# EARLY PREGNANCY DIAGNOSIS BY HORMONAL ASSAY OR ULTRASONOGRAPHY IN EGYPTIAN BUFFALOES

BY

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## ABSTRACT

*Both progesterone level and ultrasonography scanning were used for early detection of pregnant and non-pregnant buffaloes. One hundred and twenty six buffalo-cows were used for detection of pregnancy by measuring serum progesterone level 21 to 80 days post insemination (PI). Forty-one buffalo-cows were examined by ultrasonography using a 5 and 7.5 MHz linear array transrectal transducer. Progesterone concentration in positive diagnosed animals was ranged between 3.42±0.23 and 4.55±0.34 ng/ml (21-80 days PI). While its concentration in negative animals were between 0.65±0.21 and 0.97±0.10 ng/ml during the same duration. Accuracy of positive pregnancy by progesterone assay was 83.33% in 21-29 days PI , reached its highest sensitivity (100%) in 36-50 days PI. Sensitivity and specificity of early diagnosis of pregnancy by ultrasonography scanning were 77.7% and 66.6% respectively during 21-29 days PI. Its percentages increased during 30-35 days PI (83.3% and 100% respectively). It can be concluded that, either progesterone level or ultrasonography are highly accurate methods for early detection of pregnancy in buffaloes.*

## INTRODUCTION

Accurate early detection of pregnant and non-pregnant animals is an essential factor for optimizing reproductive performance in dairy animals. Several studies have assessed the accuracy of pregnancy by palpation per rectum, but palpation by it self is too difficult and precise in diagnosis early pregnancy besides its dangerous which may cause early embryonic death at early stages (Abbitt et al., 1978; Pasiely et al., 1978; Vaillancourt et al., 1979 and Franco et al., 1987). On the other hand, Alexander et al. (1995) reported that rectal palpation after four weeks of breeding could not affect on the survival of the embryo.

Hormonal assays, especially progesterone, in blood or milk are currently used to diagnose early pregnancy. The accuracy of pregnancy diagnosis by measuring blood progesterone in buffalo was 75 to 83.3% (Nada et al., 1984). By measuring milk progesterone, the accuracy ranged between 66-68% for pregnant and 90-91% for non-pregnant animals (Dionysius, 1991). Other pregnancy diagnosis testing included counting of thrombocytes in peripheral blood during follicular and after service was recorded (Knof and Schallenberger, 1988). Moreover, antibodies against the isolated antigen from the placenta or foeti were used in early pregnancy diagnosis (Noble, 1982 and Soliman et al., 1998).

Transrectal ultrasonography of the uterus, using 5 MHz linear-array or sector transducer, provides an accurate method for identifying pregnant (95 to 95%) and non-pregnant cattle (75 to 97%) at 26 to 27 days after AI under field conditions (Pieterse et

al., 1990; Szenci et al., 1990 and Hazen et al., 1991). However, Badtram et al. (1991) reported an accuracy of 69 and 72% for pregnant and non-pregnant cows, respectively, using ultrasonography between 23 and 31 days after insemination. Under controlled experimental conditions, early diagnosis using 7.5 MHz linear-array transducer was 100% accuracy by day 20 (Kastelic et al., 1991). However, Comparable results could not be obtained in a field study (Scenci et al., 1995).

The aim of this study was to evaluate the sensitivity, specificity and positive or negative predictive values of progesterone assay and ultrasonography to diagnose early pregnancy in Egyptian buffaloes.

## MATERIALS AND METHODS

### Animals:

One hundred and twenty six buffalo-cows from different farms and Governorates were used in this study. They used at time between 21-80 days Post Insemination (PI). According to PI days, animals were divided into 5 categories or groups:

1- From 21 to 29 days PI. 2-From 30 to 35 days PI.
3- From 36 to 50 days PI. 4-From 51 to 69 days PI.
5- From 70 to 80 days PI.

### Blood Sampling:

Blood samples were collected from 126 buffalo-cows via jagular veinpucture. Serum was separated and stored at –20 °C until hormonal assay.

### Hormonal assay:

Serum progesterone concentration was estimated using direct radioimmunoassay (RIA) using coat A-count kit (Diagnostic Products Corporation, Los Angeles). This method have been characterized and verified to measure progesterone with an extremely low cross reactivity to other hormones. Concentrations were calculated, as the method adopted by Kubasik (1984).

