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Enzymes Activity in Bovine Cervical Mucus Related to the Time of Ovulation And Insemination

S. Benbia, A.Kalla, M. Yahia, K. Belhadi, and A. Zidani

***Abstract*—**Forty-five dairy cows were used to compare the enzyme activity of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), **α** -amylase in the cervical mucus of cows during spontaneous and induced estrus using progestagen or PGF2 **α** and to determine whether these enzymes affect the fertility in cows with induced estrus, at the time of Al. The animals were assigned to 3 groups (no treatment, a Crestar® for 12 days, a double im injection of PGF2 **α).** The cows were artificially inseminated (AI). Cervical mucus samples were collected from all cows 3 to 5 min before the AI. The results are summarized as follows: ALP and **α** -amylase activity for spontaneous estrus were similar to those for induced estrus (P>0.05) . LDH activity levels during spontaneous and PGF2 **α** induced estrus was significantly lower ($P < 0.001$) than that in progestagene induced estrus groups. While no difference was found between the first and the third groups. Our result showed a significant difference in LDH activity levels between cows conceived with 2 or more AI and those conceived with 1 AI. The result of this study showed that the enzyme activity in cervical mucus is helpful for detection of ovulation and time of AI.

***Keywords*—**cervical mucus, dairy cow, enzyme, induced, estrus, ovulation, AI

Introduction

CERVICAL mucus is a mixture of mucins and cervical plasma [1] located between microbe-rich vagina and the normally sterile uterine cavity, it can be used only indirectly as an indicator of the stage of the cycle or of the hormonal state of the females in most domestic animal and human [2]. Therefore, it is important to know the enzyme composition of cervical mucus during estrus. Alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and **α** -amylase are present in semen and in cervical mucus from the cows and human [3]. The presence of α-amylase and lactate dehydrogenase (LDH) to the cervical mucus of women has suggested that it plays a role in sperm capacitation of spermatozoa [4]. In a previous studies the concentration of Alkaline phosphatase is inversely correlated with follicle size (Wise, 1987), decreased significantly at estrus just prior the ovulation and began to rise after ovulation when a corpus luteum is present [5,6].

All authors are from the University of Batna, Republic of Algeria Biotechnology's laboratory of the bioactive molecules and the cellular physiopathology (corresponding author to provide phone: 00 213 662 64 00 06; fax: 00 213 33 86 23 71E-mail: biasouhila@yahoo.fr.

It has been shown previously that LDH activity to be significant in cyclic changes in the cervical mucus of women, being high during the proliferative phase, gradually decreasing to the lowest level around ovulation, and then increasing markedly again [7,8]. These changes are under hormonal control [7].The present study is concerned with the determination of enzyme activity of ALP, LDH and α-amylase in the cervical mucus of dairy cows either at spontaneous estrus or induced estrus using progestagene or PGF2α at the time of artificial insemination (AI).

II. Materials And Methods

A. Animals

The experiment was conducted in eastern Algeria. Forty-five adult (n=45), reproductively normal, dairy cows aged 3-5 years (average body weight 400-500 kg), maintained under normal feeding and management conditions, were used. .All had produced at least one normal calf and had calved normally at least 80-100 days before the collection of cervical mucus. All were clinically normal as determined by rectal palpation at the time of sampling and had showed at least two periods of estrus before the treatment.

B. Synchronisation of estrus

The animals were divided into 3 treatment groups as follows:

-Animals in groups1 (n= 25): received no treatment, serving as the group of cows with normal spontaneous estrus.

-Animals in group 2(n=15): estrus was induced with an implant containing progestagene (CRESTAR ®, Intervet Schering Plough, Beaucouze, France). The day of the installation of the implant, an injection of 5 mg of estradiol valerate is managed the implant remains in place 12 days, an injection of eCG the day of the withdrawal of the implant is carried out. Five of these cows were excluded because of the inadequate quantity of cervical mucus collected.

-Animals in group 3 (n=5): were given a double injection of PGF2α, 12 days apart.

C. Cervical mucus sampling

All animals were carefully inspected for signs of oestrus. Standing to be mounted over 3 s was considered as the first sign of oestrus. Translucent cervical mucus samples were collected from all cows 5-3 min before AI, using a tube

stainless steel, 10 ml wide and 35 cm long [3]. At the other end of the tube a vacuum syringe was attached. Cervical mucus was aspirated from the external OS of the cervix and the area around it into vacuum syringes by gentle suction using a plastic, transparent tub [3]. Artificial insemination was conducted only after overt estrus. Samples were introduced into sterile plastic containers and stored frozen at -30 °C. Prior to use, they were thawed at 38 °C in a water bath.

