

Cyclic Variations of the Enzymatic Activity in Endometrial Secretions during an Estrous Cycle of the Cow

<Samira, Friha>¹, <Ramdhane, Guerri>²

¹Elhadj Lakhdar University, Batna Algeria
Samira.friha@gmail.com

²CHU Batna Algeria
Ramdhane.guerri@gmail.com

Abstract: An analysis of collected uterine secretions of the matrices of the bovine females related to five enzymes (aldolase, glucose-6-phosphodéshydrogénase (G-6-PDH), the sorbitol deshydrogenase (SDH), oxaloacetic glutamic transaminase (TGO) and pyruvic glutamic transaminase (TGP)). Our statistical analyses showed no significant cyclic variations of the activity of these enzymes in the uterine secretions; but they nevertheless have a tendency to be different between the two phases of which:

- The activity of the aldolase is slightly high during the estrogenic phase that in there progesteronic phase (8.618 ± 1.539 UI/L vs 6.253 ± 1.138 UI/L)
- The G-6-PDH presents a larger activity at the progesteronic phase (2.295 ± 0.809 UI/L vs 4.938 ± 1.761 UI/L)
- The SDH is higher during the estrogenic phase (0.2990 ± 0.0647 UI/L vs 0.220 ± 0.03 UI/L)
- Concerning transaminases (TGO and TGP) they present a light rise during the follicular phase

The variations observed can be the result of a regulation of the cel metabolism on the level of the uterus by the ovaries hormones.

Keywords: uterus; secretion; enzyme; estrus cycle.

1. Introduction

Uterine secretions constitute a medium favorable for the survival of the gamètes and of the embryo and the chemical incompatibility of its secretions are with the spermatozoides bull or with the blastocyste is accused in certain disorders of the reproduction in the mammals.

According to certain authors [1],[2],[3],[4],[5],[6] deterioration of the chemical composition of these secretions results from a uterine cellular metabolic deficiency.

The very small quantity of endometriales secretions obtained from a same animal, always constituted a factor limiting the study in vivo of cyclic variations of the elements constituting intra-uterine secretions.

The composition of the intra-uterine medium of the bovine female is considerably known. The analysis of some electrolytes, proteins and the glucose of endométriales secretions was made during the normal estrous cycle at the cow [1],[2],[3],[7],[8],[9],[5],[6]

In order to better include the enzymatic activity of uterine secretions of the cow, we chose to evaluate the activity of three enzymes of the metabolism of the glucides and of two

enzymes of the metabolism of the protids: the aldolase, glucose-6-phosphate deshydrogenase (G-6-PDH), the sorbitol deshydrogenase (SDH), oxaloacetic glutamic transaminase (TGO) and pyruvic glutamic transaminase (TGP).

Moreover, we evaluated the influence of the periods of the estrous cycle on the enzymatic activity of uterine secretions.

2. Material and Methods

2.1. Animals

Our practical study was begun December 2010 on 20 cows multipares with an average age of 3-10 years

The recovery of the samples unrolled on the level of the public slaughter-house of the town of Batna where a 1st examination on the animal upright then a 2nd after being slaughtered, this slaughter-house present the climatic conditions identical to those of the ambient environment of the town. And which receives cows of the east of Algeria where the food is based mainly on the straw, forages green and concentrated... etc.

2.2. Collect and classification of uterine secretions

The matrices of the cows, with any ovarian or uterine pathology, were collected from public slaughter-house of the town of Batna after being slaughtered and were transported

to laboratory under cold conservation. The matrices were classified in follicular and luteal phase by a visual examination of the ovaries which is based on the aspect of the cystic follicles and evolutionary stage of the yellow body according to the method of [10]

The uterine fluid was collected one gently scraping the endometrium by a scraper then one transfers secretions in tubes

dryness from 2 ml according to the method of [10]. En reason of the recovery of small quantities of secretions, we carried out dilution (a volume of 0.2 ml of uterine secretions is diluted in 3,8 ml of distilled water) then a centrifugation to 3000 G during 5 minutes for the elimination of the cellular remains. The supernatant was stored immediately with -80°C until its use.



Figure 1. Determination of the phase of the bovine reproductive cycle [10].

2.3 Enzymatic analyses

For the measurement of the catalytic activity of the enzymes: Aldolase, G-6-PDH, SDH, TGO, TGP., we used like apparatus an analyzer automat METROLAB 2300; whose distribution of the reagents and the samples thus the dilution of the latter are made in an automatic way in a programmed time. This automat has 9 wavelengths located enter 340 and 750 Nm.