### Ultrasonographic scanning:

Forty-one buffalo-cows were examined for the first time using a real time ultrasound scanner 480 Vet (Pie Medical- Maastricht- Netherland) equipped with 5 and 7.5 MHz linear array rectal transducer and thermal paper vedio printer (Metsubishi). All the ultrasonographic examinations were performed by the same person. Ultrasonographic was conducted from day 21 till day 80 PI.

After removal of feces, the transducer was inserted into the rectum. The interface between the transducer scan head and the organs was covered by ultrasonic gel to prevent air bubbles. The transducer was moved slowly from the cervix, uterine body, uterine horns and ovaries. Scanning of the uterus and interpretation of the echoscopic images have been described according to Szenci et al. (1995).

Palpation per rectum of the uterus tookplace between days 60-80 PI to all animals to confirm weather or not the baffaloes was pregnant.

Sensitivity, specificity, positive and negative predictive values were calculated as follow:

**Sensitivity of positive diagnosis (%)** = $\frac{\text{True positive cases}}{\text{True positive + false negative}} \times 100$

**Specificity of negative diagnosis (%)** = $\frac{\text{True negative cases}}{\text{True negative+ false positive}} \times 100$

**Positive predictive value (%)** = $\frac{\text{True negative cases}}{\text{True positive + false positive}} \times 100$

**Negative predictive value (%)** = $\frac{\text{True negative cases}}{\text{True negative+ false negative}} \times 100$

## RESULTS

Table (I) showed serum progesterone concentrations during the different days postinsemination (PI), where its concentration was relatively high (3.77±0.32 ng/ml) in animals diagnosed as positive pregnancy by days 21-29 PI. Increased by days 30-35 PI to 4.55±0.34 ng/ml, continued on that pattern during the rest of the experiment. On the other hand, progesterone concentration of negative diagnosis of pregnancy ranged between 0.65±0.21 and 0.97±0.10 ng/ml all over the time of the study.

The proportion of pregnancy diagnosis by progesterone concentration (table 2) classified as: true positive or negative and false positive or negative. Numbers of true positive or negative were increased with increase time post insemination. Although, the values of the intrinsic properties of the diagnostic method (sensitivity and specificity) were also increased. Sensitivity was 83.33% on 21-29 days PI, reached to 90.91% on 51-69 days PI. Specificity was 71.42% reached its maximum (l00%) on 51-69 days PI. The positive predictive value was 78.94% in 21-29 days group, then increased with increase the time post insemination. The same was recorded for the negative predictive values.

Table (3) shows the results of the pregnancy diagnosis of ultrasonographic scanning. Embryo proper and allantoic fluid recognition was used as the criterion for true positive or negative diagnosis. Results revealed that, there was no false positive diagnosis was detected after 30 days PI. The sensitivity of ultrasonographic diagnosis increased from 77.7% to 83.3% then to its maximum (100%) on 21-29, 30-35 and 36-50 days PI respectively. Also specificity of the diagnosis was 66.6% on first group then reached 100% in different duration groups. Predictive values were also shown in table (3). Fig. (1) & (2) show the ultrasonograms of various days of pregnancy as well as negative or empty uterus in some buffaloes examined.

## DISCUSSION

Early breeding in buffaloes result in more calves and higher milk production per lactation. Accurate early detection of pregnancy therefore plays a key role in the achievement of an optimal calving to conception interval. Progesterone concentrations during different duration of the experiment, ranged between 3.42±0.23 and 4.55±0.34 ng/ml, were in agreement of some studies about pregnancy diagnosis or progesterone

profile during pregnancy in buffaloes (Kamonpatana et al. 1979a&b; Pahwa and Pandey, 1983; Humblot et al., 1988 and Aly, 1989).

In the present study, the accuracy of pregnancy (sensitivity) by progesterone assay from day 21-29 and 30-35 postinsemination was 83.33% and 85.71% respectively. The false positive diagnosis may be due to early embryonic death or persistence of CL which lead to somewhat increase of progesterone concentration. While the specificity increased from 71.42% to 77.77% by days 21-29 and 30-35 respectively.

Practically under field condition, acceptable ultrasonographic scanning for multiperus cows or buffaloes for early pregnancy diagnosis could only be achieved from 21-27 days postinsemination (Peterse et al., 1990; Szenci et al., 1990; Hazen and Laurent, 1991 and Szenci et al., 1995) due to that the bovine uterus is coiled and tortuous and the shape of most embryonic vesicles in early stages of pregnancy (10-20 days) is round or oblong which make diagnosis by ultrasonography very difficult (Curran et al., 1986a). In our study, accurate results of pregnancy diagnosis by ultrasound scanning of the uterus were 83.3% on days 30-35 PI. Non pregnant animals were diagnosed more accurately on the same duration than true positive animals. Similar differences between the specificity and sensitivity of the ultrasound scanning in cows were found by Curran et al, 1986b; Bayed et al., 1990; Badtram et al., 1991 and Szenci et al., 1998).

