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STUDY OF LUTEINIZING HORMONE RECEPTORS IN OVARIAN TISSUES OF BUFFALOES

(With 2 Tables & 4 Figs.)

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دراسة على مستقبلات هرمون الإباضة فى أنسجة مبيض الجاموس

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أجريت هذه الدراسة للتعرف على المستقبلات الخاصة لهرمون الإباضة فى حويصلات جراف أثناء مراحل تطوره المختلفة فى الأجسام الصفراء أثناء تكوينها وكذلك فى المبيض الخامل· وقد تم إستخلاص هرمون الإباضة من الغدد النخامية للجاموس · وتم تقدير قوتها البيولوجيـــة وقد أستخدم هذا الهرمون فى إعداد المصل المضاد له فى الأرانب التى أستخدمت بعد ذلك فى قياس الهرمون بالطرق البيولوجية والمناعية · كما تم تحديد المستقبلات بواسطة المواد المتلألئة التى تلتصق بإستخدام المجهر الفاحص للمبيض · وقد أسفرت الدراسة عن النتائج الآتيـــة: مقدار ماتأخذه الحويصلات الجرابية من هرمون الإباضة الخاص بالجاموس تتزايد مع زيـــادة حجم الحويصلات · كما تبين الدراسات المناعية والبيولوجية على الهرمون المتبقى من تحضـــين كميات ثابتة من الهرمون مع الأحجام المختلفة من الحويصلات تتشابه هذه النتائج مع الدراسات التى أجريت بواسطة المواد المتلألئة التى تلتصق بالأجسام المناعية الخاصة لهرمون الإباضة· ـ أثناء عملية تحويل الحويصلات الجرابية إلى أجسام صفراء تقل القدرة على الإتحاد مع الهرمون ويكون معدل وجود مستقبلات الخاصة لهرمون الإباضة فى الجسم الأصفر أثناء النمو أكثر منه فى الجسم الأصفر القديم ـ كان معدل مستقبلات هرمون الإباضة فى المبايض الخاملة قليلاً بالمقارنة بحويصلات جراف الكبيرة · ويمكننا القول بأنه بزيادة حجم الحويصلة الجرابية يزداد معـــدل المستقبلات لهرمون الإباضة ليساعد على نمو الحويصلات وعملية التبويض · أما قلة المستقبـــلات أثناء تكوين الجسم الأصفر فلا يرجع إلى نشاط هرمون التبويض ·

SUMMARY

The aim of the present study is to gain information regarding the intake of LH by normal follicular and luteal cells of buffalo ovaries, with reference to tissues from inactive smooth ovaries.

Leutinizing hormone was prepared from pituitaries obtained from mature female buffaloes. Then used as antigen for preparation

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of the specific rabbit antiserum which was used for biological assay, immunological assay and immunofluorescence localization of buffalo LH receptors in ovarian tissues.

The results obtained from this investigation revealed that:
The intake of buffalo LH by follicles of different sizes showed a parallel increase during maturation. The results obtained by biological and immunological assay were identical.
Immunofluorescent technique revealed high LH intake by granulosa and thecal cells of large follicles. The LH intake was high with mature CL as compared to old ones.
LH intake by preantral follicles and inactive ovaries were less than those obtained by the large sized follicles.
It was concluded that, as the follicle matures, the receptors of the follicle to LH are increased to augment their growth and response to LH to induced ovulation. The loss of receptors during luteinizing does not involve LH activity.

INTRODUCTION

Treatment of infertility in farm animals with gonadotrophins is of great value. Several mechanisms interfere with the effectiveness of these hormones on activation of the gonads. One of the most important mechanisms is the availabiligy of gonadotrophin receptors in target cell membranes (AZHAR and MENON, 1981 and MULLER, et al. 1983). These receptors could be present or absent and if present probabilities of being mashed or unmashed can modulate potency of gonadotrophins (DANFORTH and STOUFFER, 1985).

Exposure of granulosa cells to follicle stimulating hormone (FSH) is known to induce formation of LH receptors and subsequant treatment of these primed cells with luteinizing hormone (LH) enhances granulosa cells differentiation (BICSAK, et al. 1986).

The aim of the present study is to gain informations regarding the intake of LH by normal follicular and luteal cells of buffalo ovaries, with reference to tissues from inactive smooth ovaries.

MATERIAL and METHODS

Luteinizing hormone was prepared from the pituitary glands of mature female buffaloes using the method of LI, et al. (1942). The potency of this LH was determined biologically according to the method of SOLIMAN (1960).

Antisera against the bovine LH (NIAMDD)* and the prepared buffalo LH were raised according to VAITUKAITIS, et al. (1971). The prepared antisera were then absorbed using

* Received from the National Institute of Arthritis, Metabolism and Digestive Diseases, Baltimore, Maryland, USA.

normal calf serum in 0.85% saline according to the method described by HAYASHIDA (1963).

