CONTROL OF OESTRUS AND OVULATION RATES IN YANKASA EWES

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Abstract

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Experiments were carried out in Yankasa sheep to study the efficacy of progesterone for oestrus synchronization and the effect of various gonadotrophin treatments (pregnant mare serum gonadotrophin (PMSG), human chorionic gonadotrophin (HCG) and gonadotrophin releasing hormone (GnRH)) on ovulation rates and litter size, and to elucidate responses by monitoring progesterone concentration. Preliminary studies showed normal progesterone profiles during the cycle, and a mean ovulation rate and litter size of 1.36 ± 0.34 and 1.23 respectively. Following synchronization with progesterone pessaries, PMSG dosage influenced ovulation rates, being 1.0 ± 0.0, 1.3 ± 0.3, 2.0 ± 0.0, 5.5 ± 0.5 and 7.0 ± 1.2 for ewes treated with 0, 250, 500, 750 and 1000 IU PMSG respectively. When 500 IU PMSG was given to ewes, HCG injection had an additive effect of increasing ovulation rate, being 2.0, 3.3 and 3.0 for ewes treated with HCG at 24, 48 and 72 hours after PMSG injection. For ewes treated with GnRH at 0, 24 and 48 hours following withdrawal of progestagens, the mean ovulation rates were 2.0 ± 0.6, 2.7 ± 0.3 and 1.3 ± 0.3. Plasma progesterone concentrations showed significant treatment effects, being increased in relation to an increase in ovulation rates. Ovulation in Yankasa ewes occurred between 30–32 hours from onset of oestrus. In a fertility trial, mean ovulation rates and corresponding litter size were 2.1 versus 1.8 and 1.5 versus 1.2 for PMSG treated and untreated ewes respectively.

1. INTRODUCTION

The Yankasa (a hairy sheep breed) is the predominant sheep in the northern part of Nigeria and occurs throughout the Sahel, Sudan and Guinea Savanna zones [1]. Out of a total sheep population of 13 million in Nigeria, the Yankasa form about 40%. They are therefore very important in livestock production programmes designed to meet the demands for meat in Nigeria.

Ovulation rate, litter size and twinning rate (1.36, 1.25 and 12%) of Yankasa sheep are, however comparatively low [2–4]. Thus, one area where emphasis can be placed is in the improvement of their reproductive rate. It has been suggested that

ovulation rate in sheep is the primary factor limiting their litter size [5]. Increase in ovulation rate and consequent improvement in litter size can be achieved either by genetic (long term) or non-genetic (short term) means [6], and are the objectives of the present studies.

Application of progestagens and gonadotrophin treatment for oestrus synchronization and for short term non-genetic increases in ovulation rate have been widely reported for other breeds of sheep [7].

Significant increases in litter size following treatment with gonadotrophins have been reported in breeds with genetically low litter sizes [8, 9]. However, responses to gonadotrophins are equivocal and variable, being influenced by factors such as breed [10] and treatment [11]. Similar information is lacking for Yankasa sheep and is essential if attempts to introduce artificial and accelerated breeding practices for increasing efficiency of production through improved reproductive rate are to be successful.

The following investigations were therefore designed to study the efficacy of progestagens for oestrus synchronization and the effect of various gonadotrophin treatments on ovulation and lambing rates, and to elucidate these responses by monitoring progesterone concentrations in Yankasa sheep.

2. MATERIALS AND METHODS

2.1. Experimental animals

The animals used were obtained from a Yankasa sheep flock at the institute; they were reared on pasture or fed hay plus a concentrate mixture containing equal amounts of cotton seed cake, wheat bran, maize grains and 1% mineral/vitamin premix based on body weight. Water and salt licks were provided ad libitum.

2.2. Ovulation rates and embryo survival in Yankasa ewes based on post mortem examination

In a preliminary study [2], data on ovulations and litter size were collected on 454 complete genital tracts of Yankasa ewes from two abattoirs in Zaria. The incidence of types of ovulations and live foetuses were determined.

2.3. Onset of puberty and oestrous cycle phenomena in Yankasa ewes as monitored by plasma progesterone concentrations

Because of the dearth of information on reproductive endocrinology of the Yankasa breed, there was a need to determine age at puberty, oestrus parameters and normal progesterone profiles during the oestrous cycle.

Ten Yankasa ewe lambs were weaned at 3 months of age and used in the study. The ewe lambs were checked for oestrus twice daily (a.m. and p.m.), using vasectomized rams, and blood samples for progesterone determination were collected every other day until the first behavioural oestrus (puberty). Further blood samples were collected every other day until ewes showed two subsequent oestrous periods.