It could be concluded that, both ultrasonography and progesterone concentrations are highly sensitive and specific methods for early diagnosis of pregnancy in Egyptian buffaloes from day 21 postinsemination.

## REFERENCES

**Abbitt, B; Ball,, L.; Kitto, G.P.; Wilgenburg, B.; Rain, L.W. and Seidel, G.E. (1978):**Effect of three methods of palpation for pregnancy diagnosis per rectum on embryonic and fetal attrition in cows. J Am. Vet Med. Assoc. 173: 973-977.

**Alexander, B.M.; Johnson, M.S.; Guardia, R.O.; Van de Graaf, W.L.; Senger, P.L. and sassar, R.G. (1995):**Embryonic loss from 30 to 60 post breeding and effect of palpation per rectum on pregnancy. Theriogenology, 43: 551-556.

**Aly, A.H. (1989):** Profile of some hormones of pregnancy and parturition in Egyptian buffaloes. M.V.Sc., Cairo Univ.

**Badtram, G.A.; Ganis, J.D.; Thomas, C.B. and Bosu, W.T.K. (1991):** Factors influencing the accuracy of early pregnancy detection in cattle by real-time ultrasound scanning of the uterus. Theriogenology, 35: 1153-1167.

**Boyd, J.S.; Omran, S.N. and Ayliffe, T.R. (1990):** Evaluation of real time B-mode ultrasound scanning for detecting early pregnancy in cows. Vet. Rec. 127: 350-352.

**Curran, S. Pierson, R.A. and Ginther, O.J. (1986a):** Ultrasonographic appearance of the bovine conceptus from days 10 through 20. J. Am. Vet. Med. Ass., 189: 1289-1294.

**Curran, S. Pierson, R.A. and Ginther, O.J. (1986b):** Ultrasonographic appearance of the bovine conceptus from days 20 through 60. J. Am. Vet. Med. Ass., 189: 1295-1302.

**Dioysius, D.A. (1991):** Pregnancy diagnosis in dairy goats and cows using progesterone assay kits. Australian Veterinary J., 68(1): 14-16.

**Franco, O.J.; Drost, M.; Thatcher, M.J.; Shille, V.M. and Thatcher, W.W. (1988):** Fetal survival in the cow after pregnancy diagnosis by palpation per rectum. Theriogenology, 27: 631-644.

**Hazen, C and Laurant, Y. (1991):** Application de l'echographic bidimensionnelle au diagnostic de gestation et l'evaluation de l'incidence de la mortalit, embryonnaire dans l'esece bovine. Ann Med. Vet., 134: 481-487.

**Humbolt, P.; Jeanguyot, N.; Ruder, C.A.; Leriche, I; Thibier, M. and Sassar, R.G. (1988):** Accuracy of pregnancy diagnosis by PSPB RIA in the plasma of dairy cows after AI. Proc. 11th ICAR, vol. 2, 94-106.

**kamonpatana, M.; Kunawangkrit, A.; Bodhipaksha, P and Luvira, Y. (1979a):** Effect of PGF2$\alpha$ on serum progesterone in swamp buffaloes (Babalus bubalis). J. Reprod. Fertil. 56: 445-449.

**Kamonpatana, M.; Van de Weil, D.F.M.; Koops, W.; Leenanuruksa, D.; Ngramsuriyaroj, C and Usanakornkul, S. (1979b):** Oestrous and early pregnancy diagnosis in swamp buffalo: Comparison of enzyme immunoassay and radioimmunoassay for plasma progesterone. Theriogenology 11: 399-409.

**Kastelic, J.P.; Bergflet, D.R. and Ginther, O.J. (1991):** Ultrasonic detection of the conceptus and characterization of intrauterine fluid on days 10 to 22 in heifers. Theriogenology, 35: 569-581.

**Knopf, L. and Schallenberger, E. (1988):** Thrombocyte determination and indicator for ovulation and conception in cattle. Zuchtyg., 23: 130

**Kubasik, N.P. (1984):** Evaluation of a direct solid phase radioimmunoassay for progesterone. Clin. Chem., 30: 285-286.

