# دراسة على الفشاء المبطن الرحم في حالات خمول المبايض دكتور/محمد مصطفى ايوب ، دكتور على ع مجازى ، دكتور عثمان عطا الله قسم الولادة وامراضها والتلقيح الصناعي وقسم البائولوجيا - كلية الطب البيطرى - جامعة القامرة

#### الملخص العربي

تم فحص ودراسة الغشاء المبطن للرحم لـ ١٤٠ حالة لجاموس مصاب بخمول في المبايض دراسة -مستولوجية هوستوكيمالية ومستوبالولوجية «

الم عبين أن الصورة الهستولوجية والهستوكيمائية تشابه مثيلتها في نهاية الفترة الشبقية وقبل مرحلة الاعداد لدورة شبق جديدة وقد تعزى تلك التغيرات الى عدم افراز هرمون. الاستروجين والبرجسترون من المبيض في تلك الحالات .

colour shy than I then the on it will easily the day

and the loss of the limit of the line of t

## Wes they

o to any ferious la marie a marie a ser la ferio de la ferio de la company de la company de la company de la c La company de la company de

A second of Manager County Species of the Second of the Manager County Second of the Second of the Second of the Second of the Manager County Second of the Second of t

Dept. of Pathology and Obstet. & Gynae Faculty of Vet. Med, Cairo University

Head of Obstet and Gynae Dept. prof. Dr. K. Zaki.

Head of Pathology Dept. prof. Dr. H. Shehata

# STUDIES ON THE ENDOMETRIAL PICTURE IN CASES OF OVARIAN INACTIVITY IN BUFFALOES IN EGYPT.

MITERIALS (NO METERORS

(with 6 figures)

By

M.M. Ayoub, A.A. Hegazy and O.A., Atalah (Received 28/9/1976).

#### SUMMARY

The histomorphological and histochemical studies of 140 buffaloe-endometrium affected with true ovarian inactivity revealed that the lining epithelium was devoid from hyperplasia, the uterine glands were smaller than normal and non active, no oedema in the stroma, and its blood supply was decreased. It could be concluded that both histochemical and histomorphological finding nearly simulate that in late dioestrus and this may be attributed to the absence of ovarian hormones (estrogen and progestron).

#### INTRODUCTION

One of the most important infertility problem in buffaloes in our country is the ovarian inactivity (SHALASH, 1958, SHOKEIR 1958, EL SAWAF, and. SCHMIDT. 1962, SCHMIDT et al 1963 and ZAKI et al, 1963),

Histological and histochemical studies were done on the endometrium of buffaloes in relation to the oestrus cycle (AYOUB, 1973) and pregnancy (MAHMOUD, 1973), No similar studies could be traced on the ovaries or endometrium in cases of ovarian inactivity.

PAS positive substance and glycogen were found in most of the endometrial structures during the follicular (estrogenic) phase of cycle, reaching their maximum during oestrus. In dioestrus, the glycogen was found to be much decreased (WEETH and HERMAN, 1952, SKJERVEN, 1956 and 1956 b and AYOUB, 1973).

Alkaline and acid phosphatase activity was demonstrated in the endometrial epithelium through the different phases of the cycle, but was greater at the mid of the cycle (MARINOV and LOVELL, 1968 and AYOUB, 1973).

The aim of this work is to give a detailed information on the histological and histochemical findings in the endometrium of buffaloes affected with inactive ovaries.

#### MATERIALS AND METHODS

Histological and histochemical techniques were carried out by two means:

1.—The genetalia of 80 non-pregnant (6-10 years old) buffaloes, of unknown history, showing inactive ovaries, were collected from Giza abattoir. Immed iately after the removal of the genetalia and examining both ovaries, tissue samples were taken from the middle of each uterine horn.

II- Endometrial biopsy samples were obtained from 40 buffaloes (6-10 years old) bred at Elsafarana and Elmotamadia farms belonging to the meat and milk organization, Egypt. Another samples were taken from 20 buffsloes presented to the clinic of the Obstet. & Gynae Dept., Faculty of vet. Med. Cairo Univ., Gia.

Before obtaining the samples, the case history of such animals was scruitinized, moreover they were subjected to rectal and vaginal examination several times and their ovaries proved to be inactive.