For the measurement of the catalytic activity of the enzymes : alkaline phosphatase, lactate deshydrogenase and α -amylase, we used an analyzer METROLAB 2300.

D. Statistical analyses

Data were analyzed by Graph Pad Prism 5. One-way analysis of variance (ANOVA) was used to compare means of continuous variables on estrus days within each normal and synchronization croups. Independent T test was used to compare the insemination number between groups. The results are expressed as mean ± SEM. Statistical difference was considered significant when $P < 0.05$.

III. Results And Discussion

Data showing enzyme activity in the cervical mucus of the spontaneous and induced estrus groups are presented in table I.

TABLE I
MEAN (± SEM) OF CERVICAL MUCUS ENZYME ACTIVITY OF THE SPONTANEOUS AND THE INDUCED ESTRUS GROUPS

Enzyme activity	Estrus groups 1 (n=25)	2 (n=10)	3 (n=5)	P
ALP (U/g)	0,946 ± 0,20[a]	1,310 ± 0,33[a]	0,98 ± 0,30[a]	0.63
LDH(U/L)	6,52 ± 0,66[a]	13,29 ± 2,15[b]	4,98 ± 1,59[a]	0.003
α-amylase (U/L)	14,75 ± 0,959[a]	15,13 ± 1,6[a]	13,36 ± 2,60[a]	0.85

ALP =Alkaline phosphatase, LDH = lactate dehydrogenase
a,b : Values with different superscripts in each column are those that differ significantly (P<0.05).significantly (P<0.05).significantly (P<0.05).

Enzymes of cervical mucus have an important role to play by providing energy for sperm motility, survival and transport in the female genital tract.

α-amylase, enzymes involved in the degradation of glycogen, sperm capacitation [4]. The amylase activity in the present work was similar to those found by [3]. [9] reported that α-amylase levels were relatively higher in human cervical mucus during the pre- and postovulatory phases of the cycle.

The similarity of ALP activity in cervical mucus during the spontaneous and induced estrus has been previously described [3]. Estradiol-17β levels increased in buffalo plasma after induction of estrus by PGF2α or cloprostenol and this is related to luteolysis and growth of the follicle [10].

Despite in the present study, there was no significant difference in ALP and α-amylase activities in mucus cervical among the estrus groups, while LDH activity was significantly higher (P=0.003) in cervical mucus collected from cows in the second group than in the first and third estrus groups. There was no significant ($P \geq 0.05$) difference in LDH activity between the spontaneous and PGF2α induced estrus groups.

Lactate dehydrogenase activity has been studied in human cervical mucus [7] and cows [3]. The LDH activity in human cervical mucus was found to be inversely related to luteinizing hormone (LH) [7]. Many studies reported that the oestrogens decrease the activity of the LDH, contrary to progesterone increase it [11] [8].

To determine the relationship between the LDH activity and fertility, we have grouped the cows of first and third group because there were no differences between these two groups. Those cows be then classify in two groups, the first comprise the cows conceived with one AI and the second contains those conceived with two or more AI (Table II).

TABLE II
RELATIONSHIP BETWEEN THE LDH ACTIVITY IN CERVICAL MUCUS AND THE AI NUMBER IN DAIRY COWS (MEAN ± SEM)

Enzyme activity	AI number 1 (n=10)	≥2 (n=20)	P
lactate dehydrogenase LDH (U/L)	2.83 ± 0.58[a]	8.07 ±0.51[b]	< 0.001

a,b = Values with different superscripts in each column are those that differ significantly (P<0.05).
AI= artificial inseminations

There was significant difference (P< 0.001) in LDH activity between cows conceived with 1 and 2 or more artificial inseminations. Therefore, it would be useful to study the relationship between LDH activity in mucus cervical and steroids hormones in cows.

IV. Conclusion

The results from the present study show that there is a significant difference in LDH activity in cervical mucus between the progestagene induced-estrus groups compared to those in the spontaneous and PGF2α induced estrus groups. There was no significant difference between the spontaneous and PGF2α induced estrus groups. LDH activity in cervical mucus was significantly lower in cows conceived with 1 AI compared to those conceived with 2 or more artificial inseminations. We conclude that it is important to consider enzyme mesurment and hormonal assay together for the evaluation of the time of AI and ovulation in dairy cow.

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