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SHORT COMMUNICATION

Serum Progesterone Profiles During the Estrous Cycle, Pregnancy and Postpartum Periods in Yankasa Ewes

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SUMMARY

An understanding of the reproductive phases of female livestock is necessary for the evaluation of their fertility and the application of techniques for improving their performance such as the estrus synchronization programme protocol. Blood progesterone (P4) profiles have also been shown to be clear indicators of cyclic events in the assessment of reproductive functions in domestic animals. This study was therefore aimed at determining the serum P4 profiles during the estrous cycle, gestation and early postpartum periods as a tool for assessing the reproductive status of Yankasa ewes. Fourteen ewes were used for monitoring reproductive status out of which seven were selected for monitoring of progesterone profiles at varying phases of the reproductive cycle. Blood for serum was harvested via the jugular vein puncture every other day for 40 days. This was enough to demonstrate blood progesterone (P4) profiles during at least 2 estrous cycles in cycling ewes. Using the serum samples, P4 was assayed for using the Progesterone Enzyme Immunoassay Test kits. A standard curve was obtained by plotting the concentration of the standards against the absorbance. The P4 concentration of the specimen was calculated from the standard curve and then individual graphs were plotted for each ewe, plotting the dates of collection against the P4 concentration. The interpretation of these graphs showed that 3 ewes were pregnant and one of them lambed during the course of the study with a basal P4 concentration during the post- partum period. The other 4 ewes were at different stages of the estrous cycle and exhibited long and short estrous cycles. The lengths of the short, normal and long estrous cycles were 11&14, 16 & 21, and 25 days respectively. The P4 profiles identified animals with short cycles, normal cycles, and those during the postpartum period and pregnancy. It is concluded that P4 profile can be used for the classification of the reproductive cycles/stages and can therefore be a useful tool in assessing reproductive functions in domestic livestock.

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INTRODUCTION

The ability to rapidly improve genetics and thus increase profit in livestock production is centered on the ability to use key specialized techniques e.g. artificial insemination and embryo transfer. Both of these advanced reproductive techniques require the ability to effectively manipulate the reproductive cycles (Michael et al 2004). The knowledge of the stages of the reproductive cycle is a prerequisite to synchronize estrus in domestic animals.

Blood progesterone (P4) is the key hormone of pregnancy and thus often called the "pregnancy hormone". Its importance in maintaining pregnancy can not be over emphasized as it acts to prevent the resumption of cyclicity, prepares the uterus for implantation and maintains myometrial quiescence (Lye 1996). Myometrial quiescence during pregnancy is achieved by the combined action of P4 relaxin, prostacyclin and nitric oxide (Lye, 1996).

During pregnancy, P4 remains high in circulation and declines towards parturition due to prostaglandin F2α release (Khanum et al, 2008).

P4 is also present at varying levels during the estrous cycle, being low on day 0(estrus), increases progressively to some extent and peaks at day 12 (mid cycle), maintained till day 15, and then declines thereafter (Khanum et al, 2008), due to prostaglandin induced luteolysis signaling the proestrus of the succeeding cycle.

During the postpartum period early resumption of ovarian activity is a critical event for determining the parturition interval in domestic animals. Shorter parturition intervals will increase the lamb, kid and calf crop, in sheep, goats and cattle respectively. P4 in blood can therefore be used as an indicator of commencement of cyclicity following parturition and the chances of rebreeding of the female animals. Early rebreeding will therefore result in increased life time productivity of ruminant species.

Being the most accurate indicator of cyclic activity in domestic animals and the most predominant hormone during pregnancy, P4 in serum can be used to diagnose pregnancy and determine the stage of the estrous cycle (Oyedipe et al, 1986). This study was thus aimed to determine the serum P4 profile during the estrous cycle, gestation and early postpartum period ewes and as a tool for assessing the reproductive status of Yankasa ewes.