2.4. Effect of pregnant mare serum gonadotrophin (PMSG) on oestrus parameters, ovulation rate and peripheral progesterone concentrations

In order to determine the dose-response effect of PMSG, 30 mature Yankasa sheep of approximately 32 kg liveweight were synchronized by inserting intravaginal progestagen sponges (Chronogest, Intervet, Holland) containing 45 mg Flugestone acetate for a period of 12 days. At sponge withdrawal, 6 animals each were treated with 0, 250, 500, 750 and 1000 IU PMSG (Foligon, Intervet, Holland) intramuscularly.

2.5. Effect of PMSG with or without human chorionic gonadotrophin (HCG) on ovulation rate

Results obtained from the tests described in Section 2.4 above warranted the use of HCG, in view of the frequency of unovulated mature follicles observed. Following synchronization of oestrus as described in Section 2.4, 30 ewes were treated (except controls) with 500 IU PMSG (IM) at sponge withdrawal. The experimental animals were then assigned randomly into 5 treatment groups as follows: Group 1, Progestin pessary only — control; Group 2, 0 IU HCG; Groups 3, 4, 5 were treated with 500 IU HCG (Chorulon, Intervet, Holland) at 24, 48 and 72 hours after PMSG injection respectively.

2.6. Effect of gonadotrophin releasing hormone (GnRH) on ovulation rate

Twenty ewes were synchronized as described earlier and 5 each (except controls) were treated with 10 μg GnRH (Receptal, Hoechst, Germany) at 0, 24 and 48 hours following sponge withdrawal.

2.7. Timing of ovulation in Yankasa ewes

Fourteen ewes were used to determine time of ovulation following synchronization of oestrus with progestagen pessaries. Heat was detected using vasectomized rams. The occurrence of ovulation was determined by examining the ovaries using a mid-ventral surgical approach at 6, 12, 18, 24, 30, 36 and 42 hours from the onset of oestrus.

2.8. Effect of PMSG on ovulation rate and litter size

Forty ewes were divided into two groups after synchronization as described earlier and treated with either saline or 500 IU PMSG at sponge withdrawal. Five ewes from each of the two groups were used to determine ovulation rates and intact rams were introduced with the remaining ewes for breeding at a ratio of 1 ram:5 ewes. Heat observations were made as described earlier and blood samples were collected from the day of insertion of pessaries until 30 days after service.

2.9. Heat detection

In order to determine duration of oestrus following sponge withdrawal, all experimental animals were checked for standing heat for 1 hour at 4-hourly intervals using vasectomized rams which were usually introduced at each heat detection period. Heat detection was continued until no further heat signs were observed.

2.10. Blood sample collection

Blood samples were collected into evacuated heparinized tubes every other day by jugular vein puncture. They were centrifuged and the plasma stored at -20°C until analysed for progesterone. Blood sampling commenced on the day of sponge insertion and was continued until 7 days after the animal showed the second oestrus following withdrawal of sponges.

2.11. Examination of ovaries

The ovaries of all the experimental animals were examined 8 days after the first oestrus following sponge withdrawal. The surgical procedure was by a mid-ventral approach using Versican as a local anaesthetic. The number of corpora lutea (ovulation rate) and follicles were recorded for each ewe.

2.12. Hormone analysis

For the studies described in Sections 2.3, 2.4 and 2.5, progesterone was measured in plasma by radioimmunoassay (RIA) [12, 13]. The mean (±SEM) extraction efficiency was 75 ± 4.1%. The sensitivity of the assay, defined as twice the standard deviation from the zero standard, was 0.1 ng/mL. The within- and between-assay coefficients of variation were 8.5% and 9.6% respectively. For the studies described in Sections 2.6, 2.7 and 2.8, RIA of progesterone was carried out using the FAO/IAEA solid phase direct assay kit, which has been validated for sheep [14]. The sensitivity of the assay defined as twice the standard deviation from the zero standard was 0.06 ng/mL. The within- and between-assay coefficients of variation were 5.7% and 7.2% respectively.