2.4. Statistical analyses

The statistical analysis was carried out using the software Graphpad PRISM 5. The cyclic variations of each element

in uterine secretions of the ewes were evaluated using a test of " T " (Student). The homogeneity of the variance is checked by the test F the results are expressed in (average ±SEM). The statistical difference was considered significant when P < 0,05.

3 Results and Discussion

The analysis of intra-uterine secretions made it possible to detect an enzymatic activity as presented in Table 1.

Table 1: Proportioning of the enzymes of uterine secretions during two phases of the estrous cycle.

Biochemical parameters	Follicular phase NR=10	luteal phase NR=10	P
Aldolase(UI/l)	8.618 ± 1.539	6.253 ± 1.138	0.2448
TGO(UI/l)	8.750 ± 1.836	8.040 ± 1.105	0.7472
TGP(UI/l)	1.640 ± 0.3211	1.515 ± 0.5777	0.8538
G-6-PDH(UI/l)	2.295 ± 0.8093	4.938 ± 1.761	0.3223
SDH(UI/l)	0.2990 ± 0.0647	0.220 ± 0.033	0.3604

NR: Numbers of matrix used.

P: Statistical difference between column (P<0,05).

We know that uterine secretions are composed of a relatively significant quantity of acids amines and glucose. Each enzyme which we analyzed in uterine secretions plays a role in the use of these substrates, for thus taking part in the cellular metabolism.

Transaminases (TGO, TGP) of uterine secretions of the cow during an estrous cycle

According to [11] the acids amines of uterine secretions are formed and are metabolized under the action of the TGO and of the TGP. These enzymes act, moreover, like

intermediaries in the reactions of transamination, in order to provide to the uterus the principles necessary to its physiological activity.

[11] revealed the presence of the TGO, without however presenting significant variations (P > 0.05). Also the concentration of the TGO measured in our study does not present any significant difference (P > 0.05) between the follicular phase and the luteal phase but one can note that the concentrations of the TGO are higher during the follicular phase.

The statistical analyses of our results have shows no significant cyclic variations ($P=0.8538$) of the activity of the TGP in uterine secretions; this activity is larger with the estrogenic phase. These results are not in agreement with those reported by [11] after a study in the cow in of which a significant variation of the concentrations between the two phase of the cycle with a rise during the progesteronic phase.

Uterine secretions of the ewe are very rich in amino acids, in particular acid glutamic, glutamine, serine, and especially glycine [12],[13]. It was brought back by [14] that at the stage of morula the amino acid concentration increases enormously: approximately 20 times the value found at the peri-ovulatory phase (ewe, woman).

The dioestrals period of the cycle corresponds to that where the blastocyst is descended in the uterus, whereas the glands secrete very actively. The intra-uterine medium would thus carry on, during this phase, a significant anabolic activity, transaminases taking part in these processes.

The relatively high concentrations of the free amino acids present in secretions of the uterus indicate their importance like significant sources of the nutriment for the gametes or the zygote. Therefore the concentrations raised in amino acids during the luteal phase are in relation to the significant activity of transaminases during the same phase of estrous cycle.

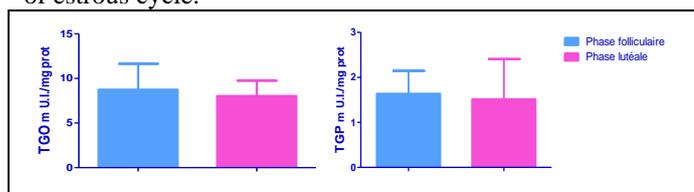


Figure 02: Rate of TGO and TGP in endometrial secretions of the cow during the estrous cycle.

Aldolase of uterine secretions of the cow during an estrous cycle

The aldolase is an enzyme which catalyses reaction 4 of glycolysis by which glucose is degraded in two molecules of pyruvate with the concomitant formation of two ATP.

The comparison of the results of the follicular period to those of the luteal period reveals us that the activity of the aldolase is stronger during the first phase of the estrous cycle but not in significant way ($P > 0.05$).

The spermatozoide of the mammals requires metabolic energy for a variety of functions, to support in particular the motility. The human spermatozoide obtains an exceptionally high proportion of their ATP starting from glycolysis [15].

One can say that the spermatozoïdes at the time of their presence in the genital tract female use the aldolase of uterine secretions for the energy production necessary for the various functions in particular the capacitation.

The Aldolase plays a significant role in the capacitation what explains the highest concentrations during the follicular phase, which agrees with the work made at the rabbit by [16] and in the cow by [11].

