



Oestrus Behaviour and Hormonal Characterization of the Oestrous Cycle in Nigerian Jennies

Hassan, R

Biotechnology Research Programme, NAPRI/ABU Shika Zaria, Nigeria *Corresponding author: Email: hraheelah@gmail.com; Tel No:+2348036479918

SUMMARY

This study was carried out to evaluate the onset of oestrus, duration of oestrus, oestrus behaviour and the hormonal profiles (Progesterone and oestrogen) of Nigerian indigenous jennies. Four (4) cycling Jennies were used in this study, cyclicity was determined based on two rectal palpations (30 days apart), ultrasound scans done twice and two blood samples were also obtained twice (30 days apart) to confirm cyclicity. Five (5) ml of blood was obtained from the jennies via jugular veni-puncture on the day the experiment commenced, then twice weekly for 8 weeks, to determine the progesterone and oestradiol using the principle of Enzyme-Linked Immunosorbent Assays (ELISA). Jennies were observed for behavioural oestrus three times daily (8-10 am, 12-2 pm and 4-8 pm) for two oestrous cycles. Parameters observed include; lowered head with neck extended forward, opening and closing of the mouth, ears back against the neck, standing to be mounted, tail raised from the perineum, vulva winking, mucous discharge and presentation of the perineum toward the jack. In addition, Jennies were exposed to a jack to aid oestrus detection. The following oestrus behaviour and characteristics were recorded: duration of oestrus, onset of oestrus, oestrus response rate, intensity of synchronization and physical manifestation of oestrus. It was established that the time to onset of oestrus in this study was 2.3 hours, the duration of oestrus was 48 hours. Tail raising, opening and closing of mouth (mouth clapping), flehmen with winking of the vulva were the most consistently observed signs of oestrus in jennies. Oestrus period was 8 days and the oestrous cycle length was 25 days in Nigerian indigenous jennies. In conclusion, it was established that the time to onset of oestrus was 2.3 hours, the duration of oestrus was 48 hours, oestrus response rate and intensity of synchronization were 50%, respectively. The characteristic oestrus behaviours were; head lowering, ears backward, standing to be mounted, vulva winking, mucous discharge, presentation of perineum, tail raising from the perineum, opening and closing of mouth (mouth clapping), flehmen with winking of the vulva.

Key words: Nigerian jennies, oestrus characteristics, oestrus behaviours, oestrous cycle.

INTRODUCTION

Donkey rearing in Nigeria started with the introduction of different donkey breeds through trans-Sahara caravan trade across

the Nile via the Sudan and Chad (Fielding and Starkey, 2004). The jenny (Jennet) is very similar in many reproductive aspects to

the mare and puberty is attained at 1-2 years (Blanchard *et al.*, 1999). Although oestrous cycle has been reported to range from 20-40 days (Fielding, 1988), oestrus usually lasts between 6 and 9 days, with ovulation occurring 5-6 days after the onset of oestrus (Vandeplassche *et al.*, 1981). Oestrus phase is the follicular phase or period where the jenny is in heat and is receptive to the jack and an ovum will ovulate, the oestrus phase can average anywhere from 6 to 8 days (Vandeplassche *et al.*, 1981).

The specific objective of this study was to evaluate; the onset of oestrus, duration of oestrus, oestrus behaviour and hormonal profiles (Progesterone and oestrogen) of Nigerian indigenous jennies.

MATERIALS AND METHODS

Experimental animals and management

This study was carried out at the donkey farm of the Equine and Camel Research Programme of the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika Zaria. Four (4) cycling Jennies aged 3.0 ± 1.0 years (The ages were confirmed based on the available farm records which was monitored properly.) with mean body weight of 90.6 ± 6.5 kg and mean body condition score of 3.5 ± 0.2 were used for this study. Cyclicity was confirmed using the following procedures (a) two rectal palpations (30 days apart), (b) ultrasound scans done twice and (c) two blood samples obtained twice (30 days apart). The jennies were kept outdoors in a group and fed *Digitaria smutsii* (woolly finger grass), concentrate rations at 1.2kg/jennies/day and hay as basal diet, water was provided *ad libitum*.

