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LACTATE DEHYDROGENASE AND PHOSPHATASES ENZYMES LEVELS IN OVIDUCAL FLUSHING FLUID OF EGYPTIAN BUFFALO-COWS WITH ACTIVE AND INACTIVE OVARIES

(With OneTable & 2 Figures)

By

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مستويات إنزيمات اللاكتيت داى هيدروجنيز والفوسفاتيز فى قناة فالوب لإناث الجاموس المصرى فى حالات نشاط وخمول المبيض

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تم فى هذه الدراسة إيجاد العلاقه بين إنزيم اللاكتيت داى هيدروجنيز وأيضا إنزيمات الفوسفاتيز فى قناة فالوب للجاموس المصرى ونشاط المبيض وكذا حاله خمول المبيض. تم إجراء عملية الغسل (باستخدام محلول KRB) لمائه جهاز تناسلى لإناث الجاموس المصرى. وتم تقسيمها حسب التركيبات المختلفه على سطح المبيض وأيضا عدم وجود أى تركيب، بعد تجميع هذا الغسول يتم وضعه فى جهاز الطرد المركزى ثم يأخذ السائل الموجود فى الطبقة العليا ويتم تقيين إنزيمات اللاكتيت داى هيدروجنيز، الفوسفاتيز الحامض والقاعدى. ووجد فى هذه الدراسة أن مستوى إنزيم اللاكتيت داى هيدروجنيز يزداد بزيادة معنوية فى حاله Follicular period بينما هناك نقص معنوى فى حالة خمول المبايض. وكذلك وجد أن فى حالة انزيم الفوسفاتيز القاعدى لا يوجد فروق معنوية بين حالات الدراسة بينما أثبتت الدراسة أن هناك زيادة معنوية لانزيم الفوسفاتيز الحامض فى حالة Luteal period بالمقارنه بباقى الحالات. أما فى حالة النسبة بين اللاكتيت والبيروفيت وجد أنها تقل معنويا فى حالة خمول المبايض عنها فى حالة نشاط المبيض وبعد هذه الدراسة تم التوصل إلى أن هناك علاقه بين الحالة الهرمونية لنشاط المبيض فى إناث الجاموس المصرى ومستوى الإنزيمات السابقة.

SUMMARY

The levels of lactate dehydrogenase (LDH) and phosphatases enzymes were studied in the oviducal flushing fluid of Egyptian buffalo-cows during cyclic and inactive ovaries. The oviducal flushing was performed by using Kreb's Ringer Bicarbonate (KRB) solution in one hundred buffalo-cows genitalia. The level of LDH was found to increase significantly during follicular period of the oestrous cycle but it decreased significantly during ovarian inactivity. The concentration of alkaline phosphatase (ALP) was observed to increase (non- significantly) with increase (significantly) in acid phosphatase (ACP) during luteal period of the oestrous cycle. However, during ovarian inactivity, the concentration of ACP decrease significantly as compared to cyclic ovaries and the observed decrease in ALP was non-significant. Lactate/pyruvate ratio was recognized to decrease significantly in static ovaries and there is no significant variation between follicular and luteal period of the oestrous cycle. In conclusion, the condition of the ovaries either cyclic (follicular and luteal periods) or inactive could influence the biochemical concentrations of LDH, ALP, ACP enzymes and the lactate/pyruvate ratio.

Key words: *Dehydrogenase-phasphatase-oviduct- buffaloes*

INTRODUCTION

The relationship of free fluid within mammalian oviduct to various aspects of the reproductive process has received little attention *(PERKINS, 1974)*. Fallopian tube is an important organ related to fertility of the animals *(PANDYA, et al., 1994)*. Fluid found within the normal oviducal lumen contains many constituent, some of which vary in concentration due to endocrine influences *(BRACKETT and MASTROIANNI, 1974)*. Mammalian oviducal fluid provides an environment for gamete transport,

maturation of the ovum, sperm capacitation and subsequent acrosome reaction, fertilization and early embryonic development. Knowledge of the composition of oviducal fluid is of fundamental importance to duplicate, in vitro, the physiological condition for fertilization *(BRACKETT and MASTROIANNI, 1974).*

