Egyptian J. Anim. Prod. (2002)39(2):87-94.

PROGESTERONE PROFILE IN RELATION TO CORPUS LUTEUM DEVELOPMENT THROUGHOUT THE NORMAL ESTROUS CYCLE OF EGYPTIAN BUFFALOES

A. H. Barkawi, Y. M. Hafez, S. A. Ibrahim, Amal K. El-Asheeri and N. Ghanem

Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt

SUMMARY

Nine non- lactating, non- pregnant buffaloes were used in this study, which extended for 12 months. Estrus was checked twice daily, 12 hours apart, starting at 06:30 hr to determine day of estrus (zero day). Estrus was recognized based on the stand of female when being mounted by male.

Daily ultrasound examination for both ovaries was performed to record size pattern of corpus luteum (CL). Blood samples were collected to determine the concentration of progesterone (P_4) and prostaglandin $F_2\alpha$ in blood plasma throughout the normal estrous cycle.

During the experimental period data of 53 normal estrous cycles were collected. The total number of detected ovulations was 106, including 19.8 % as quiet cases. Progesterone profile and ultrasound examination revealed that duration of growth; plateau and regression phases of CL size were 6.4 ± 0.4, 10.7 ± 0.6 and 4.4 ± 0.3 days, respectively. The pattern of CL growth conformed with P_4 concentration; however, the peak of P_4 occurred four days after CL reaching its maximum diameter (1.5 0.04 cm). During the first four days of the estrous cycle, progesterone concentration was at its basal level (< 1.0 ng/ml) before reaching to the level of ≥ 1.0 ng/ ml at day five. Prostaglandin $F_{2\alpha}$ increased gradually to reach its peak (12 pg/ml) on day 18 of the cycle, which coincided with P_4 decline phase. Thereafter, it declined to its basal level (< 7.0 pg/ml) up to the next heat.

Results clarified that six days should be subtracted from the day at which P_4 reaches ≥ 1.0 ng/ ml to determine day of estrus (zero day) in cases of quiet ovulation.

Keywords: ***Buffaloes, estrous cycle, progesterone, prostaglandin $F_{2\alpha}$, quiet ovulation***

INTRODUCTION

Determination of progesterone profile in peripheral blood plasma is regularly used to describe corpus luteum development (Kamonpatana *et al.*, 1976, Shafie *et al.*, 1982, Kanai and Shimizu, 1984, Barkawi *et al.*, 1986 and Aboul-Ela *et al.*, 1987). The applied regimens for blood sampling collection were either daily (Kamonpatana *et al.*, 1976 and Kanai and Shimizu, 1984) or twice weekly (Shafie *et al.*, 1982, Barkawi *et al.*, 1986 and Aboul-Ela *et al.*, 1987). In the most of the previous studies, level of ≥ 1.0 ng/ml (providing to continue for at least three consecutive samples) was considered as the main indicator for the start of corpus luteum function.

In Egyptian buffaloes, a conclusion was reached to subtract three days from the date at which progesterone concentration reaches ≥ 1.0 ng/ml to determine the approximate date of quiet ovulation incidence (Shafie *et al.*, 1982), which means that the corpus luteum starts its secretion function three days earlier than that reported in Swamp buffaloes (Kanai and Shimizu, 1984) and dairy cattle (Kanchev *et al.*, 1976).

Rare data are available concerning the pattern of prostaglandin $F_{2\alpha}$ during the estrous cycle of buffaloes. Basal level, concentration at peak and the time at peak are still obscure.

Daily profile of progesterone concentration during the estrous to determine precisely the date of quiet ovulation cases as well as to determine prostaglandin $F_{2\alpha}$ pattern in relation to corpus luteum development were the target of the present study.

MATERIALS AND METHODS

Animals, management and experimental procedure

Data of 53 normal estrous cycles (20 22 d) collected throughout 12 months from nine non- lactating, non- pregnant buffaloes were used in this study. Buffaloes were housed in a semi shaded open yard and fed according to NRC (1988) allowances.

