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دراسة بعض الانزيمات في مصل الدم وغسول الرحم لاناث الفئران الغير بالغة والبالغة خلال دورة الشبق

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أظهرت هذه الدراسة النتائج التالية :

- في مجموعة الفئران الغير بالغة زاد نشاط انزيم الاميليز، وانزيم ليوســـين أمينوبيبتيداز (ل•أ•ب)في مصل الدم • بينما لم يكن هناك نشاط لانزيم الاميليز في غسول الرحم لهذه المجموعة من الفئران•
- في مجموعة الفئران البالغة اتضح الآتـــي:

١ـ عدم وجود نشاط لانزيم الاميليز في غسول الرحم في نهاية مرحلة الشـــبق بينما زاد نشاطه جدا في مرحلة مابعد الشبق• أما بالنسبة لنشاط هـــذا الانزيم في مصل الدم فقد كان مرتفعا في مرحلة ماقبل الشبق وكذلك فـــي بداية مرحلة الشبق•

٢ـ زيادة نشاط انزيم الليبيز في غسول الرحم في نهاية مرحلة الشبق ثـــم انخفض فجأة في مرحلة مابعد الشبق• بينما كان نشاط الانزيم يـــزداد تدريجيا بدء من مرحلة ماقبل الشبق حتى وصل الى قمة نشاطه في بدايـــة مرحلة الشبق•

٣ـ انخفاض نشاط انزيم (ل•أ•ب) في غسول الرحم خلال مرحلة ماقبل الشـــبق ثم زاد نشاطه خلال مرحلة الشبق• أما بالنسبة لنشاط هذ الانزيم في مصـل الدم فقد كان كبيرا في مرحلة ماقبل الشبق ثم انخفض تدريجيا في المراحل التالية من دورة الشبق•

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STUDIES ON SOME HYDROLYTIC ENZYMES IN THE SERUM AND UTERINE WASH OF IMMATURE AND MATURE FEMALE RATS DURING ESTROUS CYCLE

(With One Table)

By
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SUMMARY

The cheracteristic features of immature rats were the high serum amylase and LAP activities. The activities of the amylase in the uterine wash of immature rats were absent. Amylase activity was absent from uterine wash during late estrus and very high during metestrus. Serum amylase was high during late proestrus and early estrus. Lipase activity was very hgih in uterine wash during late estrus then dropped suddenly during metestrus. Serum lipase showed a gradual increase from proestrus reaching its peak during early estrus.

LAP activity in uterine wash was very low during proestrus and became high during early and late estrus. Serum LAP activity was high during proestrus then declined gradually during the following stages.

INTRODUCTION

The direct analysis of enzymatic content of the uterus revealed that it has four enzyme systems capable of hydrolyzing the spermatogenic antigens, namely alpha amylase, lipase, leucine aminopeptidase (LAP) and alkaline phosphatase (KATSH et al., 1968). Moreover, it has been postulated that alpha amylase, phospholipase and lipase enzymes were almost effective in hydrolyzing spermatogenic antigens (ASA). The same authors also reported that alpha amylase and lipase are provided by the endometrium. It has been found that, amylase enzyme could be detected in the oviduct and uterus of a number of animal species (Mc GOACHIN et al., 1958 and HAFEZ and WHITE, 1968). This amylase activity in the endometrium of ewe showed cyclic changes where its level increased during follicular phase of the estrous cycle.

Lipase enzyme is present in the uteri and sera of mice, rabbits and rats (KATSH et al., 1968). This enzyme was among the most effective of the authentic enymes in hydrolyzing the sperm antigens. In addition phospholipase and lipase are effective in hydrolyzing spermatogenic antigens. The authors suggested that lipase enzyme is provided by the uterus.

KATSH et al. (1968) reported that LAP is present in the uteri and sera of Guinea pigs. Moreover, ZAVY et al. (1984) found that the enzyme LAP is also present in the uterine flushings of non-pregnant and pregnant gilts, where the activity of the enzyme increased significantly between days 10 and 12 of the estrous cycle. This enzyme is among the most

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effective enzymes in hydrolyzing the spermatogenic antigens. LAP also inactivated completely spermatogenic antigen. In additon the endometrial enzyme content is significantly different from that of serum. They suggested that the endometrium provides its own enzyme contents.

