International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:7, No:3, 2013

A Study of the Cyclic Variations of the Enzyme and the Electrolyte Activity in Uterine and Oviducal Secretions during an Estrous Cycle of the Ewe

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***Abstract*—**Uterine and oviducal fluids are necessary for capacitation of the spermatozoa and early embryonic development. The aim of the present study was to determine the effects of estrous cycle phases (follicular and luteal) on some biological parameters (enzymes, electrolytes and total proteins) in uterine and oviducal secretions of ewes. Oviducal and uterine fluids were collected, diluted and centrifuged. According to our results, concentrations of GPT, G6PDH, total proteins, K and Na were significantly ($P < 0.05$) higher at the luteal phase, however, the levels of aldolase, Mg, Ca and P were significantly ($P < 0.05$) higher at the follicular phase in uterine secretions. While, only oviducal K and Ca were significantly ($P < 0.05$) higher at the follicular phase. Our study revealed the existence of significant cyclic variations for some uterine and oviducal parameters which indicates the effect of ovarian hormones on the components of genital secretions.

***Keywords*—**Biochemical parameters, estrous cycle, ewe, genital secretion.

I. Introduction

THE collection of female reproductive tract fluids and attempts to investigate the role of these fluids in gamete transport and metabolism have been the subjects of several studies [1]–[2]. The deterioration of the chemical composition of these secretions results from the deficiency of uterine and oviducal metabolism. The very small quantity of these secretions constituted always a factor limiting the study in vivo of the cyclic variations of the elements constituting these secretions.

The oviducal and the uterine fluids represent the aqueous medium in which male and female gametes and the embryos are suspended. These fluids are a complex mixture of ions and macromolecules solubilized in water. Some components are derived from the serum; while others are synthesized in the epithelium and secreted in the lumen [3].

Rather complete data on secretion rates and certain chemical components are available for oviducal and uterine fluids from rabbits [4]. Less complete data are available for oviducal and uterine fluids in ewes [5] and uterine fluids in cows [6].Intensive research was carried out on the cyclic changes which occur in the female during the estrous cycle. Studies on some species (rabbit, sow, cow and ewe) bring back the existence of the possible cyclic variations of proteins, carbohydrates, and minerals of genital secretions.

The aim of the present work was to determine the composition of these secretions and the influence of the periods of the estrous cycle (follicular and luteal) on some components (electrolytes, enzymes and total proteins) of these secretions.

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II. Material and Methods

A. Animals

A number of 20 reproductive tracts collected from mature local breed ewes slaughtered in the public abattoir of Batna (North of Algeria) immediately after slaughter and brought to the laboratory in ice. The matrices were classified in follicular and luteal phase by a visual examination of the ovaries surface.

B. Collection of Uterine and Oviducal Secretion

The uterine fluid was collected by gentle scraping the endometrium by a curette, transferred in 2ml sec tubes, diluted then centrifuged. However, the oviducal fluid was collected by aspiration with an automatic pipette, diluted and centrifuged. The samples stored in a -80° C freezer until examination.

C. Serum

The serum was collected with heparinized tubes of 5ml in one of the jugular veins of the ewe before slaughtering.

D. Determination of the Biochemical Parameters

For the determination of the levels of ions (calcium, phosphorus), the enzyme activity (Aldolase, glucose-6-phosphodeshydrogenase G-6-PDH, sorbitol deshydrogenase SDH, glutamic oxaloacetic transaminase GOT, glutamic pyruvic transaminase GPT) and the total proteins concentrations, we used an analyzer automat. Whereas, for the determination of sodium, chlorinate and potassium concentrations, we used an ionogramme. Concerning the determination of magnesium levels, we used the spectrophotometer with atomic absorption.

E. Statistical Analyses

The statistical analysis was carried out using the software Graphpad PRISM 5. The cyclic variation of each element in serum and genital secretions was evaluated using a test of Student "T". Data are presented as the mean ± s.e.m. P <0.05 was taken to denote statistical significance. The relation between the concentration of a given element in the serum and genital secretions was obtained by the R or coefficient of regression according to the test of correlation.

III. Results and Discussion

The results concerning enzyme, electrolyte activity and total proteins in uterine and oviducal secretions and even in serum during the two phases of the estrous cycle are presented in the Table I.