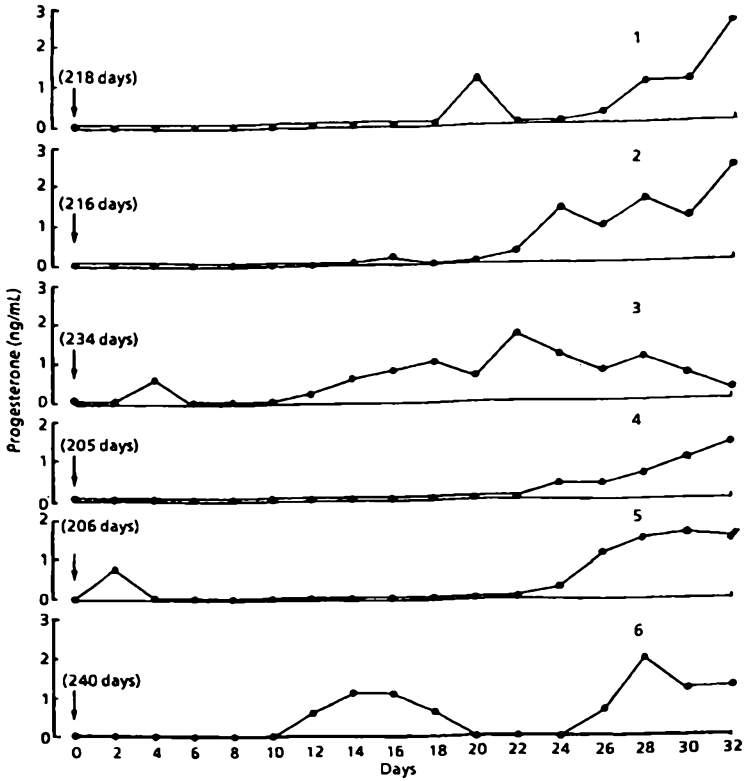

FIG. 1. Plasma progesterone concentrations before and after puberty in Yankasa ewes (E indicates puberty or first oestrus; numbers in parentheses represent age of ewe lambs).

3. RESULTS

3.1. Puberty and oestrus phenomena

Age and body weight at puberty averaged 238 ± 23.4 days and 18.4 ± 0.4 kg respectively. Occurrence of first behavioural oestrus (puberty) was generally followed by normal ovulation and corpus luteum development as shown by progesterone profiles during the oestrous cycles following puberty (Fig. 1). Short eleva-

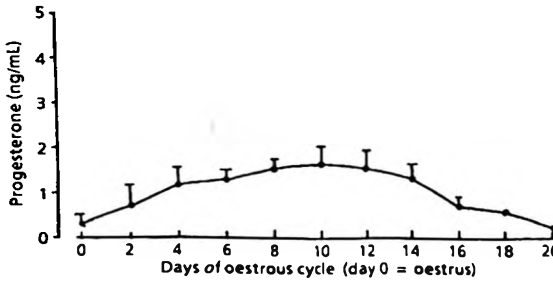

FIG. 2. Plasma progesterone concentration during the oestrous cycle of Yankasa sheep.

tions of progesterone were seen in some ewes, indicating the occurrence of short oestrous cycles (Fig. 1, No. 6). Mean oestrous cycle length after puberty was 18.1 ± 1.7 days while the mean duration of oestrus was 25 ± 2.2 h.

Progesterone values during corresponding days of the two oestrous cycles following puberty were pooled to obtain the oestrous cycle progesterone profile shown in Fig. 2. Mean (±SEM) plasma progesterone concentrations ranged from 0.12 ng/mL at oestrus to a peak of 1.86 ± 0.38 ng/mL at midcycle.

3.2. Normal ovulation rates and litter size from post mortem examinations

The mean (±SD) ovulation rate for Yankasa ewes was 1.36 ± 0.34 and the per cent incidence of single, twin, triple and multiple (>3) ovulations was 67.8, 28.9, 2.9 and 0.4 respectively. More corpora lutea were observed on the right than the left ovaries but the difference was not statistically significant. The overall probability of embryo survival for the twin ovulators was 0.82. The mean foetal number per pregnant ewe was 1.23.

3.3. Relationship of PMSG dose, ovulation rate and progesterone concentrations

Oestrous cycle length of the experimental animals before commencement of the study ranged from 16–18 days and plasma progesterone showed normal profiles, being similar to those reported earlier [5].

