Ascorbic acid in buffalo ovary in relation to oestrous cycle

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Received 22 May 1998; revised 30 November 1998

Concentration of ascorbic acid was determined in different parts of buffalo ovary at four different stages of oestrous cycle viz. early luteal, mid luteal, late luteal and follicular. The stages were decided from the physical and morphological examinations of corpora lutea. The ovary was dissected in three components viz. corpus luteum, follicular fluid and ovarian stromal tissue for ascorbic acid assay. Corpus luteum showed significant change in concentration of ascorbic acid with the advancement of oestrous cycle, value being highest in late luteal stage. Follicular fluid and ovarian stromal tissue did not show significant changes in ascorbic acid at any stage of the oestrous cycle. Small follicles, irrespective of the stage of oestrous cycle had, however, significantly higher ascorbic acid content than large follicles.

Ascorbic acid occurs at several times higher concentration in endocrine glands than in serum or other organs of the animals1. The significance of such high amount of this vitamin in those glands is not clear. Gonads are also rich sources of ascorbic acid. Ovarian slices have been known to concentrate radio-labelled ascorbate in culture2. The present paper describes the distribution profile of ascorbic acid in corpus luteum (CL), follicular fluid (FF) and ovarian stromal tissue (OST) at four different stages of oestrous cycle in buffalo. Changes during follicular growth and comparison between healthy and artetic follicles are also presented.

Buffalo genitalia were obtained from local abattoir and brought to laboratory in ice. Uterine horns were checked for pregnancy and ovaries from non-gravid uteri were removed. CL were dissected out and classified into four categories of the oestrous cycle on the basis of appearance, consistency, vascularization, diameter and weight. The four categories were termed as stage I (early luteal), stage II (mid luteal), stage III (late luteal) and stage IV (follicular) of oestrous cycle. The tissues were washed with chilled saline, blotted dry, weighed and kept at -20°C till use.

Follicular fluid was collected from all surface follicles of each ovary by aspiration and pooled stagewise in four groups. The pooled FF samples were centrifuged at 2000 rpm for 10 min at 4°C to remove granulosa cells and cell debris. The supernatant was used for ascorbic acid assay. A portion of stromal tissue was also dissected out from each ovary and washed thoroughly with chilled saline and stored at -20°C. Ascorbic acid was assayed spectrophotometrically by the method of Zannoni et al.3 in CL, FF and OST. In a separate experiment, irrespective of the stage of oestrous cycle, FF was pooled on the basis of size of the follicles. The diameters of the surface follicles were measured and grouped as small (2-4.9 mm), medium (5-9.9 mm) and large (≥10 mm) follicles. Each pool of FF was then assayed for ascorbic acid as earlier.

In farm animals like cattle, the physical characteristics of CL have been found to indicate successfully different stages of oestrous cycle4. Although information on buffalo CL is relatively less as compared to that of cattle, identification of stage of oestrous cycle is possible in buffaloes by studying the physical appearance of CL. Our observation has further revealed that the concentration of ascorbic acid in CL changes significantly with maturation and regression of CL (Table 1). The concentration of ascorbic acid increased rapidly from stage I to II and II to III when it reached its peak. This was followed by sharp decline in stage IV. The concentration of ascorbic acid in FF and OST remained more or less similar throughout the oestrous cycle. Moreover, the concentration of ascorbic acid was several fold higher in CL of stage II and III than those in stage I and IV as well as in FF and OST. This indicates that the high rate of synthesis or accumulation of ascorbic acid may be related to the growing luteal tissue. The concentration of ascorbic acid in FF decreased significantly with increase in follicle size from small to medium and nonsignificantly from medium to large (Table 2). This is probably due to rapid increase in fluid volume in the growing follicles. Large follicles are known to be more dependent on LH than small follicles. This may also be a reason for low ascorbic acid in large follicles since depletion of ascorbic acid.
found a positive correlation between ascorbic acid and hydroxyproline concentration in bovine CL. In addition, CL produces both, oxytocin, a peptide hormone and progesterone a steroid hormone in large quantities. Thus, it is likely that ascorbic acid may have some role in regulation of synthesis of these hormones. Progesterone profile of buffalo CL[12] shows a pattern of increase and decrease with different stages of oestrous cycle as we have observed (Table 1) in ascorbic acid concentration. Unlike CL, granulosa cells of growing follicles synthesize estrogen in large quantities. Whether this different steroidogenic behaviour of CL and follicle is related to different ascorbic acid content in these two components of ovary needs to be investigated. We have shown here that CL in its functional stage is a rich source of ascorbic acid while the other parts of ovary contain comparatively much lower amounts. Ascorbic acid is perhaps not involved in follicular development or atresia but in growth and function of luteal cells only.

References
1 Levine M & Morita K (1985) Vitamins Hormones 42, 1-64

Table 1—Distribution of ascorbic acid in different parts of buffalo ovary at different stages of oestrous cycle

<table>
<thead>
<tr>
<th>Parts of ovary</th>
<th>Stage of oestrous cycle</th>
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<tr>
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<td>I</td>
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<tr>
<td>Corpus luteum (mg/g tissue)</td>
<td>0.34</td>
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<tr>
<td>Follicular fluid (µg/ml)</td>
<td>18.79</td>
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<tr>
<td>Ovarian stromal tissue (µg/g tissue)</td>
<td>127.78</td>
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</tbody>
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Values within row with different superscripts differ significantly from each other (p<0.05).

Table 2—Concentration of ascorbic acid in FF collected from variable sized follicles

<table>
<thead>
<tr>
<th>Size of follicle</th>
<th>Concentration (µg/ml)</th>
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<tbody>
<tr>
<td>Small (n=288)</td>
<td>25.41±3.94*a</td>
</tr>
<tr>
<td>Medium (n=90)</td>
<td>16.82±1.51b</td>
</tr>
<tr>
<td>Large (n=30)</td>
<td>14.52±3.15c</td>
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Figures within column with different superscripts differ significantly (p<0.05)