**Nada, A.S.; Takkar, O.P. and Sharma, R.D. (1984):** Serum progesterone levels as an index of pregnancy in buffaloes. Anim. Reprod. Sci., 7(5): 447-450.

**Noble, R.C. (1982):** The isolation and utilization of a pregnancy specific antigen for early pregnancy detection in bovine species. Abstracts International, B (Sciences and Engineering), 43(3): 558.

**Pahwa, G.S. and Pandey, R.S. (1983):** Gonadal steroid hormone level in blood plasma and milk of primiparous and multiparous non-pregnant and pregnant buffaloes. Theriogenology 19: 491-505.

**Pasiley, G.L.; Mickelsen, W.D. and Frost, O.L. (1978):** A survey of the incidence of prenatal mortality in cattle following pregnancy diagnosis by rectal palpation. Theriogenology, 9: 481-491.

**Pieterse, M.C.; Szence, O. Willemse A.H.; Bajcsy, A.Cs.; Dieleman, S.J. and Taverne, M.A.M. (1990):** Early pregnancy diagnosis in cattle by means of linear array real rectal time ultrasound scanning of the uterus and quantitative and qualitative milk progesterone test. Theriogenology 33: 697-707.

**Soliman, M.M.; Younis, M.; Otteifa, A.M.; Essawy, G.S.; Abass, H.I. and Essawy, S.A. (1998):** Validity of some immunological and thrombocytopenia tests for diagnosis early pregnancy in buffaloes. Alex. J. Vet. Sci., 14(1): 45-51.

**Szenci, O.; Piros, A. and Kovacs, L. (1990):** Early bovine pregnancy diagnosis by a battery operated portable ultrasonic scanner the "ultra-Scan". Proc.16th World Buiatrics Congress. 219-223. (Abstract).

**Szenci, O.; Gyulai, G.Y.; Nagy, P.; Kovacs, L.; Varga, J. and Taverne, M.A.M. (1995):** Effect of uterus position relative to the pelvic inlet on the accuracy of early bovine pregnancy diagnosis by means of ultrasonography. Vet. Quart, 17: 37-39.
**Szenci, O.; Beckers, J.F.; Humblot, P.; Sulon, J.; Sasser, G.; Taverne, M.A.M..; Varga, J., Baltusen, R. and Schekk, G.Y. (1998):** Comparison of ultrasonography, bovine pregnancy-specific protein B and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology, 50: 77-88.
**Vaillancourt, D.; Bierschwal, C.J.; Ogwu, D.; Elmore, R.G.; Martin, C.E.; Sharp, A.J. and Youngquist, R.S. (1979):** Correlation between pregnancy diagnosis by membrane slip and embryonic mortality. J. Am. Vet. Assoc. 175: 466-468.

**Table (1): Progesterone concentrations (ng/ml) in positive and negative pregnant buffalo-cows 21 to 80 days post insemination.**

| | Days post insemination | | | | |
|---|---|---|---|---|---|
| | **21-29** n=32 | **30-35** n=23 | **36-50** n=28 | **51-69** n=23 | **70-80** n=20 |
| No. of positive pregnancy | 18 | 14 | 22 | 22 | 19 |
| Progesterone conc. (ng/ml)* | 3.77±0.32 | 4.55±0.34 | 3.94±0.28 | 3.83±0.32 | 3.42±0.23 |
| No. of negative pregnancy | 14 | 9 | 6 | 1 | 1 |
| Progesterone conc. (ng/ml)* | 0.77±0.13 | 0.97±0.10 | 0.65±0.21 | 0.93±0.00 | 0.84±0.00 |

* Values represented as Mean ± S.E.