Following synchronization and treatment with different doses of PMSG, mean intervals from sponge withdrawal to onset of oestrus (h) were 44.5 (0 IU), 32.9 (250 IU), 37.8 (500 IU), 26.9 (750 IU) and 27.1 (1000 IU) (see Table I). Mean

TABLE I. OESTRUS PARAMETERS[a] AND OVULATION RATES OF PROGESTAGEN TREATED YANKASA EWES GIVEN VARYING DOSES OF PMSG

Treatment group	Interval from sponge withdrawal to onset of oestrus (h)	Mean duration of oestrus (h)	Ovulation rates (Mean No. of corpora lutea)	No. of mounts per oestrus period	Oestrous cycle length (d)	Total progesterone concentration (ng/mL)[b]
0 PMSG (control)	44.5 ± 4.5	25.0 ± 0.6	1.0 ± 0.0	8.5 ± 2.5	16.5 ± 0.5	15.3 ± 2.5
250 IU PMSG	32.9 ± 1.7	61.3 ± 3.9	1.3 ± 0.3	5.3 ± 1.3	17.0 ± 0.6	36.6 ± 10.9
500 IU PMSG	37.8 ± 1.1	12.0 ± 4.5	2.0 ± 0.0	4.7 ± 0.3	18.0 ± 0.6	56.1 ± 7.9
750 IU PMSG	26.9 ± 3.2	49.9 ± 7.0	5.5 ± 0.5	9.8 ± 1.8	19.5 ± 0.5	82.2 ± 5.6
1000 IU PMSG	27.1 ± 2.6	44.5 ± 11.7	7.0 ± 1.2	12.0 ± 3.8	23.8 ± 0.3	125.7 ± 9.0

[a] Mean ± SEM.

[b] Sum of the daily values throughout the oestrous cycle.

duration of oestrus (h) was 25.0 (0 IU), 61.3 (250 IU), 12.0 (500 IU), 49.9 (750 IU) and 44.5 (1000 IU). Ovulation rates (based on number of corpora lutea) averaged 1.0 ± 0.0, 1.3 ± 0.3, 2.0 ± 0.0, 5.5 ± 0.5 and 7.0 ± 1.2 for ewes treated with 0, 250, 500, 750 and 1000 IU PMSG respectively.

After synchronization, oestrous cycle lengths were longer for PMSG, treated than for control ewes and increased linearly with dose of PMSG, being highest for animals treated with 1000 IU PMSG.

Plasma progesterone values for the controls during the oestrous cycle following sponge withdrawal were generally not significantly higher than the values prior to sponge insertion (Fig. 3). Plasma progesterone concentrations showed significant treatment effect (Table I, Fig. 4), being dose dependent. Generally, progesterone profiles of animals treated with PMSG showed two peaks (Fig. 5). Peak and total progesterone concentrations (sum of daily values throughout the oestrous cycle) increased in a linear fashion and were highest for animals treated with 1000 IU PMSG, which also had the highest mean ovulation rate (7.0). Thus, an increase in ovulation rate appeared to have caused a significant increase in total progesterone concentration in plasma.

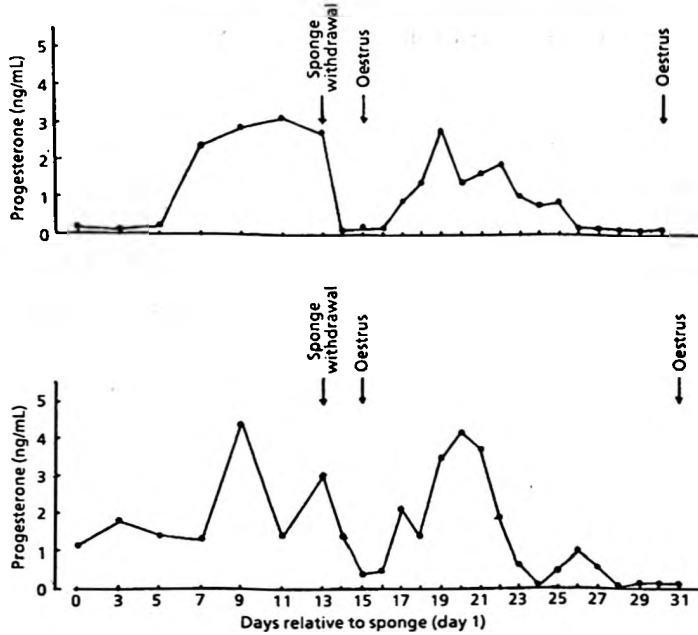

FIG. 3. Plasma progesterone concentrations in Yankasa ewes during the oestrous cycle following sponge withdrawal (N = 1 in each case).

3.4. PMSG–HCG interaction on ovulation rates

Intervals from sponge withdrawal to onset of oestrus (h) for the controls and PMSG treated ewes were 53.3 ± 9.2 and 36.3 ± 6.1 respectively, while the values were between 26–28 h for ewes treated with combinations of PMSG and HCG. Mean duration of oestrus for the control ewes (25.0 h) was shorter than that for those given PMSG only (38.3 h), PMSG–HCG 24 (51.0 h), PMSG-HCG 48 (50.0 h) and PMSG–HCG 72 (59.2 h).