Estrus was checked twice daily, 12 hours apart, starting at 06:30 hr to determine day of estrus (zero day). Mutual behavior between bull and female (standing behavior) was the principle sign to recognize buffaloes on heat.

Daily examination for both ovaries was proceeded to characterize the developmental features (growth and regression rates) of the corpus luteum (CL) using ultrasound technique (B mode linear array ultrasound scanner, Pie Medical, Masstricht, Netherlands). The scanner is provided with a dual frequency (6.0 and 8.0 M Hz) trans-rectal transducer (Probe) for measuring the diameter of CL.

Blood sampling and hormonal assay

Blood samples were collected daily before drinking in the morning (7:30 hr) from the jugular vein into heparinized tubes to determine the progesterone and prostaglandin $F_2\alpha$ concentrations. After collection, samples were centrifuged at 3000 rpm for 15 minutes for plasma separation. Harvested plasma was stored at - until the time of assay.

Progesterone (P_4) concentration (ng/ml) was measured using antibody coated tube kits (coat-A-count, DPC, Los Angeles, USA) by radioimmunoassay technique. The standard curve ranged between 0.1 and 50 ng/ml. According to the manufacturer's information sensitivity value was reported to be 0.1 ng/ml while, values of cross reaction were reported to be 8.8 % for 5α-Hydroxy progesterone, 7.1% for 5- di-Hydroxy progesterone and <1% with the other steroids and metabolites.

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) concentration was measured using Correlate-EIA assay (A competitive immunoassay kit, Assay Designs Inc., USA). The values of $PGF_2\alpha$ standard curve ranged from 3.0 pg/ml to 50 ng/ml. Sensitivity value of the assay was reported to be 6.8 pg/ml. Cross reaction was 3.6 and 0.8 % against PGD_2 and PGE_2, respectively and < 0.1% for the other metabolites. The intra- and inter-assay precision coefficients were 8.2 and 6.1, respectively.

Measurements and statistical analysis

Ovulation cases that were not accompanied by standing behavior were considered as quiet ovulation. In the case of quiet ovulation, day of estrus (zero day) was determined when ultrasound scanner, providing that this disappearance was replaced by a formation of corpus haemorrhagicum, detected after an abrupt disappearance of the largest follicle. The growth or regression rate of CL was calculated as the increase or decrease in its diameter in a particular phase divided by the length of that phase. Diameter of corpus luteum was determined as follows:

$$\text{Corpus luteum diameter} = \frac{\text{Largest diameter} + \text{smallest diameter}}{2}$$

Means $\pm$ SE were calculated using simple statistical method of analysis (SAS, 1998).

RESULTS

Ovulation was detected on day one of the cycle, followed by the formation of corpus haemorrhagicum (CH). Corpus haemorrhagicum lifespan extended for about four days, thereafter, the corpus luteum was existed. At the end of the cycle the regressed corpus luteum continued till day two of the next cycle (corpus albicans) (Table 1 and Figure 1).

Uterus on the day of estrus looked turgid and contained sacks filled with fluids (Plate 1.a) compared to the condition of uterus in the mid-cycle (Plate 1.b). Out of detected ovulations (106), the percentage of quiet ovulation incidence represented 19.8% (21 cases).

After ovulation, corpus haemorrhagicum was formed with a diameter of 0.5 cm. Corpus luteum started to develop in three distinct phases, growth, plateau (maintenance) and regression, lapsing 6.4 $\pm$ 0.4, 10.7 $\pm$ 0.6 and 4.4 $\pm$ 0.3 days, respectively (Figure 1). Corpus luteum grew and regressed at similar rate averaging 0.1 cm/day. Corpus luteum reached its maximum diameter (1.5 $\pm$ 0.04 cm, Table 1) around day seven of the estrous cycle (Figure 1), and at the end of the cycle had a diameter $\leq$ 1.0 cm (Table 1).