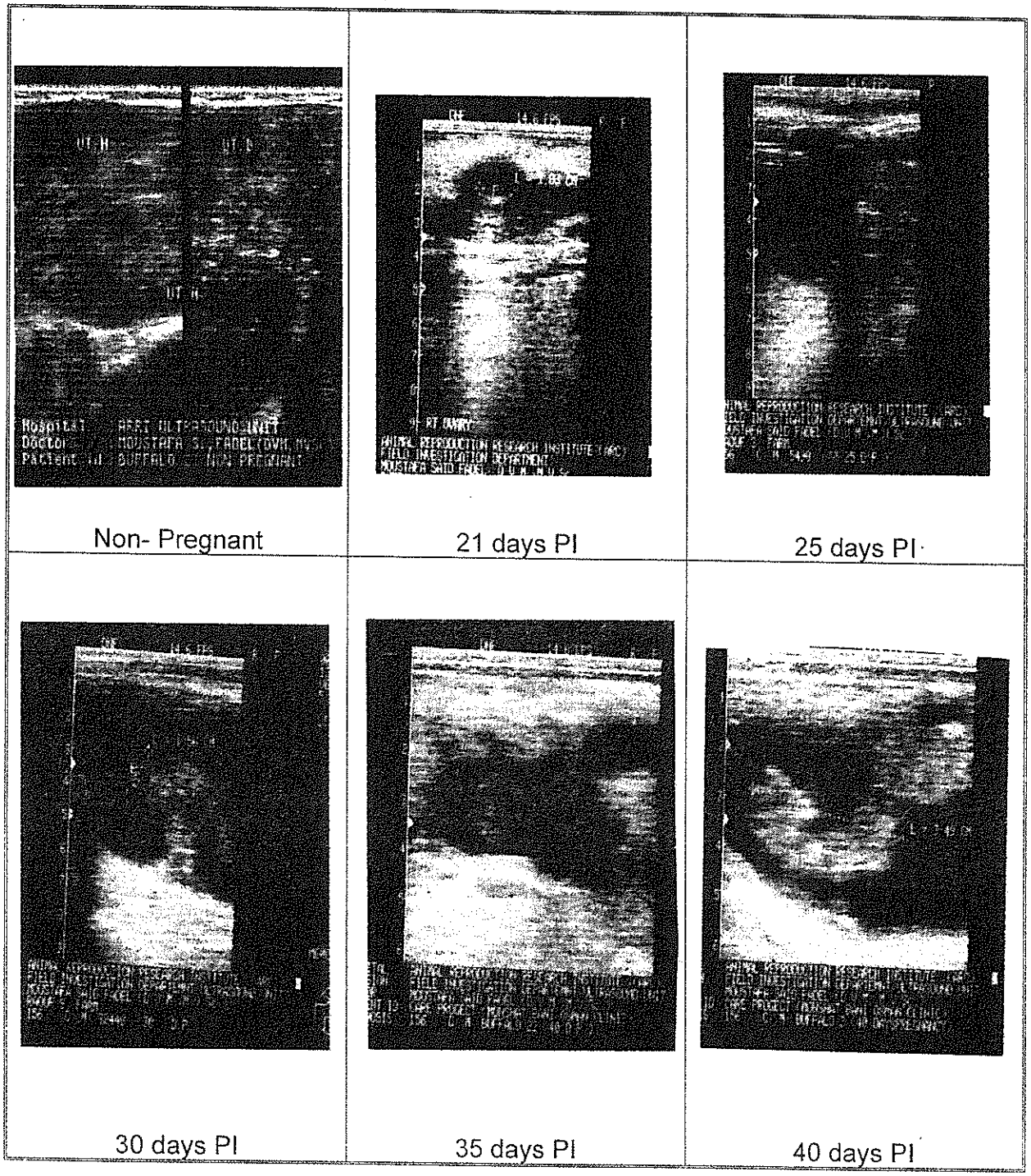
**Table (2): Early pregnancy diagnosis in Egyptian buffaloes performed by the measurements of serum progesterone concentrations.**

| | Days post insemination | | | | |
|---|---|---|---|---|---|
| | **21-29** n=32 | **30-35** n=23 | **36-50** n=28 | **51-69** n=23 | **70-80** n=20 |
| **True positive** | 15 | 12 | 20 | 22 | 19 |
| **False positive** | 4 | 2 | 0 | 0 | 0 |
| **True negative** | 10 | 7 | 6 | 1 | 1 |
| **False negative** | 3 | 2 | 2 | 0 | 0 |
| **Sensitivity (%)** | 83.33 | 85.71 | 90.91 | 100 | 100 |
| **Specificity (%)** | 71.42 | 77.77 | 100 | 100 | 100 |
| **+ve predictive value (%)** | 78.94 | 85.71 | 100 | 100 | 100 |
| **-ve predictive value (%)** | 76.92 | 77.77 | 75 | 100 | 100 |