MATERIALS AND METHODS

Location

The experiment was conducted using the ewes from the Livestock Unit of the Research and Teaching Farm of the University of Agriculture, Makurdi. For the blood profile study, seven ewes were randomly selected and moved from the University Farm to the Veterinary Teaching Hospital (VTH) which is located at the North Bank Makurdi (off campus) with a latitude of 07° 43 59" N and a longitude of 08° 31 59" E at the elevation of 106m above sea level. The climate of the area is characterized by an average annual minimum and maximum temperature of 21 to 35°c respectively. The annual rainfall ranges from 1270 to 1397mm and average annual temperature ranges from 22.43°c to 33.41°c (Abu, 2002).

Experimental Animals

Seven matured ewes of unknown parity were selected at random from the flock of sheep in the University of Agriculture Makurdi animal farm. The ewes were tagged with the following numbers: 994, 995, 996, 997, 998, 999 and 1000. They were aged 4, 3, 4, 4, 2 ½, 4, and 4 years, and had body weights of 27, 25, 25, 14, 16, 32 and 28kg respectively.

Management of the Animals

While at the University farm the ewes were managed under semi intensive system

where they were allowed to graze natural pastures in the day and return to their pen at night to rest. Reproductive records were not properly kept making selection for estrus synchronization using PGF2α uncertain.

For the study, the ewes were confined at the VTH, to ease sample collection and observing for estrus and possible lambing. The animals were kept to acclimatize for a week before the start of the experiment, during this period the animals were given routine treatment e.g. deworming with Albendazole at 25mg/kg and antibiotic treatment with oxytetracycline LA at 20mg/kg

Blood Sample Collection

Blood samples were harvested twice weekly via the jugular venipuncture from the seven ewes involved in the study into sample bottles without anticoagulant. The samples were placed in the refrigerator and allowed to clot at 4°c for 24hrs. Serum was separated by centrifugation, decanted into sample bottles and stored at -20oc until analyzed using the P4 Enzyme Immunoassay Test Kit catalog number: PROG-96.

For 6 ewes, blood collection spanned between 24/12/2010 to 28/01/2011. Collection of blood samples from the 7th ewe followed the same pattern but continued following lambing on the 10/01/2011, every other day until day 30 post partum.

Assay Method

Serum P4 concentration was determined using the P4 Enzyme Immunoassay Test Kit, Catalog number; PROG-96. (http□www.caymanchem.com)

The stepwise procedure for the serum assay is as follows;

· -the desired number of coated wells was secured in the holder.

· -it was incubated at room temperature (18-25oc for 90 minutes.

· -it was incubated at room temperature (18-25oc for 20 minutes.

· -the micro wells were flicked and rinsed 5 times with distilled water.

· -100µl of the substrate was dispensed into each well and was mixed gently on the titertek shaker for ten seconds.

· -it was incubated at room temperature (18-25oc for 20 minutes.

· -the reaction was stopped by adding 100µl of stop solution to each well.

-it was gently mixed for 30seconds on the titertek shaker; it was observed that the entire blue colour changed to yellow colour completely.

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· -absorbance was read with a micro titer well reader at 450nm within 15 minutes.

Data Handling

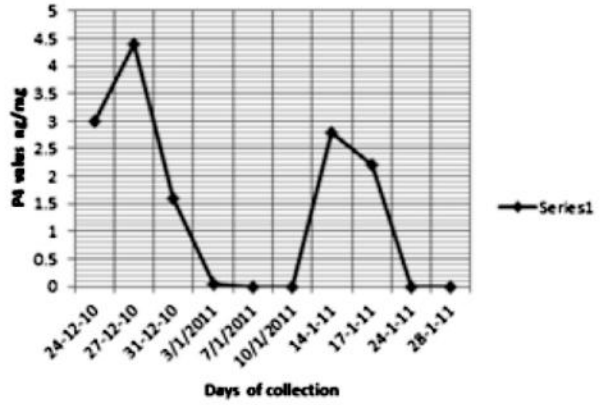
The mean absorbance value for each set of reference standards, controls and samples were calculated. A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on a graph, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis. The mean absorbance values for each specimen were used to determine the corresponding concentration of progesterone in ng/ml from the standard curve and individual graphs were plotted for each ewe using the P4 concentrations and the days of collection.