At the beginning of the estrous cycle (day zero to day four) progesterone concentration level was between 0.1 and 0.9 ng/ml. Progesterone concentration increased to reach the level of $\geq$ 1.0 ng / ml at day five of the cycle, before increasing exponentially to reach its maximum concentration at day 11 (Figure 2). At the end of the cycle (day 18), progesterone concentration declined abruptly reaching its basal level after four days. This coincided with the regression phase of the corpus luteum (Figure 1) reaching corpus albicans. Progesterone level was similar in trend to corpus luteum development pattern however, its peak occurred four days after the corpus luteum size peak time.

Prostaglandin $F_{2\alpha}$ showed a gradual increase in its concentration up to day 17 of the estrous cycle (between 4.3 and 7.0 pg/ml). Thereafter, abrupt increase occurred at day 18 of the cycle, coinciding with the decline in progesterone concentration and regression of CL (Figure 1).

Table 1. Characteristics of corpus luteum development stages throughout the normal estrous cycle of Egyptian buffaloes

Trait	Corpus Haemorrhagicum	Corpus Luteum	Regressed Corpus Luteum
Life time (day)	3.6 ± 0.57	11.2 ± 0.83	4.4 ± 0.47
Diameter (cm)	0.5 ± 0.03	1.5 ± 0.04	
Progesterone (ng/ml)	0.51 ± 0.45	4.8 ± 1.2	0.85 ± 0.93
Prostaglandin $F_{2\alpha}$ (pg/ml)	6.3 ± 0.19	6.4 ± 0.18	8.0 ± 0.92

DISCUSSION

The pattern of progesterone in the present study agrees with those reported previously by Shafie *et al.* (1982), Barkawi *et al.* (1986) and Aboul-Ela *et al.* (1987) in Egyptian buffaloes, and with Kanai and Shimizu (1984) in Swamp buffaloes.

Kanai and Shimizu (1984) reported that the first significant increase of progesterone ($\geq$ 1.0 ng/ml) and date of peak occurred at day 6 and 14, respectively, which match with the findings of the present study. However, the concentration at peak (2.4 ng/ml) was half of the present study (4.8 ng/ml). On the contrary, Kamonpatana *et al.* (1976) reported earlier secretion activity of corpus luteum, where the first increase of progesterone occurred at two days after estrus.

In Friesian cattle, progesterone starts to increase in similar manner of the present study (around day five, Kanchev *et al.*, 1976), however the time of progesterone peak occurs earlier (within the first 10 days) (Kanchev *et al.*, 1976 and Barkawi *et al.*, 1994) than in buffaloes.

The low level of progesterone during the first five days of the estrous cycle clarified that within that period the corpus luteum has no ability to secrete a significant concentration of progesterone. Accordingly,s five days; instead of three days as reported by Shafie *et al.* (1982); should be subtracted from the date at which progesterone concentration reached $\geq$1.0 ng/ml to determine date of quiet ovulation; in normal estrous cycle.

Reaching the peak of progesterone concentration later than that of the corpus luteum size means that the development of corpus luteum size does not coincide with its functional activity. Therefore, the present results indicated that the maximum diameter of the corpus luteum does not denote its maximum secretion activity. This is contradicting with the finding of Jainudeen and Hafez (1992) in which progesterone level peaked earlier than reaching the maximum diameter of corpus luteum.

This difference between the growth pattern of corpus luteum and the concentration pattern of progesterone raises a question concerning the cellular activity of the corpus luteum particularly during the growth phase. Further histological and histochemical studies are needed to clarify the relation between corpus luteum development and its function.