The collected samples (biopsy and necropsy) were immediately fixed in 10% neutral formaline, Bouins fluid, and Carnoy's fluid. The routine histological and histochemical methods for dehydration, clearing, embedding and sectioning were applied. The obtained sections were stained with the following stains:

- 1. Harris haematoxyline and eosin, for general histological examination (CARLTON et al., 1967).
- Alcian blue method (STEEDMAN, 1950) for acid mucopolysaccharide.
- 3. Best's Carmine method (Best, 1906) for glycogen.
- 4. PAS + ve technique (PEARSE, 1968) for glycogen and neutral mucopolysaccharides.
- 5. Calcium cobalt method (GOMORI, 1950) for alkaline phsophatase activity.
- 6. Lead nitrate method (GOMORI, 1950) for acid phosphatase activity.

#### RESULTS

## 1 Histological Findings:

# A) Surface epithelium .:

In most of cases the epithelium lining of the endometrium was of a pseudostratified columnar type with oval nuclei occupying the basal half of the cells. In some cases the endometrium was lined with low columnar cells with dark round or oval nuclei (Fig. 1), while in the other few cases the endometrium was lined with cuboidal epithelium which changed to strtified squamous in some area.

#### B) Uterine glands:

The uterine glands were fairly coild of small to average size. They were lined with simple columnar epithelium with oval nuclei which occupied the basal layer of the cell.

In some cases the uterine glands were lined with pseudostratified columnar epithelium and their lumen contained desquamated epithelial cells, while others were obliterated (Fig. II).

The uterine glands were generally inactive and showed no secretion.

#### C) Endometrial Stroma:

The stratum compactum was nearly free from uterine glands but it was highly cellular. The most predominant cell was the fibroblast, however in some cases few lymphocytic infiltrations were observed. The stratum spongiosum was consisted of connective tissue characterized by having few cells and more fibers. Coild arterioles with thick hyalinized wall and almost obliterated lumen were present in endometrial stroma which was free from congested blood vessels or oedema and contained a minimum blood supply (Fig. 111). In most of the cases the endometrium was found to be relatively thin.

#### 11 Histochemical Findings

#### A) PAS positive substances:

The apical border of the epithelial lining of the endometrium reacted slightly for PAS + ve technique while strong + ve reaction was found in the endometrial stroma. The wall of the endometrial arteris possessed a faint reaction.

# B) Glycogen:

Most of the examined cases gave negative results. But in few cases fine glycogen granules were present in uterine glands (Fig. IV).

# C) Acid Mucopolysaccharides:

Epithelial lining of the endometrium revealed a diffuse faint reaction with alcian blue, while the uterine glands showed a very faint reaction in their luminal border (Fig V),

# D) Alkaline and acid phosphatase enzymes:

The surface epithelial cells showed a strong activity for alkaline phosphatase in their apical portions, silmilar reaction was also present in uterine glands specially those near the surface (Fig. VI). The activity of acid phosphatase in the examined cases gave negative results, except in few cases the stratum compactum possessed a moderate activity of this enzyme.

#### DISCUSSION

Estrogen and progestrone hormones are responsible for the cyclical changes in the endometrium (ASDELL, 1955, MCDONALD, 1969 and AYOUB, 1973). The surface epithelium of endometrium in cases of ovarian inactivity

resembles the epithelium lining in uterus in late dioestrus, this may be explained by the fact that in both conditions estrogen hormone is minimum or defecient.

The histological examination of uterine glands, in ovarian inactivity, gave a picture simulating that in late dioestrus.

Progestrone provide the chief stimulus for glandular activity in bovine as has been proved by ASDELL (1955). This may be hold true for buffaloes, since ABDO (1962) has reported a significant rise in their progestrone level in early dioestrus. AYOUB (1973) reported that the uterine glands in buffaloes, undergo hypertrophy as judged by the maximal cross sectional diameter, he concluded that this finding denotes evidence of secretion which is clearly seen in the glands shortly after ovulation till early dioestrus.

In ovarian inactivity, the ovarian progestrone and estrogen are lacking, so the uterine glands showed no evidence of secretions, hyperplasia or activity,

It was proved from the present investigation that the thickness of the endometrium simulate that in late dioestrus and early proestrus. HAMMOUD (1927), HANASEL (1959), CUPPS et al (1969) and AYOUB (1973) reported that the increase in thickness of the endometrial stroma at the middle of the cycle may be attributed to the highest degree of oedema and glandular hyperplasia; these may give an explanation why endometrial stroma is relatively thin in cases of inactive ovaries simulating that present in early proestrus and late dioestrus.