Oviducal cells have been shown to secrete factors benifecial to embryo development *(GANDOLFI, et al., 1989)* and the conditioned medium has been show to support the embryo development at rates similar to those obtained with co-culture *(EYESTONE and FIRST, 1989).* Oviducal fluid is a combination of secretion and transudate *(LEESE, 1988).* Therefore, it is produced by selective transudation of blood constituents and secretion of specific constituents by the endosalpinx *(FEIGELSON and KAY, 1972).* all mammalian species, the oviducal epithelium secretes lipids, glycogen, enzymes which controlled by steroid hormones *(WITKOWSKA, 1979 and VERHAGE, et al., 1990).* In addition, the oviducal secretion is high in lactate, pyruvate, sodium and calcium ions *(PINEDA, 1989).*

The oviducal energy sources are pyruvate, glucose and lactic acid which found in the oviducal fluid of the cow *(ELLIOTT, 1974).* Lactic acid utilized directly by the embryo or after conversion to pyruvate by the LDH present *(GIBSON and MASTERS, 1970).* LDH is an oxidoreductase enzyme whose activity is necessary for the reversible reaction in which pyruvate and lactate are interconverted *(WARD and COCKAYNE, 1993).* The same authors added that LDH catalyzed the conversion of lactate to pyruvate.

Interestingly, the activity of LDH in oviducal fluid of the rabbit, guinea pig, rat and mouse is of the same magnitude as in some of the greatest mammalian tissue sources, such as the heart *(GIBSON and MASTERS, 1970).* Various enzyme activities have been reported in oviducal fluid of the rabbit *(GEORGIEV, et al., 1970 and FRLEY, et al., 1990)* and the specific activity of LDH is regulated by progesterone or a metabolic product of progesterone.

In addition, oestrogen levels may be important for stimulating lipid production in the oviducal tube at the time of fertilization *(ELLINGTON, 1991).*

Furthermore, *KILLIAN et al., (1989)* noted that, during oestrus the levels of phospholipid in tubal fluid were elevated and reached to peak. Alkaline phosphatase (ALP) is an enzyme existing in the tissue and body fluids of all vertebrates *(FERNLEY, 1971)* and several isoenzymes were distributed in different tissue with different frequencies values *(FORSCHER and NAGODA, 1979).* There have been a number of studies which described the levels of ALP and ACP in uterine fluid during oestrous cycle *(BOOS, et al., 1988 and SCHULTZ, et al., 1971)* but the available literatures are not described those enzymes in the oviducal fluid. In recent years, there has been an increasing interest in defining the biochemical nature of the oviducal fluid of buffalo-cows which very important for understanding the culture media used for in vitro fertilization and led to successful in vitro development of embryos. From the above point, this study was undertaken in order to determine the concentrations of LDH, ALP, ACP and lactate\pyruvate ratio of oviducl fluid during different stages of the oestrous cycle and inactive in the Egyptian buffalo-cows.

MATERIAL and METHOD

One hundred reproductive tracts of Egyptian buffalo-cows were obtained from a local slaughterhouse. The female reproductive tracts were excised from each animal at the time of slaughter and kept in cool box, then transferred immediately to the laboratory. The female genitalia were examined specially with regard to the status of the ovaries which classified into active (follicular and luteal phases) and inactive.

Fallopian tube flushing:-

Both oviducts were excised free from each reproductive tract. Each oviduct was blotted on sterile gauze and flushed with 2.5 ml of KRB solution by inserting a 22- gauge needle attached to 5 ml

sterile syringe into the ampullary end and collecting the lavage from the isthmic end. KRB solution consisted of 6.95 gm NaCI, 0.35 gm KCL, 0.16 gm $KH_2 PO_4$, 0.14 gm $MgSO_4$, 0.90 gm glucose, 2.1 gm $NaHCO_3$ and 0.19 gm $CaCL_2$ per liter *(FARLEY, et al., 1990)*. The pH was adjusted to 7.4 Flushings obtained from both oviducts in each animal were pooled and centrifuged at 3000 rpm for 15 min. then stored at-20°C until needed.

Biochemical analysis:-

1- Determination of LDH

The LDH concentrations of fallopian tube flushing were determined by using LDH, UV-rate kit (stanbio, Texas, USA) according to *BUHI and JACKSON (1978).*

2- Determination of ALP

The ALP concentrations were determined in oviducal flushing by using alkaline phosphatase kit (Sclavo, Diagnostica, Italia) according to *MOSS (1984).*

3- Determination of ACP

The ACP concentrations were determined by using acid phosphatase P-NPP Kit (Quimica, Clinica Aplicada, Spain) according to *AMADOR and WACKER (1965).*

4- Determination of lactate-pyruvate ratio

This ratio was determined according to *MARBACH and WEIL (1967).*

Statistics:-

The results were analyzed by Analysis Of Variance (ANOVA) with repeatedmeasures on all factors (PC-state, 1985). Data were considered to be significantly different at $P< 0.05$.