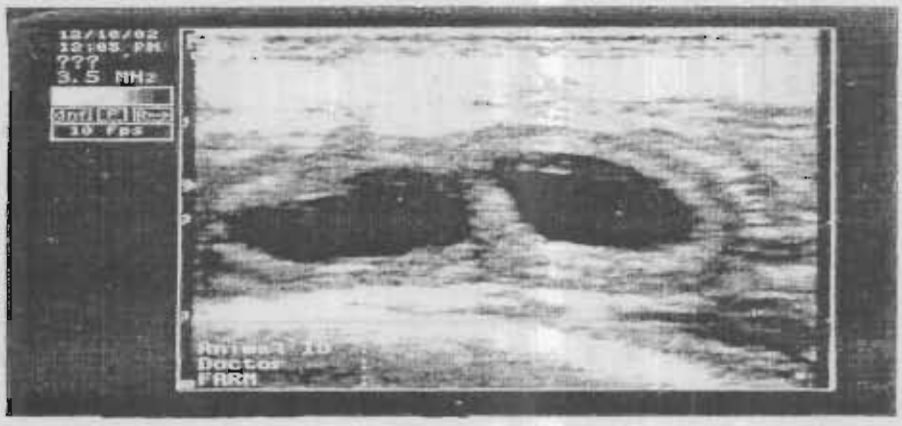

Plate 1.a.: Water sacs inside the uterus during estrus

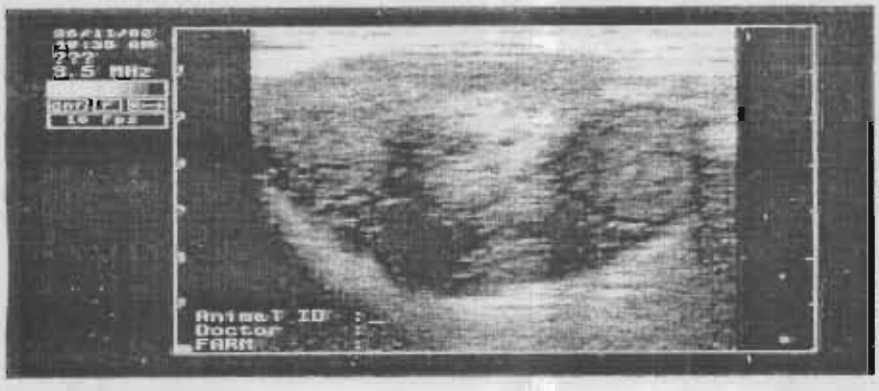

Plate 1. b. The uterus during diestrus

Plate 1. Uterus condition as shown by ultrasonography during the estrus day (1a) and diestrous (1b)

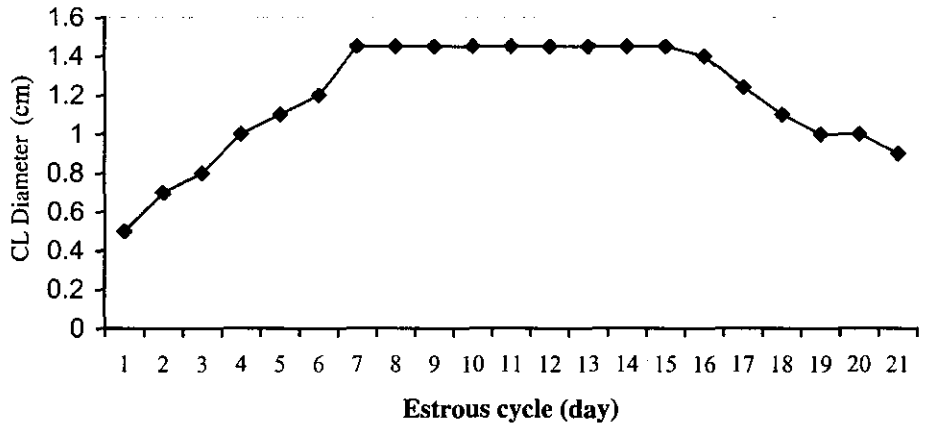

Figure 1. Development of corpus luteum during the normal estrous cycle of Egyptian buffaloes