RESULTS

The P4 profiles of the ewes showed that the ewes were at varying stages of the reproductive cycle. The profiles are shown in [Fig. 1 -7]: Ewe No 994, Showed evidence of two normal cycles WIth cyclic activities observed from the commencement of the study: (Fig 1). At the commencement of the study the estrous cycle appeared to be at the

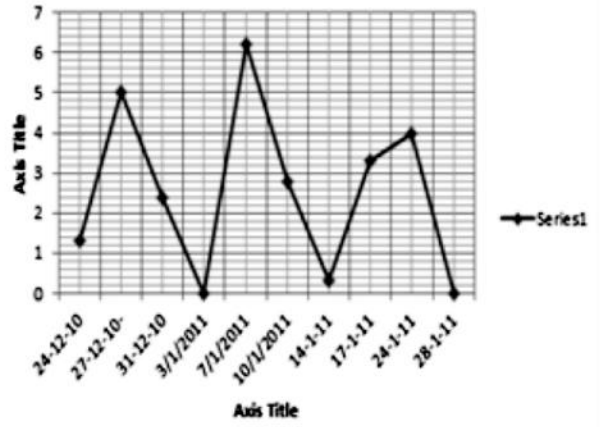
luteal phase. The P4 level fell to basal level 3 to 10th Jan 2011 and was followed by a normal cycle which occurred from 10/01/2011 to 28/01/2011 lasting 16 days. However, the P4 levels were low throughout the study. Estrus was also observed in this ewe during the stud.

Fig.1. Progesterone profile of ewe No 994 showing normal cycles



Estimated day of Estrous Cycle (0= Estrus)

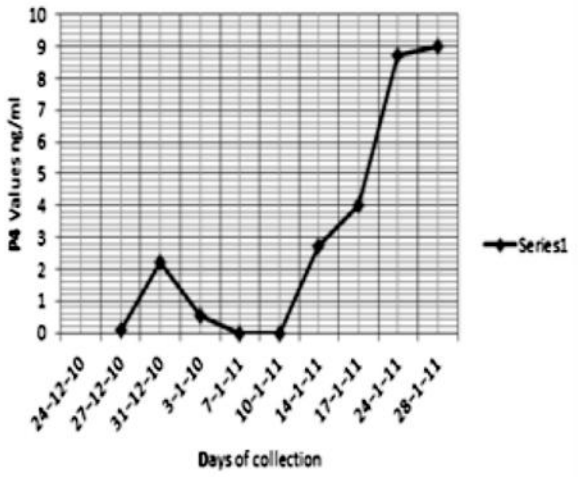
Fig.2. Progesterone profile of Ewe No 995 with short cycles



Estimated day of Estrous Cycle (0= Estrus)

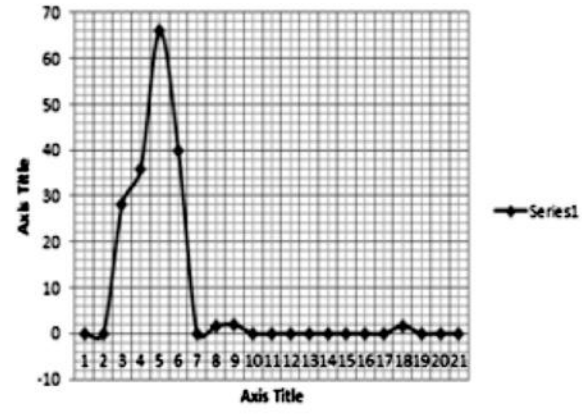
Ewe No 995: Showed three consecutive short cycles during the study period: [Fig.2] Progesterone value from 24/12/2010 to 3/01/2011 showed a cycle lasting about 14 days. This was followed by another cycle lasting 11days (3/01/2011 to 14/01/2011), and a third cycle lasting 14 days 14/01/2011 to 28/01/2011 Ewe No 996:Progesterone profiles of ewe No 996 with a short cycle preceding a normal cycle showed a cycle from 24/12/2010 to 9/01/2011 lasting 14 days. Progesterone level appeared low during this cycle. It exhibited another cycle commencing on the 9/01/2011 with an increase in P4 still at peak level by 17days after the previous basal level on about 9th Jan: [Fig.3].