RESULTS

The concentrations of LDH, ALP, ACP and lactate/pyruvate ratio in oviducal flushing fluid of buffalo-cows are presented in Table 1 and Figures 1 and 2. The mean values of the LDH enzyme in the oviducal flushing fluid were varied according to the ovarian conditions. The LDH level was increased significantly ($P< 0.018$)

in follicular period than the other condition. Moreover, the level of LDH decreased significantly ($P< 0.5$) during static ovaries when compared to the luteal and follicular periods.

The variation of the ALP level differed non-significantly among cyclic and inactive ovaries.

Differences of the ACP levels were significant ($P< 0.019$) among ovarian conditions. Its level increased significantly ($P<0.05$) in luteal period.

The lowest level ($P< 0.05$) was shown during inactive ovaries.

The lactate/pyruvate ratio in the oviducal flushing fluid decreased significantly ($P< 0.009$) in static ovaries when compared with luteal period and there was no significant difference between it and follicular phase.

DISCUSSION

The significant difference in the level of LDH between follicular and luteal periods is in agreement with that reported by *BOUSQUET et al. (1976)* in uterine secretions. This result could be attributed to that reported by *WITKOWSKA (1979) and ELLINGTON (1991)* who concluded that oviducal cells responded to exogenous estradiol by producing high level of LDH. The activity of LDH is higher during follicular period of the oestrous cycle. It is believed that, the oviduct can use glucose in an aerobic situation by conversion of pyruvate to lactate and this pyruvate conversion could supply energy for the cellular metabolism *(SCHAPIRA, 1973)*. At this time, the glucose metabolism is accompanied by the production of lactate as LDH activity rises *(BOUSQUET, et al., 1976)*. However, the obtained results are in disagreement with *GEORGIEV et al. (1970) and FARLEY et al. (1990)* who reported that the specific activity of LDH in oviducal fluid of the rabbit is increased by progesterone. The interpretation of this variation is due to the genetic and non-genetic factors *(ASHA MAZUMBER and MAZUMBER, 1985)*.

The observed low level of LDH during inactive ovaries in the oviducal fluid is reported by *LEESE, (1988)* to be the main derive of LDH in blood. This may lead to the maintenance of LDH at a rather constant level usually below that of the follicular or luteal periods of the oestrous cycle *(McDONALD and BELLVE, 1969 and PERKINS, 1974)*. Moreover, there is an inhibitory effect (in case of inactive ovaries) upon pituitary-thyroid axis and the production of thyroid hormones are not enough *(KPATEL, et al., 1994 and MEGAHED, et al., 1995)* to regulate available energy and heat production which very important for reproductive function *(SHI and BARRELL, 1994)*.

In the present study, there is a non-significant variation in alkaline phosphatase activity between inactive ovaries, luteal and follicular periods of the oestrous cycle in Egyptian buffalo-cows. The mean concentration of this enzyme in oviducal fluid during luteal period was higher than the follicular period. This results are similar to those recorded by *BOOS et al. (1988) and SCHULTZ et al. (1971)* in the uterine flushing. They concluded that alkaline phosphatase was regulated by ovarian steroids, where progesterone seems to increase it and oestrogen was thought to decrease its activity. Furthermore, *KPATEL et al. 1994 and VADODARIA (1978)* reported significantly lower values of serum alkaline phosphatase activity at oestrus than luteal period. Because of the high relative of alkaline phosphatase found during luteal phase in oviducal fluid and in uterine fluid (which recorded by above mentioned authors), it is believed that a small flow of the oviducal fluid through the utro-tubal junction, which increased at the time of embryo passage into the uterus *(BELLVE and DONALD, 1968 and LEESE, 1988)*.