Blood Sampling

Five (5) ml of blood was obtained from the jennies via jugular veni-puncture using a 5 ml syringe (18 Gauge needle) on the day the experiment commenced, then twice weekly for 8 weeks, to determine the progesterone and oestradiol concentrations. The blood samples were decanted into vacutainers and

transported in an ice pack to the Biotechnology Research programme laboratory NAPRI, samaru A.B.U Zaria Blood samples collected were centrifuged at $2000 \times G$ and serum harvested. Serum samples obtained were appropriately stored at $-20^{\circ}C$ until analysis, for determining progesterone and oestradiol concentration using the principle of Enzyme-linked immunosorbent assays (ELISA).

Hormone assays

Concentrations of Progesterone and Oestradiol were measured in the serum using enzyme-linked immunosorbent assays (ELISA). The ELISA kits were obtained from Monobind[®] Inc, USA. The kits were used according to the manufacturer's specifications. The oestradiol AccuBind[™] Microplate (EIA) test system has a sensitivity of 8.2 pg/ml serum calibrator and using the 95% certainty statistic to calculate the minimum dose. The between assay precision coefficient of variation for the low, normal and high pooled controlled serum samples were 9.9%, 8.5% and 7.5%, respectively.

The progesterone AccuBind[®] ELISA test system has a sensitivity of 0.105 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 95% certainty statistic to calculate the minimum dose. The between assay precision coefficient of variation for the low, normal and high pooled controlled serum samples were 8.9%, 7.5% and 6.4%, respectively. The within assay precision coefficient of variation for the low, normal and high pooled controlled serum samples were 15.3%, 3.8% and 6.1%, respectively.

Oestrus detection

Jennies were observed for behavioural oestrus beginning on the day the experiment commenced, then twice weekly for 8 weeks. The observations were for two hours, three times daily (8-10 am, 12-2 pm and 4-6 pm) for the period of the experiment.

Parameters observed include; lowered head with neck extended forward, opening and closing of the mouth, ears back against the neck, standing to be mounted, tail raised from the perineum, vulva winking, mucous discharge and presentation of the perineum toward the jack. In addition, Jennies were exposed to a jack to aid oestrus detection. The following oestrus characteristics were recorded: duration of oestrus, onset of oestrus, oestrus response rate, intensity of synchrony and physical manifestation of oestrus.

Data analysis

Oestrus response rate, intensity of synchrony and physical manifestation of oestrus were expressed as percentages. Data on progesterone and oestradiol profile, values of the oestrous cycle, duration of oestrus and time to onset of oestrus, were expressed as Mean \pm S.E.M. The differences were considered significant when $P < 0.05$, highly significant when $P < 0.01$ and not significant when $P > 0.05$. SAS system for windows 9.0 was used for the analysis.

Table 1: Oestrus characteristic of jennies

| Parameters | Frequency | LS |
|-----------------------------|-----------------|-----|
| Onset of oestrus (hours) | 2.34 \pm 0.32 | NS |
| Duration of oestrus (hours) | 48 \pm 24.0 | ** |
| Oestrus response rate (%) | 50 | *** |
| Intensity of synchrony (%) | 50 | *** |

Significance ($P < 0.01$). LS= Level of Significance

Table 2: Oestrus Parameters of jennies

| Parameters | (%) | LS |
|--------------------------|-----|-----|
| Head lowering | 60 | *** |
| Mouth clapping | 70 | *** |
| Ears backward | 60 | *** |
| Standing to be mounted | 60 | *** |
| Tails raising | 70 | *** |
| Vulva winking | 60 | *** |
| Mucous discharge | 20 | *** |
| Presentation of perineum | 10 | *** |
| Flehmen | 70 | *** |

Significance ($P < 0.01$). LS= Level of Significance

RESULTS

Oestrus characteristics and percentage of frequency for oestrus behaviours in jennies

Oestrus characteristics and percentages of frequency for oestrus behaviours are shown in Tables 1 and 2. The characteristic oestrus behaviour of jennies were; head lowering, ears backward, standing to be mounted, vulva winking, mucous discharge, presentation of perineum, tail raising from the perineum, opening and closing of mouth (mouth clapping), flehmen with winking of the vulva. Percentage of frequency for oestrus behaviour had highly statistical significant differences ($P < 0.01$).