Fig.3. Progesterone profile of Ewe No 996 with short cycles preceding a normal cycle



Estimated day of Estrous Cycle (0= Estrus)

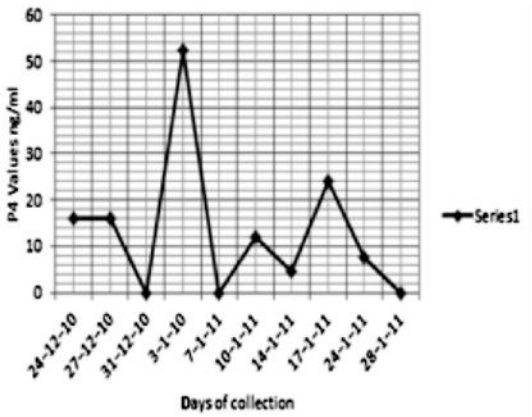
Fig.4. Progesterone profiles of ewe No 997 during pre and postpartum period



Estimated day of Estrous Cycle (0= Estrus)

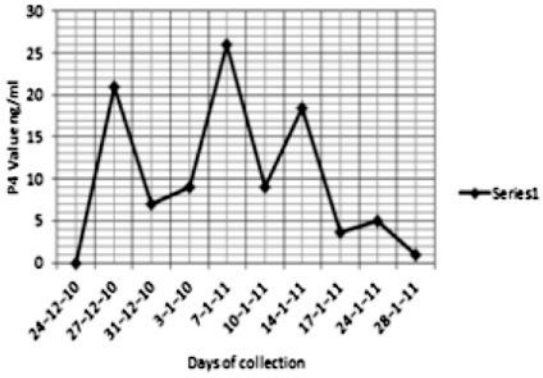
Ewe No 997: progesterone profiles during pre and postpartum periods, Showed a very high P4 value from commencement of the study, till 11/01/11 when the ewe lambed as shown in Fig 4.
Serum progesterone appeared to decline rapidly prior to lambing and remained on a basal level throughout the post partum period from 11/01/2011 to 01/02/2011. Serum P4 appeared to have increased from 2nd-3rd February, and this increase in P4 value could be a sign of resumption of normal cyclic activity post partum period from 11/01/2011 to 01/02/2011. Serum P4 appeared to have increased from 2nd-3rd February, and this increase in P4 value could be a sign of resumption of normal cyclic activity post partum

Fig.5. Progesterone profile of ewe No 998 with a long cycle



Estimated day of Estrous Cycle (0= Estrus)

Fig.6. Progesterone profiles of ewe No 999 with high P4 value throughout the experimental period.



Estimated day of Estrous Cycle (0= Estrus)

Ewe No 998:progesterone profile with a long cycle-At the commencement of the study ewe No 998 appeared to be at the luteal phase of the estrous cycle. The P4 level fell to the basal level on the 31/12/2010 and a second cycle commenced on the 3/01/2011 and ended on the 28/1/2011. This cycle lasted for 25days. During the luteal phase of this cycle the P4 values were high as shown in Fig 5.

Ewe No 999: showed a high level of P4 from the commencement of the study to the end. High P4 values were seen from day 27/12/10 to 14/01/11 with the highest value of 15ng/ml:[Fig 6]. On day 17/01/11 P4 value declined but not to a zero level, the lowest value observed was 1.5ng/ml. On physical examination this ewe appeared pregnant: the mammary glands were enlarged and the abdomen appeared markedly enlarged and on palpation the fetus was felt at the right side of the

Fig.7. Progesterone profiles of ewe No 1000 with a long luteal phase.

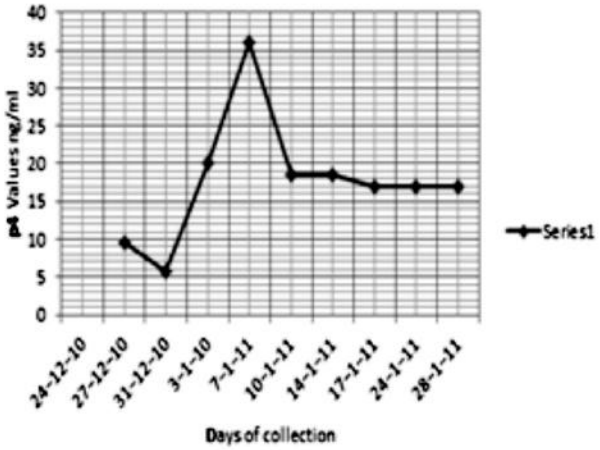


Fig.7. Progesterone profiles of ewe No 1000 with a long luteal phase.

abdomen.
Ewe No 1,000:Progesterone profiles of with a long luteal phase-showed high P4 concentration from the commencement of the study to the end with a peak value of 20ng/ml. This ewe appeared to be

pregnant, as observed in Fig 7 and there was no return to estrus. On physical examination the ewe appeared pregnant judging from the size of the mammary gland and abdomen.