The role of alpha amylase, lipase and aminopeptidase (LAP) in the inactivation of sperm antigens called for further investigations to evaluate these enzymes in the serum and uterine wash of the female rats during different phases of the estrous cycle.

MATERIAL and METHODS

Seventy mature and ten immature female Albino rats were used in this experiment. Vaginal smears were obtained from each mature rat twice daily; early in the morning and at late afternoon. The smears were dried with a flame and stained for one minute with 1% methylene blue and examined under the microscope. When the females showed two successive regular (4 days) estrous cycles they were selected and classified according to vaginal smear picture into seven groups of ten rats each, representing early proestrus (P_1 at 09.00 hr.), late proestrus (P_2 at 16.00 hr.), early estrus (E_1 at 09.00 hr.), late estrus (E_2 at 16.00 hr.), metestrus (M at 0.9.00 hr.), early diestrus (D_1 at 0.9.00 hr.) and late diestrus (D_2 at 16.00hr.).

At the end of the experimental period, all rats were kept starved for 12 hours. Blood samples were collected from each rat, by intraoccular puncture, allowed to clot and serum was obtained by centrifugation. The animals were sacrified and each uterus was ligated at its free end and removed quickly. Uterine wash was obtained by reverse flushing of the horns using 0.5 ml phosphate buffer saline (pH 7.4) (ALBERS et al., 1961).

Serum samples and uterine washes were kept frozen at -20 C. Amylase activity in the serum and uterine wash was estimated following the method of SMITH and ROE (1949). Lipase activity of the serum or uterine wash was determined following the technique of ZAKARIYA et al. (1971). Leucine aminopeptidase (LAP) activity of the serum or uterine wash was estimated using the method of NAGEL et al. (1964).

RESULTS

Amylase, lipase and leucine aminopeptidase (LAP) activities in the serum and uterine wash of immature and mature female rats during the estrous cycle are presented in table (1).

It can be noticed that amylase level in the serum was significantly high in immature rats. The analysis of variance showed that there are significant differences ($P < 0.01$) between the amylase activity during the different phases of the estrous cycle. The highest level in mature rats occurred at early estrus and the lowest level occurred during late diestrus.

Ragarding amylase activity in the uterine wash it was obvious that it was low during early estrus and completely absent during late estrus, whereas, it was very high during metestrus.

As shown in table 1, lipase activity in the serum of immature rats was significantly lower ($P < 0.05$) than its activity throughout the different phases of the estrous cycle in mature rats. The analysis of variance showed highly significant differences between lipase activities in the serum during the different phases of the estrous cycle, with the lowest level recorded during late proestrus and the highest level during early estrus.

Lipase activity in the uterine wash was completely absent in immature rats as well as in mature rats during proestrus and diestrus (table 1). Analysis of variance revealed the

presence of highly significant differences between lipase activity during the different phases of the estrous cycle. It reached a high level during late estrus; decreased abruptly during metestrus and disappeared completely during early and late diestrus.

It was clear that LAP activity in the serum decreased with the progress of the stages of the estrous cycle starting from proestrus toward diestrus. Serum enzyme activity at early estrus, late estrus, metestrus, early diestrus and late diestrus were significantly (P/ 0.05) lower than that of immature rats. LAP activity during early proestrus was significantly (P / 0.05) higher than at any other phase of the cycle except at late proestrus. LAP activity in the uterine wash was completely absent in immature rats. In mature rats the LAP activity was at its lowest level during early and late proestrus then it increased during early and late estrus and metestrus. At last it tended to decrease again during early and late diestrus (table 1).

DISCUSSION

The results obtained in the present study revealed that, the uterine wash content of amylase showed cyclic variations. Maximal concentration was recorded during metestrus (table 1). This correlated with a significant drop in its level in the serum. HAFEZ and WHITE (1968) noticed that endometrial homogenate of the ewe during the period of implantation, that is the postovulatory stage, contained very large amounts of amylase. This refers to progesterone which is available at this time of the cycle to be responsible for the accumulation of amylase in the uterus.