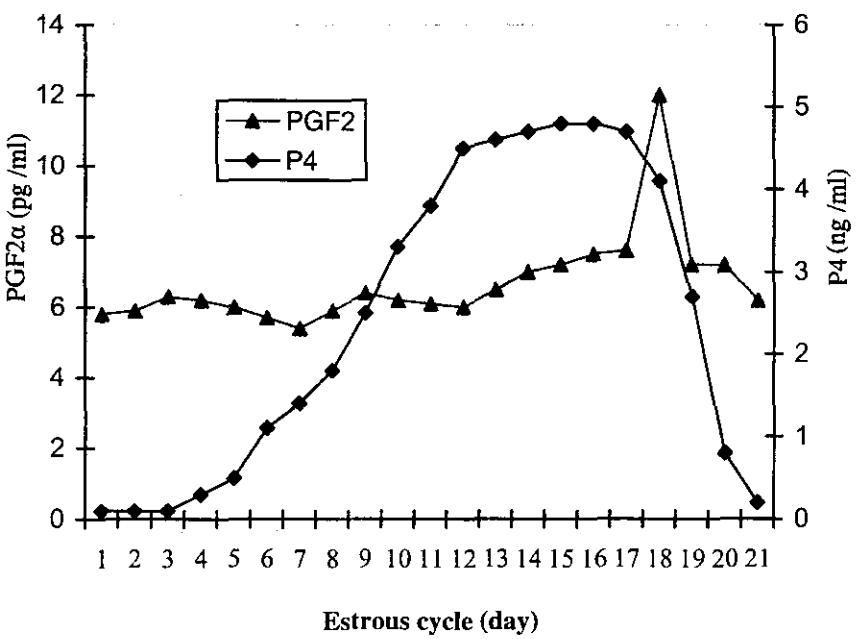

Figure 2. Progesterone (ng/ml) and prostaglandin $F_{2\alpha}$ (pg/ml) concentrations during the normal estrous cycle of Egyptian buffaloes

The abrupt increase in $PGF_{2\alpha}$ on day 18 of the cycle (Figure 2) emphasizes its role as luteolysis promoter. The reported concentration of $PGF_{2\alpha}$ in the present study, had normal pattern but it was lower (12 pg/ml) than that reported previously (68.8-95.2 pg /ml) by Beg and Totey (1999) in cattle. Since it is the first study to determine $PGF_{2\alpha}$ in Egyptian buffaloes, it is not clear whether this difference is attributed to the type of animal or the method of determination applied in the present study (ELISA).

ACKNOWLEDGEMENT

The authors would like to thank the Academy of Science and Technology, Egypt for financing this work through the project of *"Using New Techniques for Improving the Reproductive Performance of Egyptian Buffaloes"*, which had been conducted at Animal Physiology Lab., Faculty of Agriculture, Cairo University.

REFERENCES

Aboul-Ela, M. B., R. M. Khattab, F.E. El-Keraby, M.M. Shafie and L.H. Bedier, 1987. Patterns of ovarian and oestrous activity and induction of cyclic activity during the post-partum in Egyptian buffaloes. Proceedings of the Final Research Coordination Meeting on Optimization of Grazing Animal Productivity in the Mediterranean and North Africa Region with the Aid of Nuclear Techniques. Rabat, Morocco, March 23-27. pp: 239-254.

Barkawi, A. H.; F. E. El-Keraby, and S. A. Sweify, 1994. Puberty of Friesian heifers in subtropical zone, Egypt. Egyptian J. Anim. Prod., 31 (1): 27- 41.

Barkawi, A. H.; M.M. Shafie, Y. Mekawy, and M.B. Aboul-Ela, 1986. The use of serum and milk progesterone concentration to monitor post-partum ovarian activity in Egyptian buffaloes. Buffalo Journal, 2: 125-134.

Beg, M. A. and S. M. Toty, 1999. The oestrous cycle, oestrous behaviour and the endocrinology of the oestrous cycle in the buffalo (*Bubalus bubalis*). Animal Breeding Abstracts, 67 (5): 329- 337.

Jainudeen, M.R. and E.S.E. Hafez, 1992. Reproductive cycles: cattle and water buffalo in: Reproduction in Farm Animals, Edit. E.S.E. Hafez, Lea and Febiger, Philadelphia, USA. PP: 307-314.

Kamonpatana, M.; Y. Luvira; P. Bodhipaksha; and A. Kunawongkrii, 1976. Serum progesterone, 17-hydroxy progesterone and 17 -oestradiol during the oestrous cycle in the Swamp buffalo in Thailand. Nuclear Techniques in Animal Production and Health (Proc.Symp. Vienna), IAEA, Vienna, 569.