DISCUSSION

The results of this study show that estrous cycle lengths in the Yankasa ewes can be short, normal and long depending on factors such as nutrition, diseases, and observation is similar to reports by Garci et al, (1989). The incidence of the long and short luteal cycles among Yankasa in Nigeria had been reported by Oyedipe (1986), and could be among the major problems responsible for reductions in herd fertility and reproductive management in Africa (Rekwot et al, 2000).

A prolonged phase can reflect occurrence of early embryonic mortality (Bulman et al, 1978) as was observed in the P4 profiles of ewe No. 998 and ewe No. 994 where they showed longer cycles of 25 and 21days respectively. Early embryonic death can be indicated when P4 declines beyond expected day of estrus following breeding in the previous estrus. This could also be as a result of season (dry season when feed is scarce) and housing, Llewelyn et al, (1992). The latter reported that both season and housing may have influenced the pattern of ovarian activity during the year, with the proportion of normal cycles being highest in the winter months (June, July and August) when the goats were in single pens, and lowest during the ensuring hot spring (September, October and November) and summer (December, January and February) months. The ewes were kept together in one pen during the study period which could also have disrupted follicular development. Moberg (1991) also reported the possibility that stress caused by fighting could disrupt follicular development. These extended cycles may also be as a result of degree of genetic variability in the mechanisms controlling follicular dynamics in this unselected population. Llewelyn et al, (1992) had also reported that the major source of variation in cycle length was the length of the peri-ovulatory period could be estimated to be about 20 days as seen from the result of ewe No 997; which showed a very high P4 value from commencement of the study, till 11/01/2011 when the ewe lambed as shown in Fig 4.The Serum progesterone appeared to decline rapidly prior to lambing and remained on a basal level. The results in this study showed that the post partum period in Yankasa ewe throughout the post partum period from 11/01/2011 to 01/02/2011. Serum P4 appeared to have increased from 2nd-3rd February, and this increase in P4 value could be a sign of resumption of normal cyclic activity post partum.

This finding is similar to the findings of Sharpe and King (1981) where they compared the onset of ovarian activity in Holstein and Jamaica Hope cattle raised in the tropics. They found that cyclic levels of P4 indicative of ovarian activity appeared as early as 20 days post partum and concluded that Holstein cattle kept under tropical conditions are capable of returning to a cycling, re-breeding state soon after calving as can be reasonably expected. The interval between parturition and ovulation is characterized by sexual quiescence (postpartum anoestrus). By implication, the ewe cannot be bred during this period. During the postpartum period, uterine involution occurs, following expulsion of the fetal membranes, the uterine contraction and peristalsis continue as strong rhythmical waves that gradually, diminish through the fourth day, shortening of the uterine muscle cells, discharge of lochia, from the fourth to eight days with only irregular contractions of the horn.

This study showed that the ewes were in different stages of the estrous cycle and reproductive phases indicating the

absolute need for a thorough reproductive evaluation before administration of PGF2α for synchronization. Selection of non-pregnant and normally cycling ewes are essential to achieve consistent results to reproductive manipulations.

CONCLUSION

The findings of this research indicates that P4 enzyme immunoassay test kit for P4 in serum is of practical value in determining the serum P4 profiles during the estrous cycle, gestation and early post-partum period as a tool for assessing the reproductive status of Yankasa ewes. Pregnant ewes can be diagnosed using this assay method and may be more efficient when combined with ultrasound and physical examination. This study also could aid in planning of breeding among flocks and herds with irregular reproductive patterns especially following parturition and during the peri-pubertal periods and thus help to achieve better economic returns to farmers.

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