Preparation of tissue slices:

Ovaries of normal healthy mature female buffaloes were collected from slaughter house and kept in a thermos bottle containing ice bags. Follicles of different sizes (preantral, medium "0.5-1.0 cm" and large sized "more than 1.0 cm") were scraped with a spatula to obtain granulosa and thecal tissues as described by IRELAND and ROCHE (1982). Thirty mg of these tissues were used. In addition 50 mg of very fine slices, approximately 0.05 mm thickness, were obtained from mature and newly formed corpora lutea, corpora albicans and tissues from inactive ovaries by a sharp microtome knife as described by LEE and RYAN (1971).

The intake of luteinizing hormone by different buffalo ovarian tissue was then determined using two different methods.

A) Biological and Immunological methods:

These methods are based upon the presence of different quantities of receptors for LH in the ovarian tissues. When the tissues are incubated with known amount of LH, it gets combined with some of the hormone as described by COLE, et al. (1973) and the rest will remain free in the perfusion media. The free hormone was measured by the biological assay technique of SOLIMAN (1960) and the immunoassay technique of SCHUURS (1969). The micrograms of LH taken bymilligram of different ovarian tissues were then calculated.

B) Immunofluorescent localization of LH in the ovarian tissue of buffaloes:

In this method the ovaries of buffaloes were removed quickely after slaughter, split in the mid sagital plane was made and placed in neutral buffer formalin 10% as a fixative at room temperature for 24 h. After fixation, the tissues were trimmed, embeded in paraffin and sectioned at 6 microns and mounted onglass slides with egg albumin. Sections were used for localization of LH in the cells. Direct immunofluorescent technique of CHADWICK and FOTHERGILL (1962) was used for this purpose.

RESULTS

The biological potency of the prepared buffalo pituitary LH was 107.7 i.u/mg on the average.

Results obtained by biological and immunoassay revealed that, the intake of LH by large follicle was significantly higher than the other tissues studied (Tables, 1 & 2). The mean values of LH intake were 1.23 ± 0.11, 1.28 ± 0.00 and 1.88 ± 0.19 ug/mg tissue as measured by biological assay and 0.60 ± 0.1, 0.95 ± 0.31 and 1.30 ± 0.20 ug/mg tissue as measured by immuno assay in the preantral, medium sized and large sized follicles, respectively.

The intake of buffalo LH by newly formed Cl did not differ significantly from the intake by mature ones as indicated by biological assay (0.93±0.16 and 0.75±0.24 ug/mg tissue) and immunoassay (0.23±0.01 and 0.30±0.06 mg tissue), respectively.

When LH intake was measured by biological and immunological assay, it revealed that, the LH intake by preantral follicles and inactive ovaries were less than that obtained by the large sized follicles.

The results obtained by immunofluorescent technique revealed localization of fluorescence around the granulosa cells (Fig. 1) and thecal cells (Fig. 2) of large follicles indicating high LH intake by these cells. Sections prepared from mature corpus luteum treated with LH then followed by fluorescein labelled antisera showed great brilliance on the periphery of luteal cells (Fig. 3) indicating higher intake of mature corpus luteum as compared to old ones which showed poor areas of brilliance on these cells (Fig. 4).

DISCUSSION

The degree of LH intake by the graafian follicle as measured by biological and immunological assay was proportional with the stage of its maturity. These results are in accordance with the results of IRELAND and ROCHE (1982 & 1983) in cows. They demonstrated that LH receptors were observed in theca and granulosa cells of large follicles and as the follicle matures, th granulosa cells bind more LH.

The immunofluorescent studies confirm these results. Sections obtained from large graafian follicles of buffaloes became occupied with buffalo LH, then treated by specific LH fluorescein labelled antisera showed indication of the presence of LH receptors at the membranes of granulosa and theca cell layers indicating gradual increase in LH receptors as the follicle matures (Fig. 1 and 2).

Regarding corpus luteum, fluorescence was found to be very pronounced around mature luteal cells and weak around the old ones, indicating poor intake by old luteal cells.

The LH intake by small or preantral follicles did not differ significantly from the intake by inactive ovarian tissue slices as measured by biological and immunological assays. The reason why small follicles fix small amount of LH and did not differ significantly from inactive ovary could be attributed either to saturation of gonadotrophin receptor sites in small follicle with endogenous LH (CHANNING and KAMMERMAN, 1974) or to the presence of luteinization inhibitor. This was isolated from the follicular fluid of immature follicles of bovines (SHEMSH, 1979). The amount of luteinzation inhibitor was found to be more in small follicles than in the medium and large sized follicles as reported in porcine ovary (OSTEEN, et al. 1985). This fraction decreased with follicular development suggesting its role in regulation of formation LH receptors in the granulosa and theca cells layers.