The mean value of acid phosphatase, in the present study, at luteal period was higher than follicular period. This is in agreement with those of *SCHULTZ et al. (1971)* who reported that this enzyme activity was highest significantly in the uterine fluid during luteal period of the oestrous cycle. Among the present study, the overall means of lactate/pyruvate ratio were

significantly difference ($P< 0.009$). The obtained findings were in agreement with those of *HOLMDAHI and MASTROIANNI (1965) and FARLEY et al. (1990).* Also during maturation of the fertilized ova, *KHURANA and NIEMAN (1992), LEESE and BARTON (1985) and RIEGER and LOSKUTOFF (1994)* observed a significant increase in the metabolism of pyruvate. Moreover, glucose is converted to lactate and secreted from oocyte in early cleavage-stage *(LEESE, et al., 1993).*

From the present results, it is concluded that variations in ovarian conditions could influence the function of the fallopian tube with special reference to the levels of LDH, ALP, ACP and the ratio of Lactate/pyruvate.

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Table (1): LDH, ALP, ACP levels and lactate/pyruvate ratio (mean ± S.D) in the oviducal flushing in relation to ovarian conditions of Egyptian buffalo-cows.

	Follicular period	**Luteal period**	**Inactive ovaries**	**Prob.> F**
LDH (U/L)	76.47±47.56[1,2]	50.09±20.19[2]	31.53±21.74[1]	0.018
ALP (U/L)	53.64±13.99	56.64± 8.26	49.89± 8.17	n.s
ACP (U/L)	7.32± 3.45[1,2]	10.91± 6.04[1]	4.44± 2.98[2]	0.019
Lactate/pyruvate ratio	23.83± 3.01[2]	30.13± 6.85[1]	22.75± 8.92[2]	0.009

n.s = non-significant

Prob.> F = The probability of ANOVA

Means which are not significantly different are followed by the same number (significance level = 0.05).