Progesterone and oestradiol profiles

The serum progesterone (P_4) and oestrogen (E_2) concentration of the sample collected is shown in Figure 1. Mean P_4 concentration rose from 1.35 \pm 1.08 ng/ml on day 0 to 2.01 \pm 1.80 ng/ml on day 3 then declined to 0.18 \pm 0.07 ng/ml on day 10 increasing to 2.44 \pm 2.22 ng/ml on day 18, while E_2 concentration decreased from 11.81 \pm 4.94 pg/ml on day 0 to 8.55 \pm 1.63 pg/ml on day 14 then increasing to 11.10 \pm 3.05 pg/ml on day 25. However, P_4 and E_2 are highly significant ($P < 0.01$).

DISCUSSION

It was established that the time to onset of oestrus in this study was 2.3 hours which was shorter than the work of Getachew (2014) who observed that the onset of oestrus was 86.4 hours, the duration of oestrus was 48 hours which differs from the report of Henry *et al.* (1991) who reported a longer duration of oestrus. The difference between the results in this study and the earlier studies could be as a result of breed differences, environment (tropics and temperate) and nutrition. From this study, tail raising, opening and closing of mouth (mouth clapping), flehmen with winking of the vulva were the most consistently observed signs of oestrus in

jennies, corroborating the works of Henry *et al.* (1991) and Taberner *et al.* (2008). The jennies used for this study were in their different reproductive phases, the progesterone and oestradiol levels were inversely related, as progesterone peaks oestradiol decline and this agrees with the normal physiology of these hormones

On day 14 progesterone concentration starts increasing and decline on day 21, this indicates that the corpus luteum that produces progesterone has undergone lysis therefore causing a decline in progesterone concentration from day 21

to 32. However, oestrogen and progesterone concentration are low between day 21 and 32, this signals the pituitary gland to produce follicle stimulating hormone as a result of the reduction in progesterone concentration, follicle stimulating hormone begins the process of maturing a follicle, then the follicle produces more oestrogen, therefore the oestrogen concentration begin to rise and gets to its peak on day 35.

It was established from this study that the oestrus period was 8 days and the oestrous cycle length was 25 days, these findings are similar to the work of Henry *et al.* (1987), Blanchard *et al.* (1999), Taberner *et al.* (2008) and McKinnon and Voss (2003), who established that the oestrus period can occur between 6 to 8 days, and the oestrous cycle from 25 to 26 days.

In conclusion, it was established that the time to onset of oestrus was 2.3 hours, duration of oestrus 48 hours, oestrus response rate and intensity of synchronization were 50%, respectively. The characteristic oestrus behaviour of jennies included head lowering, ears backward, standing to be mounted, vulva winking, mucous discharge, presentation of perineum, tail raising from the perineum, opening and closing of mouth (mouth clapping), flehmen with winking of the vulva, it was observed that tail raising,

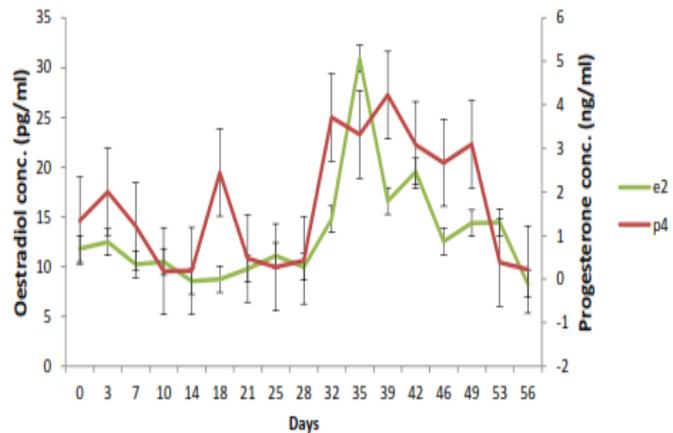


Figure 1: Progesterone and oestradiol profiles

opening and closing of mouth (mouth clapping), flehmen with winking of the vulva were the most consistently observed signs of oestrus. Oestrus period was 8 days and the oestrous cycle length was 25 days in Nigerian indigenous jennies.

ACKNOWLEDGEMENT

We thank the Executive Director, National Animal Production research Institute (NAPRI), A.B.U, Shika Zaria, for providing the animals and facilities used for this work, all members of Biotechnology Research programme and NAPRI Laboratory Samaru, members of Equine and Camel Research programme NAPRI, for their support and contribution into the success of this work.

REFERENCES

- BLANCHARD, T. L., TAYLOR, T. S., and LOVE, C. L. (1999). Oestrus cycle characteristics and response to oestrus synchronization in Mammoth Asses (*Equus asinus americanus*). *Theriogenology*, 52:827–834.
- BLENCH, R. (2004). Natural resource conflicts in northwest Nigeria. A handbook and case studies. Mallam Dendo Ltd Cambridge, United Kingdom. P.106.