#### HISTOCHEMICAL OBSERVATIONS:

PAS + ve substance:

The present investigation revealed that there are slight faint + ve reaction for PAS substance in the apical border of the epithelial lining the endometrium. The investigation of these substances in the cells during the follicular phase (MARINOV and LOVELL, 1968) may indicate that there is a connection betwen oestrogen level and PAS positive material. The absence of ovarian oestrogen in case of true inactive ovaries may explain the cause of decrease of PAS + ve substance in the epithelial lining of the endometrium.

Glycogen:

In the present work, most of examined cases gave a negative result, while in few cases fine glycogen granules were present in uterine glands.

WEETH and HERMMAN (1952), SKJERVEN (1953, 1956 a & b) MOSS et al (1954) and AYOUB (1973); demonstrated that the glycogen contents increase in the epithelial cells of the surface epithelial and uterine glands during proestrus and metoestrus, while in dioestrus the glycogen content was found to be much decreased.

There is no doubt that the distribution of glycogen in the endometrium is the result of physiological response to hormonal stimulation (ovarian hormones) SKYES et al. (1955) and SOLIMAN and SAID (1966).

It is quite clear that the glycogen content of epithelial lining of endometrium increase under the effect of estrogen. The minimum level of both oestrogen and progestrone in case of true inactive ovaries, may throw a light. on the cause of decrease of the glycogen content of epithelial lining of endometrium.

Alkaline and acid phosphatase:

Alkaline and acid phosphatase enzymes in endometrium of buffaloes show clear cycilical variations as they increased at the oestrus reaching thier maximum concentration during metoestrus and decrease in late, dioestrus (AYOUB, 1973).

Changes in phosphatase activity reflect changes in the metabolic activity of the cells, the latter is infeluenced by sex hormones (MARINOV and LOVELL, 1968).

The present work indicates that the surface epithelium of the uterus in case of inactive ovaries has got a strong positive reaction to alkaline phosphatase and a negative reaction to acid. one. MARINOV, and LOVELL (1968) reported that the activity of these enzymes can be described throughout the different phases of the cycle, but MOSS et al (1954) concluded that the enzymes are only found during dioestrus. The negative acid phosphatase reaction may be attributed to the low metabolic activity of the cells as a result of nearly abscence of sexual hormones.

#### REFERENCES

- Abdo, M.S. (1962).—Hormonal variation in the blood of buffaloes during pregnancy.

  M.D. vet. Cairo University.
- Asdell, S.A. (1955).—Cattle fertility and sterility. Little; Brown Company (London).
- Ayoub, M.M. (1973).—Morphogynaecological studies on the uterus of buffaloes in Egypt. M.D. Vet. Cairo Univ.
- Cupps, P.T., Anderson, L.L. and Cole, H.H. (1969).—The oestrus cycle, cited in Cole, H.H. and Cupps, P.T. (1969) Reproduction in domestic animals. 2 nd Ed. Academic Press, N.Y. and London.
- Hammond, J. (1972).—The physiology of reproduction in the cow. Univ. Press. Cambridge.
- Hanasel, W. (1959).—In Reproduction in Domestic animals By Cole, H.H. and Cupps, P.T. (1959); Vol. 1, Academic Press, N.Y. and London.
- El Sawaf, S. and Schmidt, K. (1962).—Morphological in normal and abnormal ovaries of buffaloes with special references to their function. Vet. Med. J. Giza. 9, 249.
- Mahmoud, S.A. (1973).—Some studies on the endometrium and placenta of Egyptian buffaloes during pregnacy. M.V.Sc. Cairo Univ.
- Marinov, M. and Lovell, J. E. (1968).—Cytology of bovine uterine epithelium during the oestrus cycle. Am. J. Vet. Res. 29, 13.