The newly formed CL showed fixed smaller amount of LH than other tissues studied except inactive ovary, indicating that ovulation and luteinization of granulosa cells were

accompanied by loss of LH receptors. similar observation was previously reported by LEE and TAKAHASHI (1977) who showed that heavy luteinization of rat ovary was accompanied by loss of LH receptors which could be due to loss of LH sensitive adenylate cyclase activity.

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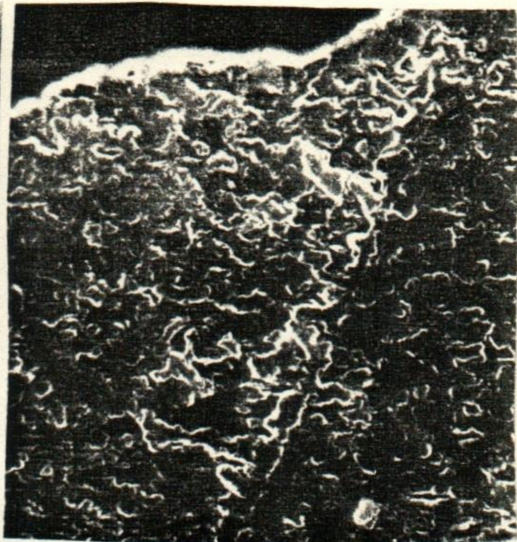
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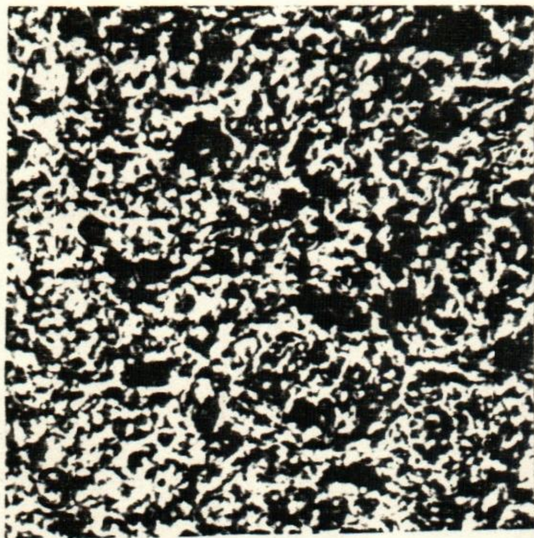
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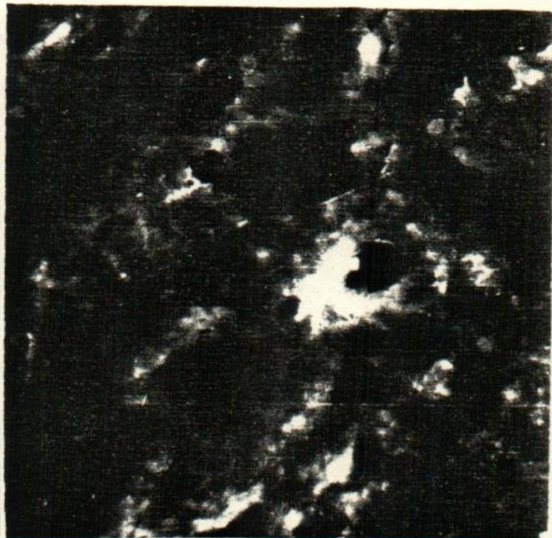
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Table (1) : Intake of buffalo LH by ovarian tissue (Biological assay)

Ovarian tissues	Ug hormone/mg tissue
Cortical tissue contain Preantral follicle	1.23 ± 0.11
0.5-1.0 cm follicle (medium sized follicle)	1.28 ± 0.00
Over 1.0 cm follicle (large sized follicle)	1.88* ± 0.19
Newly formed corpus luteum	0.93 ± 0.16
Mature corpus luteum	0.75 ± 0.24
Corpus albicans	1.41 ± 0.00
Inactive ovary	1.13 ± 0.13

± Standard error

* Significantly differs from other tissues at $P < 0.01$.

Table (2) : Intake of buffalo LH by ovarian tissues (Immunoassay)

Ovarian tissues	Ug intake mg/tissue
Cortical tissue contain Preantral follicle	0.60 ± 0.10
0.5-1.0 cm fillicle (medium sized follicle)	0.95 + 0.31
Over 1.0 cm follicle (large sized follicle)	1.30* ± 0.20
Newly formed corpus luteum	0.23 ± 0.01
Mature corpus luteum	0.30 ± 0.06
Corpus albicans	0.28 ± 0.07
Inactive ovary	0.55 ± 0.16

± Standard error

* Significantly differs from other tissues at $P<0.01$.