The physiological significance of alpha amylase attracted the attention, because of its possible role in capacitation of spermatozoa (KIRTON and HAFS, 1965). These investigators found that incubation of rabbit sperms with beta amylase in phosphate buffered Lock's solution resulted in capacitation just as the capacitation induced by uterine fluids obtained during estrus in vivo and in vitro.

The activity of lipase in the uterine wash was completely absent during the stages of early and late proestrus, then it started to appear at a low level during early estrus, then showed a pronounced increase during late estrus. This was followed by a rapid drop during metestrus and disappeared again during early and late diestrus (table 1). The serum levels of lipase showed a significant increase in the different phases of estrous cycle when compared with that of immature rats.

The cyclic variation in the activity of lipase in the serum and lumen fluids of the uterus seems to be linked with the blood level of estrogen and progestins. NEMETALLA (1979) showed that incubation of spermatozoa with lipase for 18 hr. at 37 C resulted in detachment of their heads. This indicated that the site of action of lipase was localized to the neck region and the two fragments remain intact. This might be a factor which favours separation of the sperms head at syngamy. Thus the appearance of lipase in the uterine wash during late estrus for a short duration seems to be favourable for the fertilization process.

LAP activity increases in the uterine tissues of rats during estrus (SCHMIDT et al., 1968). In the present study it was found that LAP contents of the uterine wash was relatively high during early and late estrus, table (1). This high level extended during metestrus then it declined suddenly during diestrus and remained low as during proestrus. This seems to be an indication of a dual function of this enzyme. First it is expected to play a role in starting and completing the processes of capacitation and acrosome reaction. It is supposed also to play an imporatant role in liquifaction of the postcoital sperm plug. Finally after fertilization

it is expected to remove the sperm antigens called aspermatogenic antigen (KATSH and KATSH, 1966). By this way, formation of antibodies aainst spermatozoa in the female is avoided (KATSH et al., 1986). Thus LAP could be considered as an activator to sperms during the phase of estrus and when its action is prolonged it becomes an inactivator. It provides continuous fertility of the female despite repeated exposure to foreign antigens, which would prevent reproduction of the species.

The levels of LAP in the serum of female rats also showed cyclic variations as shown in table (1). Maximum activity of LAP was observed in the serum during the two phases of proestrus then it declined to almost half of its concentration during the remaining stages of the cycle. It appears that there is a reverse relation between the serum level of LAP and its level in the uterine fluids. This opens the discussion of whether the enzyme is innate of the uterine tissues or is under the control of a pump directing it from the blood to the uterus. The present findings seem to indicate that LAP is formed in the uterus rather than being collected from blood.

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Table (1): Amylase, lipase and leucine-aminopeptidase activities in the serum and uterine wash of immacture and mature rats during the estrous cycle. (i.u./L).

Item	Amylase		Lipase		Leucine-aminopeptidase	
	Serum	Uterine wash	Serum	Uterine wash	Serum	Uterine wash
Immature rats :	520.27±20.36	46.52±14.80	9.78± 2.13	00.00±0.00	49.40±6.70	00.00±0.00
Mature rats :						
Early proestrus	413.15± 8.40**	92.49±31.45	53.45±11.08**	00.00±0.00	38.60±2.27	3.33±0.88**
Late proestrus	447.76±28.25	48.30± 6.54	27.90± 3.65**	00.00±0.00	37.60±1.16	3.00±0.58**
Early estrus	524.60±28.41	31.40± 7.30**	56.38± 6.73**	2.84±0.57**	26.60±1.63*	13.86±3.69**
Late estrus	497.35±30.81	00.00±00.00	51.10±10.39**	28.82±4.22**	28.20±2.26*	13.50±2.74**
Metestrus	437.50±10.36**	191.26±29.37**	42.76± 3.91**	6.05±2.74*	30.37±2.84*	11.50±1.77**
Early diestrus	460.60±18.18	104.11±29.37*	30.83± 7.83*	00.00±0.00	23.40±3.44**	5.00±1.16**
Late diestrus	359.99+10.41**	54.71+ 4.91	35.53+ 4.34**	00.00+0.00	24.40+4.03*	7.00+2.31*

± : Standard error.
* : Significantly different from immature group at (P/ 0.05).
** : Significantly different from immature group at (P/ 0.01).