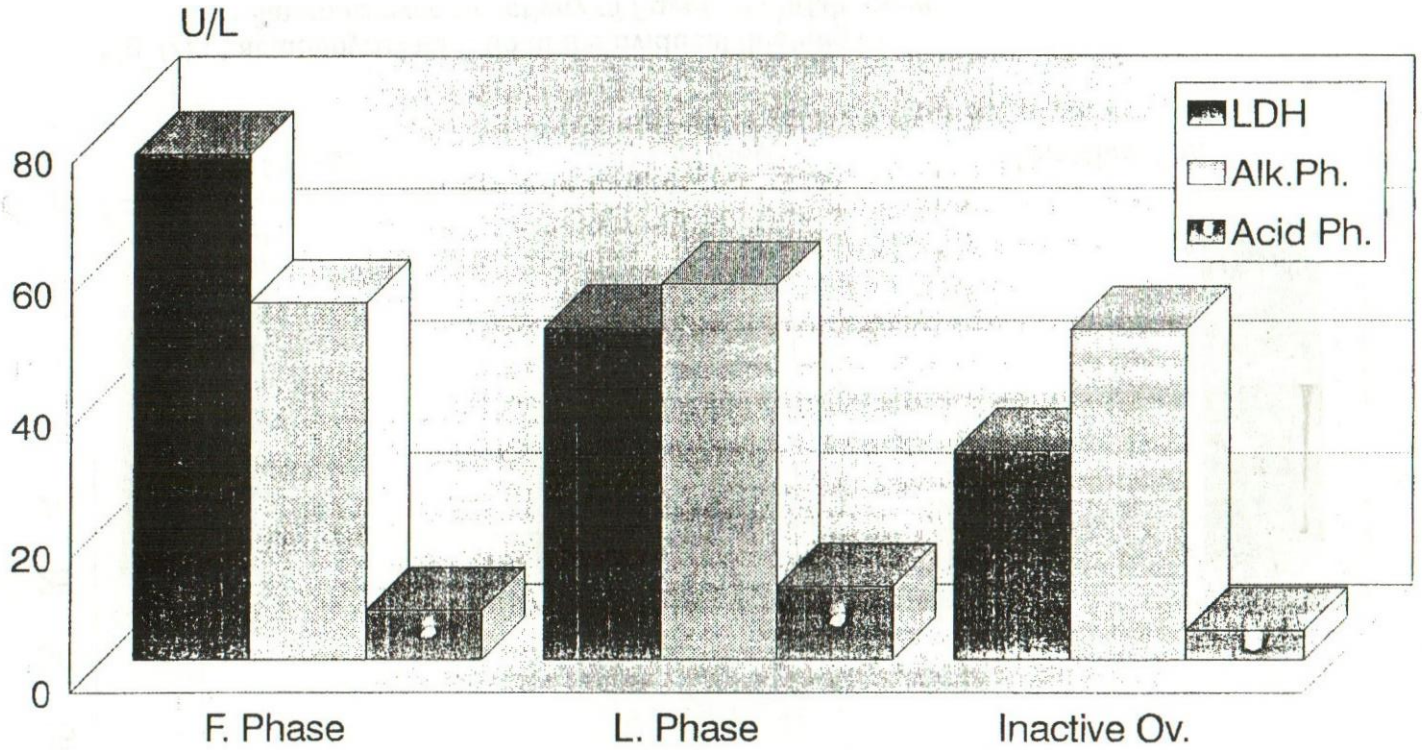

Fig. (1) : Concentrations of LDH and Phosphatases enzymes in the oviducal flushing in relation to ovarian activity of Egyptian buffalo-cows.

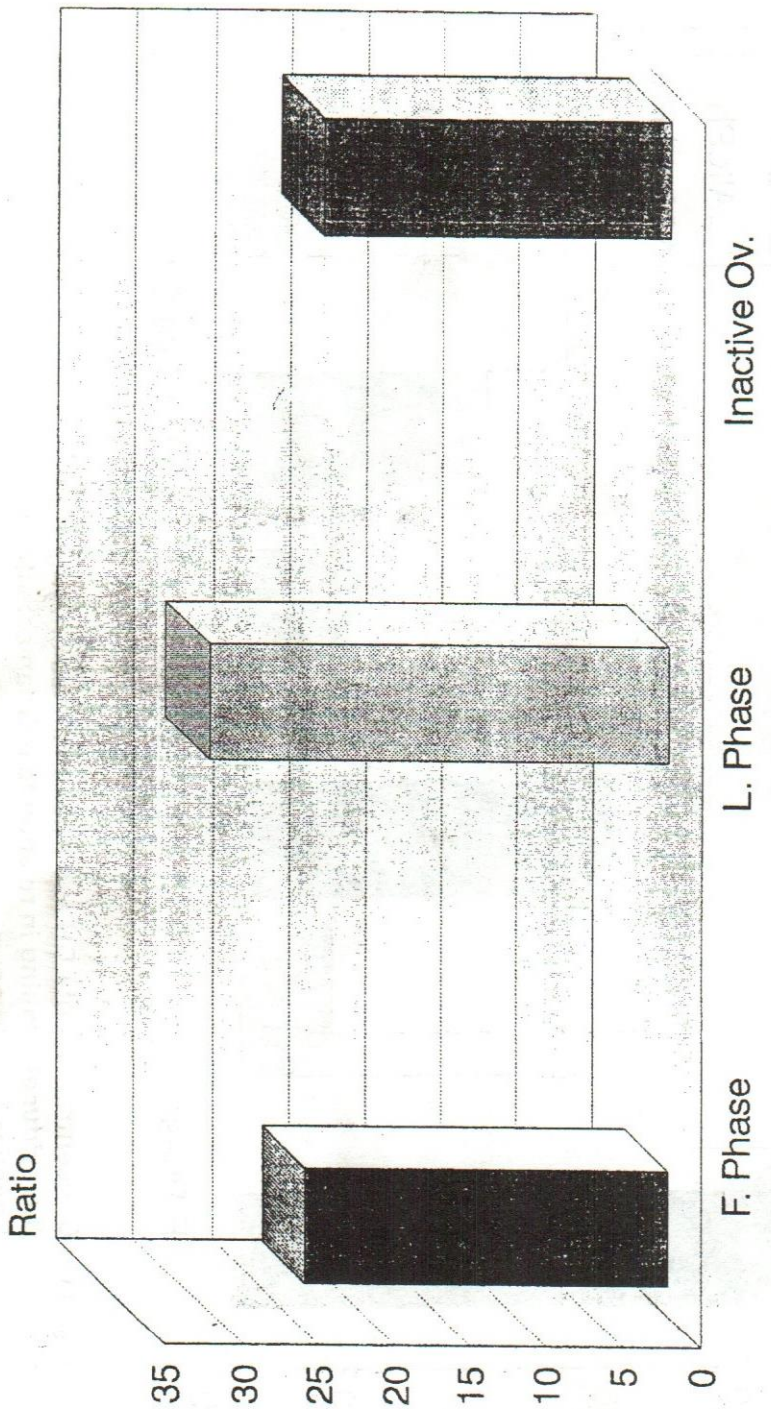

Fig. (2) : Lactate/pyruvate ratio in the oviducal flushing in relation to ovarian activity of Egyptian buffalo-cows.