**Table (3): Early pregnancy diagnosis in Egyptian buffaloes performed by real time ultrasonography.**

| | Days post insemination | | | | |
|---|---|---|---|---|---|
| | **21-29** n=32 | **30-35** n=23 | **36-50** n=28 | **51-69** n=23 | **70-80** n=20 |
| **True positive** | 7 | 5 | 5 | 5 | 3 |
| **False positive** | 2 | 0 | 0 | 0 | 0 |
| **True negative** | 4 | 2 | 3 | 1 | 1 |
| **False negative** | 2 | 1 | 0 | 0 | 0 |
| **Sensitivity (%)** | 77.7 | 83.3 | 100 | 100 | 100 |
| **Specificity (%)** | 66.6 | 100 | 100 | 100 | 100 |
| **+ve predictive value (%)** | 77.7 | 100 | 100 | 100 | 100 |
| **-ve predictive value (%)** | 66.6 | 66.6 | 100 | 100 | 100 |

Fig. (1)



Non- Pregnant

21 days PI

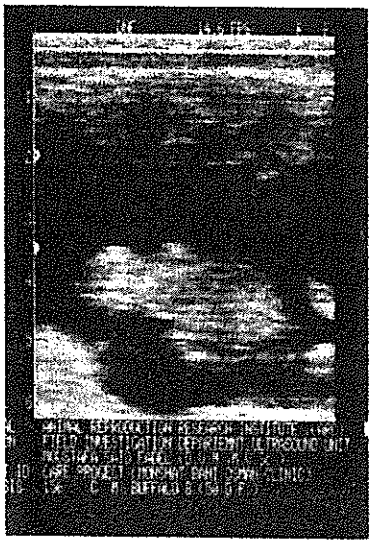
25 days PI

30 days PI

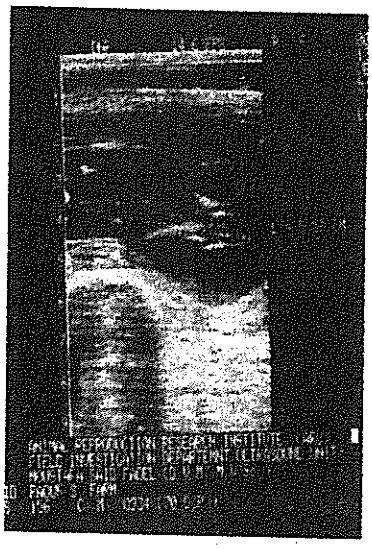
35 days PI

40 days PI

Fig (2):



50 days PI



70 days PI

78 days PI

80 days PI

الملخص العربى

## تشخيص الحمل المبكر بواسطة قياس الهرمونات أو الموجات الفوق صوتية في الجاموس المصري

أيمن حسن علي – طارق عبد السلام حسن – سيد علي أحمد عيسوي

معهد بحوث التناسليات الحيواني مركز البحوث الزراعية

في هذه الدراسة تم الاستعانة بقياس هرمون البروجيستيرون بطريقة المناعة الاشعاعية وكذلك جهاز الموجات الفوق صوتية ( السونار) وذلك لاستكشاف حالات الحمل المبكر في الجاموس. و قد تم قياس هرمون الروجيستيرون في عدد ١٢٦ أنثى جاموس في خلال الفترة من ٢١ وحتى ٨٠ يوما بعد التلقيح. كما تم أيضا فحص عدد ٤٢ أنثى جاموس عن طريق جهاز السونار في نفس الفترات المذكورة.

وقد أوضحت النتائج أن تركيزات هرمون البروجستيرون في حالات الحمل المبكر تراوحت بين ٤٢, ٣±٥٥, ٤ نانوجرام/مللي في الفترة من ٢١ وحتى ٨٠ يوما من التلقيح. بينما كانت النسبة تتراوح بين ٦٥, . ±٢١ , . نانوجرام/مللي في الحالات السلبية للحمل. وقد كانت حساسية تحديد الحمل بقياس البروجيستيرون ٣, ٨٣ % في فترة ٢١ وحتى ٢٩ يوما من التلقيح زادت إلي ١٠٠% بعد ٣٦-٥٠ يوما من التلقيح.

كما اظهرت نتائج الفحص بالسونار أن حساسيته لتحديد الحمل الإيجابي كانت ٧, ٧٧ % في فترة ٢١-٢٩ يوما من التلقيح زادت إلي ٣, ٨٣ % بعد ٣٠-٣٥ من التلقيح وفي نفس الوقت كانت دقة تحديد عدم وجود حمل ٦, ٦٦ % في فترة ٢١-٢٩ يوما وصلت إلي ١٠٠ % بعد ٣٠-٣٥ يوما من التلقيح.

من هذه النتائج يتضح أن كل من هرمون البروجيستيرون وكذلك الفحص بالموجات فوق الصوتية يعتبرا طريقتان شديدة الحساسية لمعرفة الحمل المبكر في الجاموس المصري.