Glucose-6-phosphate deshydrogenase of uterine secretions of the cow during an estrous cycle

The glucose-6-phosphate deshydrogenase is the first specific enzyme of the way of pentoses phosphates. This way provides two precursors significant for anabolic ways: the $\text{NADPH} + \text{H}^+$ used for example for the biosynthesis of the fatty acids, and ribose 5 phosphates, a precursor of the biosynthesis of the nucleotides [17].

The activity of the G-6-PDH indicates to us that the intra-uterine medium, in addition to metabolizing glucose like direct source of energy, can degrade it by the way of pentoses and thus take part in the synthesis of the fatty acids and nucleotides.

The statistical analyses of our results showed no significant cyclic variations ($P > 0.05$) of the activity of this enzyme in uterine secretions; this activity is larger with the luteal, phase which agrees with the work made in the rabbit by [16] and the cow by [11] which found variation significant ($P < 0.05$).

According to [12], the embryo uses the way of pentoses to form the nucleic acids, and the lipids. The $\text{NADPH} + \text{H}^+$ used for the biosynthesis of the fatty acids, and ribose 5 phosphates, a precursor of the biosynthesis of nucleotides.

Therefore during the luteal phase, the activity of the way of pentoses increases to ensure the needs for the embryo in lipids and nucleic acids, which explains the high concentrations of glucose 6-phosphate deshydrogenase during the luteal phase.

Sorbitol deshydrogenase of uterine secretions of the cow during an oestrous cycle

The sorbitol was a long time known to be present in the fluids of the genital tracts females and males. Two major monosaccharides, sorbitol and fructose, are present in sperm [18]. Glucose is initially tiny room in sorbitol by aldose reductase, the sorbitol being then oxidized by the sorbitol deshydrogenase in fructose, with parallel reduction of cofactor NAD^+ in NADH [19];[20].

We noted the presence of sorbitol deshydrogenase in uterine secretions with concentrations which are varied from a phase to another. Therefore the intra-uterine medium of the bovine female can transform glucose into fructose by the action of the SDH on the sorbitol.

The sorbitol is the principal carbohydrate in the uterine fluids of the bovines. A function of SDH maintained in the spermatozoïdes seems to convert the sorbitol in seminal plasma and the uterine fluid into fructose, which represents a source of energy [18].

Although the SDH does not have present significant variations ($P > 0.05$), it nevertheless has a tendency to be higher during the estrogenic phase; it is at this time that the spermatozoïde could use the SDH of uterine secretions for the transformation of glucose into fructose and its to enter the glycolytic way; from there, a possible source of energy for the cells. Therefore the activity of SDH would provide the fructose necessary not only for the metabolism of the spermatozoïdes, but also for their capacitation and their advance until A the ovule [21].

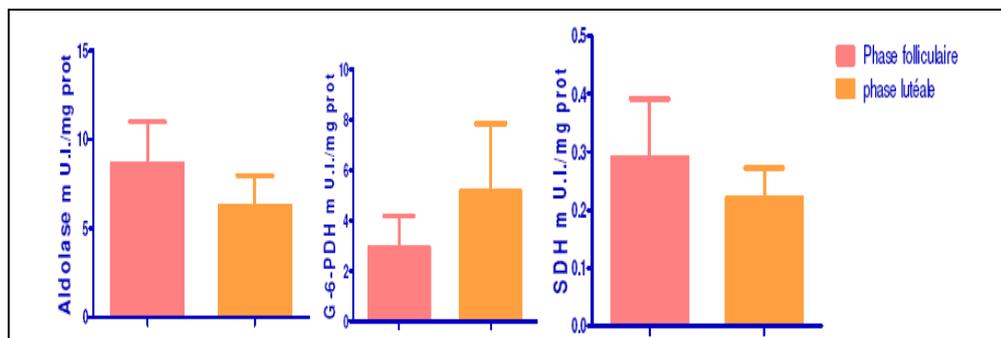


Figure 03: Rate of the aldolase, G-6-PDH, SDH in endometrial secretions of the cow during the estrous cycle.

3. Conclusion

We know now that the metabolism in the intra-uterine medium is active thanks to the enzymes present and that aldolase, the G-6-PDH, SDH and transaminases (TGO, TGP) present considerable cyclic variations even non significant.

These results indicate a uterine metabolic activity at the time of the various phases of estrous cycle. Therefore the ovarian hormones have an effect on the enzymatic activity of the uterus. It acts there of significant uterine functions, at the time of development of the biological environment necessary to the need for the spermatozoïdes, the ovule and the blastocyste.

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