Kanai, Y., and H. Shimizu, 1984. Plasma concentration of LH, progesterone and oestradiol during the oestrous cycle in swamp buffaloes (*Bubalus bubalis*). Journal of Reproduction and Fertility, 70: 507-510.

Kanchev, L. N.; Hilary Dopson, W. R. Ward and R. J. Fitzpatricki, 1976. Concentration of steroids in bovine peripheral plasma during the oestrous cycle and effect of betamethasone treatment. J. Reprod. Fert. 48: 341-345.

NRC, 1988. Nutrient requirements of dairy cattle. National Academy of Science. National Research Council, 6th revised ed. Washington DC., USA.

Shafie, M. M., H. M. Mourad, A.H. Barkawi, M.B. Aboul-Ela and Y. Mekawy, 1982. Serum progesterone and oestradiol concentration in the cyclic buffalo. Tropical Animal Production, 7: 283-289.

SAS, 1998. SAS user's guide for personal computers, SAS Institute, Inc., Cary, NC. USA.

مستوى البروجسترون وعلاقته بتطور الجسم الأصفر خلال دورة الشبق الطبيعية للجاموس المصري

أشرف هشام برقاوى ، ياسين محمد حافظ ، صالح عبد الحميد إبراهيم ، آمال كمال العشيري ، ناصر غانم

قسم الإنتاج الحيواني – كلية الزراعة – جامعة القاهرة – الجيزة –ج.م.ع

استخدم في هذه الدراسة تسعة من إناث الجاموس غير الحوامل في تجربة استمرت اثنا عشر شهراً ، كانت الحيوانات تراقب خلالها لتحديد الشبق بواقع مرتين يومياً وبفاصل زمني اثنتا عشرة ساعة . وقد تم تحديد الشبق اعتماداً على استسلام الأنثى للذكر عند محاولة الاعتلاء . وقد تم فحص الحيوانات يومياً باستخدام الموجات فوق الصوتية لتحديد التبويض وسمات تطور حجم الجسم الأصفر . كما تم جمع عينات دم يومية لتقدير مستوى هرموني البروجستيرون والبروستجلاندين خلال دورة الشبق .

خلال فترة التجربة تم جمع بيانات عن ٥٣ دورة شبق طبيعية (٢٠-٢٢ يوم) وبلغ عدد التبويضات ١٠٦ تبويض ، كانت نسبة التبويضات الصامتة منها ١٩,٨% كما بلغت فترات نمو واستمرار واضمحلال الجسم الأصفر ٦,٤ ± ٠,٤ ، ١٠,٧ ± ٠,٦ ، ٤,٤ ± ٠,٣ أيام على التوالي ، تطابقت مراحل تطور الجسم الأصفر مع تركيز البروجستيرون في الدم المحيطي للحيوانات تحت الدراسة ، إلا أن الجسم الأصفر وصل إلى أقصي نمو له (١,٥ سم) قبل وصول البروجستيرون لأقصي تركيز له بعد أربعة أيام .

خلال الأيام الأربع الأولى من دورة الشبق كان تركيز هرمون البروجستيرون في المستوى القاعدي (٠,١- ٠,٩ نانوجرام /سم^3) قبل أن يصل إلى تركيز ≤ ١ نانوجرام / سم^3 في اليوم الخامس.

أرتفع مستوى هرمون البروستجلاندين تدريجياً خلال الأيام السبع عشرة الأولى من دورة الشبق قبل أن يرتفع فجائياً إلى أعلى تركيز له (١٢ بيكوجرام/ سم^3) في اليوم الثامن عشر الذي توافق مع بداية اضمحلال الجسم الأصفر وانخفاض مستوى هرمون البروجستيرون إلى المستوى القاعدي. وقد استمر هرمون البروستجلاندين في مستواه القاعدي حتى الشبق التالي.

وقد أوضحت النتائج أنه يجب طرح خمسة أيام من تاريخ وصول تركيز هرمون البروجستيرون إلى مستوى ≤ ١ نانوجرام/ سم^3 لتحديد يوم الشبق في حالات التبويض الصامت.