- McDonald, L.M. (1969).—Veterinary endocrinology and reproduction. Lea and Febiger, Philadelphia.
  - Moss, S. Wreen, T.R. and Skykes, J.E. (1954).—Alkaline phosphatase, glycogen, PAS+ ve substances in the bovine uterus during the oestrous cylce. Endocr. 35, 261.
- Schmidt, K., El Sawaf, S. and Gharib, (1636).—Some studies on diagnosis in herd problems with regard to pregnancy and infertilityin buffaloes. Vet. Med. J. Giza, 9,113
- Shalash, M.R. (1958).—Physiology of reproduction in buffalocow. Internat. J. Fert., 3, 425.
- Shokeir, A.A. (1958).—Observations on disease conditions and malformation of reproductive organs of slaghtered buffaloes. Vet. Med. J. Giza 5, 265.
- Skjerven, O. (1953).—PAS + ve material in the endometrium of the cow during the sexnal cycle. A preliminary report. Nord., Vet. Med. 5, 395.
- Skjerven, O. (1956 a).—Endometrial biopsy studies in reproductivity normal cattle. Acta Endocr. 26, 22.
- Skjerven, O. (956 b).—Phosphatase, fat and carbohydrate content of normal bovine endomet rium. Biopsy studies of cyclic variation. J. Fert. and Steril. 7, 31.
- Soliman, F.A. and Said, A.H. (1966).—Changes in the glycogen content of reproductive tract of the buffalo during the oestrus cycle. U.A.R. physiological soc. 2, 101
- Sykes, J.F., Moss, S. and Ween, T.R. (1955).—Cited by Lareson et al (1970).
- Weeth, H.J. and Herman. H.A. (1952).—A histological and histochemical study of the bovine oviduct, uterus and placenta. Miss. Agri. Exp. Sta. Bull. Res. 501.
- Zaki, K. El Wishy, A.A. Osman, A. M. and Afffy, M.M. (1963 a).— Gynaecological diagnosisin buffaloes and native cows Vet. Med. J. Giza, 9, 281.

#### Author's Address :

Dr. M.M. AYOUB Dept of Obstet. & Gynae. Fac. Vet. Med., Cairo Univ. Giza.

Acons Vets Mich. J. Vol. 4 No. 7

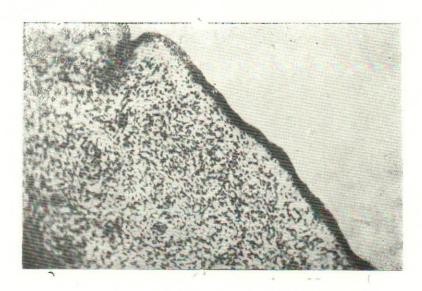


Fig. I. - Low columnar epithelium lining the endometrium. (H & E × 200)

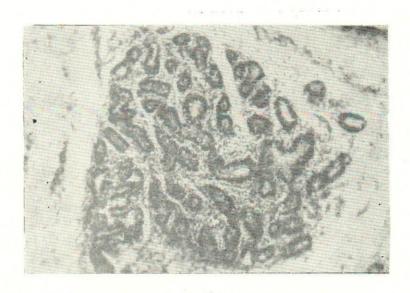


Fig. II. - Ooliterated lumen of most of the small-sized endometrial gland. (H & E  $\times$  200)



#### ENDOMETRIAL PICTURE IN OVARIAN INACTIVITY

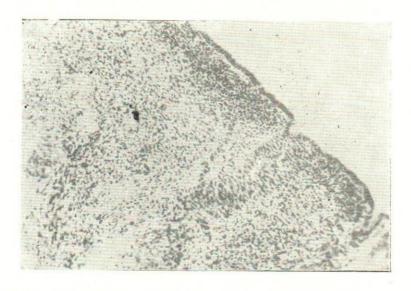


Fig. III.—Minimal blood supply for endometrial stroma (H & E  $\times$  100)

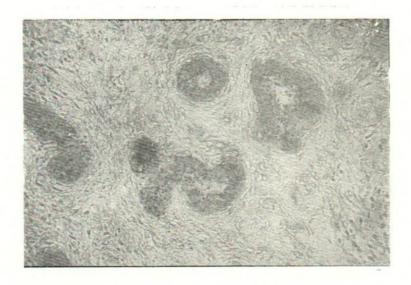


Fig. IV.—Glycogen granules in the endometrial glands. (PAS  $\,\times\,$  450).





Fig. V. — Epith. lining of the endometrium, revealed Faint reaction with alcian blue) (Alcian blue  $\times$  200),

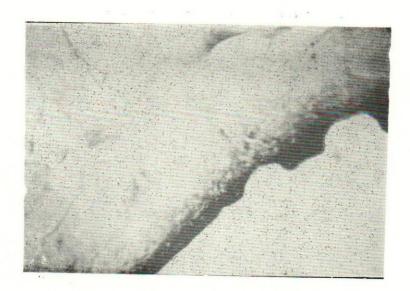


Fig. VI. — Alkaline phosphatas + ve reaction in surface epith. (Gomeri' method  $\times$  200).