TABLE I
CONCENTRATIONS OF THE DIFFERENT BIOCHEMICAL PARAMETERS IN UTERINE, OVIDUCAL SECRETIONS AND IN THE SERUM

N=20	Parameters	Follicular	Luteal	p
Uterine secretion	Glutamic oxaloacetic transaminase(UI/l)	144.5 ± 34.52	274.9 ± 76.54	0.14
	Glutamic pyruvic transaminase*(UI/l)	39.88 ± 10.53	146.0 ± 34.76	0.01*
	Aldolase(UI/l)	273.1±58.69	95.53 ± 17.75	0.04*
	G6PDH (UI/l)	69.71±9.41	134.1± 17.85	0.01*
	SDH(UI/l)	61.01±16.32	20.47± 5.38	0.11
	Mg(mg/l)	9.05 ± 2.54	2.04 ± 0.38	0.01*
	Ca(mg/l)	59.57 ± 17.57	4.13 ± 1.18	0.0009*
	K(mmol/l)	25.80 ± 2.61	44.60 ± 6.25	0.02*
	Na(mmol/l)	115.2 ± 7.88	159.8 ± 7.96	0.003*
	Cl (mmol/l)	198.9 ± 5.70	273.1 ± 45.98	0.13
	P(mg/l)	90.38 ± 1.72	84.17 ± 2.05	0.03*
	Total proteins(g/l)	25.16 ± 5.18	124.5 ± 43.04	0.03*
Oviducal secretion	Mg(mg/l)	1.44 ± 0.64	2.71 ± 1.10	0.37
	Ca(mg/l)	232.1 ± 51.68	95.09 ± 16.05	0,01*
	K(mmol/l)	83.80 ± 5.00	56.81 ± 6.84	0,006*
	Na(mmol/l)	125.2 ± 19.60	115.9 ± 8.38	0.67
	Cl(mmol/l)	135.5 ± 7.03	150.9 ± 7.12	0.15
	P(mg/l)	100.2 ± 6.95	95.37 ± 3.57	0.54
	Total proteins (g/l)	37.88 ± 17.08	16.44 ± 7.86	0.31
Serum	Mg (mg/l)	1.54 ± 0.591	1.28 ± 0.59	0.77
	Ca(mg/l)	81.24 ± 5.73	56.95 ± 8.30	0,02*
	K(mmol/l)	3.62 ± 0.33	4.66 ± 0.40	0.14
	Na(mmol/l)	142.6 ± 1.25	143.0 ± 1.04	0.83
	Cl(mmol/l)	135.5 ± 7.03	109.2 ± 0.66	0.50
	P(mg/l)	76.49 ± 10.59	66.68 ± 5.76	0.50
	Total proteins (g/l)	64.82 ± 16.05	67.55 ± 2.79	0.87

*: Significant variation; N: A number of ewes used; P: Statistical difference between columns (P < 0.05).

A. Enzyme Activity of the Uterine Secretion

Reference [7] revealed the presence of GOT, without however showing significant variations (P > 0.05). Also the concentration of GOT measured in our study did not show any significant difference (P > 0.05) between the follicular and the luteal phase. However, the statistical analyses of our results showed a very significant cyclic variations (P=0.01) of GPT activity in uterine secretions, this activity was larger at the luteal phase. Reference [7] showed the same results after a study on the cow. Uterine secretions of the ewe are very rich in amino acids, in particular acid glutamic, glutamine, serine, and especially glycine [8].

The comparison of the results of the follicular period to those of the luteal period revealed that the activity of the aldolase was significantly higher (P < 0.05) during the first phase of the estrous cycle.

The statistical analyses of our results showed significant cyclic variations (P < 0.05) of the activity of this enzyme in uterine secretions; this activity was higher at the luteal phase, which agrees with the work made on the rabbit [6] and on the cow [7].

We noted the presence of sorbitol deshydrogenase in the uterine secretions with concentrations which were varied from a phase to another. Although the SDH did not show significant variation (P > 0.05).

B. Serum, Uterine and Oviducal Total Proteins

According to our results, the concentrations of uterine total proteins of the ewe were significantly (P < 0. 05) greater at the luteal phase. These results are similar to those reported in the studies on the cow [2] and on the woman and the ewe [3]. According to the reference [2], the exact interpretation of these variations is problematic, but it is thought that it is an important nutritional factor in the composition of the uterine milk: the protein catabolism and anabolism are larger at this period.

However, the statistical analysis of our results indicates that the concentration of oviducal total proteins was not significantly (P > 0.05) different between the two phases of the estrous cycle.