- CLAYTON, H. M., LINDSAY, F. E. F., FORBES, A. C. and HAY, L. A. (1981). Some studies of comparative aspects of sexual behaviour in ponies and donkeys. *Applied Animal Ethology*, 7:169-174.
- FIELDING, D. (1988) Reproductive characteristics of the jenny donkey—*Equus asinus*: a review. *Tropical Animal Health Production*, 20: 161–166.
- FIELDING, D. and STARKEY, P. (2004). Donkeys, people and development. *A resource book of the Animal Traction Network for Eastern and Southern Africa (ATNESA). Technical Centre for Agricultural and Rural Cooperation (CTA), Wageningen, The Netherlands. P.211 ISBN 92-9081-219-2.*
- GETACHEW, T. (2014):*Response of Abyssinian Jennies to treatment with Prostaglandin F₂ alpha Ethiopian Veterinary Journal* 18(1), 99-103
- GINTHER, O.J., SCRABA, S.T., and BERGFELT, D.R. (1987). Reproductive seasonality of the jenny. *Theriogenology*, 27:587–592.
- GRAHAM, D.J and CLARKE, C.L (1997). Physiological action of progesterone in target tissues. *Endocrine Reviews*. 18: 502-519.
- HENRY, M., MCDONNELL, S.M., LODI, L.D. and GASTAL, E.L. (1991). Pasture mating behaviour of donkeys (*Equus asinus*) at natural and induced oestrus. *Journal of Reproduction Fertility*, 44: 77-86.
- HUTZ, R. J, DIERSHKE, D. J and WOLF, R. C (1988). Induction of atresia of the dominant follicle in rhesus monkeys by the local application of estradiol-17 β . *American Journal of Primatology*, 15: 69-77
- KATZENELLENBOGEN, B. (1996). Oestrogen receptors: Bioactives and interactions with cell signaling pathways. *Biology of Reproduction*, 54: 287-293
- MCCORT, W.D. (1980). The behaviour and social organization of feral asses (*Equus asinus*) on Ossabaw Island, Georgia. *Unpublished Doctoral Dissertation, the Pennsylvania State University, State College, PA.*
- MCKINNON, A. O., and VOSS, J. L. (2003). *Equine Reproduction*. Blackwell Publishing, Ames, Iowa. Pp. 130, 131, and 535.
- OSBORNE, C. K., ZHAO, H. and FUQUA, S.A.W. (2000). Selective oestrogen receptor modulator: structure, function and clinical use. *Journal of clinical oncology*, 18:3172-3186
- RICHARDS, J. S., and HEDIN, L.(1988). Molecular aspects of hormone action in ovarian follicular development, ovulation and luteinization. *Annual Reviews Physiology*, 50: 441-463
- RUEDA, B.R., HENDRY, I.R., HENDRY III, W.J., STORMSHAK, F., SLAYDEN, O.D. and DAVIS, J.S. (2000). Decreased progesterone levels and progesterone receptor antagonist promote apoptotic cell death in bovine luteal cells. *Biology of Reproduction*, 62: 269-276
- SPICER, L.J and ECHTERNKAMP, S.E (1986). Ovarian follicular growth, function and turnover in cattle: a review. *Journal of Animal Science*, 62: 428-451.
- TABERNER, E., MEDRANO, A., PENˆA, A., RIGAU, T. and MIRO´ J. (2008): Oestrus cycle characteristics and prediction of ovulation in Catalonian jennies *Theriogenology*, 70: 1489–1497.
- VANDEPLASSCHE, G. M., WESSON, J. A. and GINTHER, O. J. (1981). Behavioural, follicular and gonadotropin changes during the oestrus cycle in donkeys. *Theriogenology*, 16: 239-249.

WILLIAMS, S. P. and SIGLER, P. B.
(1998). Atomic structure of
progesterone complexed with its
receptor. *Nature*, 393: 392-396.

YASIN, M., DALGIN, A. C.,
HAISENLEDAR, D. J.,
KERRIGAN, J. R. and

MARSHALL, J. C (1995).
Gonadotropin-releasing hormone
pulse pattern regulates GnRH
receptor gene expression:
augmentation by estradiol.
Endocrinology, 136: 1559-1564.