 198.15, 168.27, 149.64, 118.83, 75.26, 50.33, 46.09, 37.69, 32.96 and 21.05 kDa was 100, 100, 80.76, 73.07, 26.92, 11.53, 15.38, 19.28, 3.84 and 3.84% respectively during the follicular phase of the estrous cycle. High molecular weight proteins were estrus specific. In all samples of follicular phase high molecular weight proteins were obtained but in few samples low molecular weight proteins also present.

Uterine fluid proteins during luteal phase of estrus cycle
The proportion of proteins bands with their molecular weight 198.15, 168.27, 149.64, 118.83, 75.26, 50.33, 46.09, 37.69, 32.96 and 21.05 kDa was 90.47, 84.21, 68.42, 57.89, 36.84, 26.31, 31.57, 15.78 and 10.52% respectively during the luteal phase. Low molecular weight proteins were obtained during the luteal phase but also appear during the follicular phase. Protein with molecular weights 46.09 kilodalton was absent during luteal phase. High molecular weight protein bands were also obtained during luteal phase of estrus cycle. High molecular weight protein bands were also obtained during luteal phase of estrus cycle in the present study.

Uterine fluid proteins during early pregnancy
The proportion of proteins bands with their molecular weight 198.15, 50.33, 37.69, 32.96 and 15.76 kDa was 15.38, 84.61, 100, 100 and 100% respectively during early pregnancy. During early pregnancy, mainly lower molecular weight proteins were found. A high molecular weight (198.15 kDa) proteins band also observed during early pregnancy. Protein with molecular weights 15.76 kilodalton was absent during early pregnancy. In all samples of early pregnancy low molecular weight proteins were obtained but in few samples high molecular weight proteins were also present.
Discussion

In the present study proteins with molecular weight 46.09 kDa was found during the follicular phases only when the plasma concentration of estrogens is high. Murphy and Ballejo (1994) [30] reported that vascular permeability factor (VPF), isolated from many conditioned medium of a variety of cell lines, is a 40–45 kDa disulfide-linked, homodimeric, heparin-binding glycoprotein that promotes endothelial cell growth and appears to be a good candidate for mediating the increase in vascular permeability and blood vessel growth induced by estrogens in the uterus.

High molecular weight proteins were estrus specific. The 198.15 and 168.27 kDa fractions, which were found in large proportion during the follicular phase are probably released under the influence of estrogens. Kumar and Purohit (2018) [26] studied buffalo ULFP and recorded the presence of protein with mean molecular weight 207.28±6.65 and 160.28±2.53 and during proestrus and estrus (follicular phase). Studies in ewes showed that certain proteins are more abundant during follicular phase and this altered secretory patterns regulate various reproductive process (Soleilhavoap et al., 2016) [39]. In the present study ULF was obtained by gentle scraping the endometrium by a curette which was considered more appropriate in previous studies (Alavi-Shoushtari et al., 2006; Kumar and Purohit, 2018) [1,26].

The observation of higher frequency of 21.06 kDa protein bands during the luteal phase compared to the follicular phase in the present study on goats is similar to previous studies on buffalo (Roy et al., 2006; Kumar and Purohit, 2018) [16,26]. In the present study 32.96 kDa protein bands were higher during early pregnancy and lower in luteal phase and follicular phase. In a study Schlosnagle et al. (1974) [37] purified a basic progesterone-induced glycoprotein (32 kDa molecular weight polypeptide) from the uterine fluids of ovariectomized female pigs. In our study 15.76 kDa protein bands were present only during early pregnancy. The study by Weise et al. (1993) [42] on caprine endometrial tissues recorded the presence of most prominent a very basic 14 kDa protein transiently produced between Days 15 and 24 of pregnancy. Two other acidic proteins with molecular masses of 14 kDa and 15 kDa were also associated with this time period.

Intrauterine infusion of conceptus-derived proteins or recombinant IFN-τ from Days 14 to 18 of the estrous cycle results in an extension of luteal life span until approximately Day 28 in sheep (Vallet et al., 1988) [41] and goats (Newton et al., 1996) [33]. However, a large number of embryos are lost even though luteal function has been maintained (Ayalon, 1978) [4]. These losses occur during a stage of pregnancy when embryonic and uterine tissues are closely apposed, cellular adhesions are forming and definitive attachment is achieved through microvillous interdigitations between trophoblast and uterine epithelium, culminating in placenta formation. Cellular interactions leading to attachment and initiation of placenta are likely mediated by developmentally regulated changes in the apical plasma membranes of uterine luminal epithelial (ULE) and trophoblast cells (Powell et al., 2000) [35].

Conclusion

It was concluded that there is a preponderance of high molecular weight proteins during follicular phase and higher proportion of low molecular weight proteins appear during early pregnancy in goat uterus.

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Conflict of interest: The authors have no conflict of interest.

Authors Contribution

Part of MVSc research work carried out by Surya Prakash Pannu under the guidance of Prof. Govind Narayan Purohit.

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