According to our results, no significant cyclic variation (P > 0. 05) was noted for the serum total proteins. [2] reported similar results, which indicates to us that the ovarian hormones don’t influence the rate of serum total proteins.

In the present study, we have searched the relation between serum total proteins and their uterine and oviducal counterparts during the two phases of the estrous cycle using a test of correlation. According to our results, no significant correlation was noted between serum and uterine total proteins (R=0.86 and P=0.33 follicular phase, R=0.94 and P=0.21 luteal phase) (P > 0.05) or oviducal total proteins (R=-0.83 and P=0.36 follicular phase, R = 0.87 and P = 0.35 luteal phase) (P > 0.05) during the two phases of the estrous cycle.

C. Serum, Uterine and Oviducal Ions

According to our results, except of calcium, all the other studied minerals (Na, K, P, Mg and Cl) were in higher concentration in uterine secretions than in serum. While, in oviducal secretion, only K, P and Ca were higher than in serum. II thus seems logical to believe that the liquid collected in the uterus and the oviduct doesn't constitute by a transsudat, but that it is well the product of an active secretion of uterine and oviduct cells.

In the present study, we noted that uterine potassium was significantly ($P<0.05$) higher at the luteal phase. It is necessary to take care not to carry a final judgment from the conclusions of only one author; the bibliography provides data which seem often contradictory.

Potassium levels in cows were considerably higher in tubal fluid than in plasma being the highest at or near estrus. High potassium levels in tubal fluid seem to be constant across species and it seems necessary for fertilization and the embryonic development [9]. According to our results, oviducal potassium concentrations varied in a cyclic and significant way ($P < 0.05$). The highest concentrations were observed during the follicular phase.

We can note in the present study that the potassium concentration in follicular phase and luteal phase was almost of approximately ten times higher in the uterus than in the serum.

According to our results, uterine sodium showed a very significant cyclic variation ($P<0.005$) with a marked increase at the luteal phase.

Concerning oviducal sodium, there was no significant difference ($P>0.05$) in the concentrations between the follicular and the luteal phase. According to the reference [10], in the buffalo, concentrations of the oviducal sodium were not different among the phases.

The phosphorus varied in a cyclic and significant way ($P<0.05$) in uterine secretions of normal cows. In the present study, phosphorus showed significant cyclic variations ($P<0.05$) with higher concentrations at the follicular phase. These cyclic variations would be then necessary to the "capacitation" of the spermatozoa.

However, oviducal phosphorus did not show any significant cyclic variation ($P>0.05$). Oviducal phosphorus concentrations are higher than in serum, this indicates an active secretion by the cells of the oviduct.

Serum phosphorus did not vary significantly ($p>0.05$) during the estrous cycle in the cow [11].

According to our results, uterine calcium showed high significant cyclic variations ($P < 0.0005$).We can explain this high concentration at the follicular phase by the important role which the calcium plays in the capacitation. The spermatozoa during its presence in the female genital tract use the calcium of the genital secretions for varieties of functions especially for the capacitation.

The concentrations of the ions calcium during the follicular phase were higher than the luteal phase and also higher than the serum concentrations luteal phase. Whereas, at the luteal phase, concentrations were close in the two compartments serum and oviduct.

According to our results no significant correlation was noted between serum and uterine calcium during the two phases of the estrous cycle. Also, no significant correlation was noted between serum and oviducal calcium.

In the present study, the concentrations of the ions magnesium were close in the two compartments blood and oviduct.

According to our results, blood magnesium did not have any significant cyclic variation ($p > 0.05$). Also no significant correlation was noted between blood and uterine magnesium. We also noted, as in the uterine secretions, the absence of the significant correlation between oviduct and serum magnesium.

In our study, uterine Cl showed cyclic but not significant variations ($p>0.05$) with higher concentrations at the luteal phase.

In the present study, the concentrations of the ions Cl^- were close in the two compartments blood and oviduct.

Serum chlorinate did not have any significant cyclic variation ($P>0.05$) [3]. Same observation was reported in the present study. Also no significant correlation was noted between serum and uterine chlorinate. The absence of significant correlations was also between serum and oviduct chlorinate.

IV. Conclusion

Some constituent of uterine and oviduct secretions varied with the stages of estrous cycle. This may be a part of ovine uterus and oviduct modifications, which favor its physiological functions during the estrous cycle and is under the influence of the ovarian steroid hormones.

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