Oestrous cycle lengths following sponge removal and treatment were within the normal range (16–21 days). However, oestrous cycle lengths for HCG treated ewes were generally of longer duration than for the PMSG treated and control ewes.

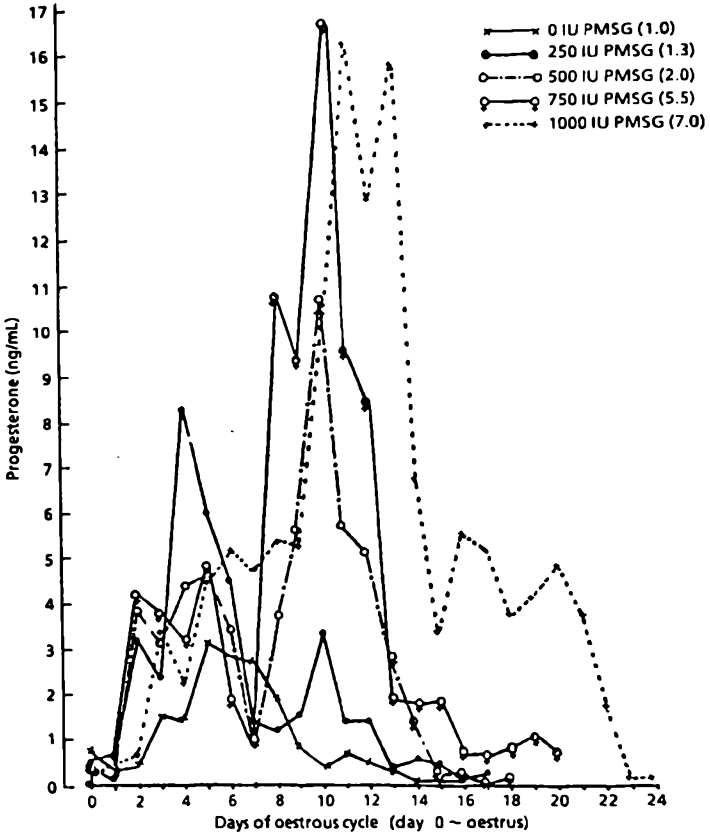

FIG. 4. Mean plasma progesterone concentrations in Yankasa ewes treated with varying doses of PMSG.

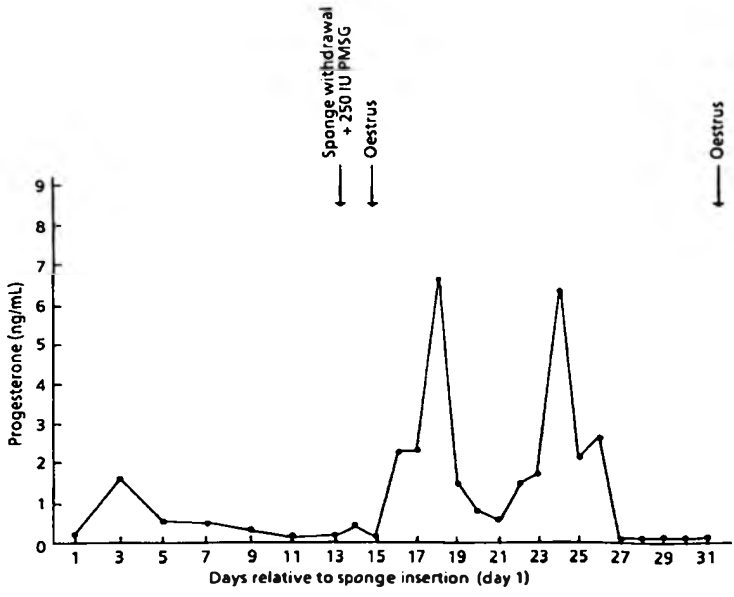

FIG. 5. Plasma progesterone profiles of Yankasa ewes showing two peaks following treatment with PMSG.

Mean ovulation rates were 1.0, 1.7, 2.0, 5.3 and 3.0 for the control, PMSG only, PMSG–HCG 24, PMSG–HCG 48 and PMSG–HCG 72 respectively (Table II).

Plasma progesterone profiles for all the groups appeared normal but showed significant treatment effects. Values for plasma progesterone for control ewes during the oestrous cycle following sponge withdrawal were generally not significantly higher than the values prior to sponge insertion. Progesterone concentrations for the ewes treated with PMSG only were not significantly higher than for the PMSG–HCG 24 ewes (Fig. 6).

Peak plasma progesterone concentrations appeared to be correlated with number of corpora lutea, being highest in the HCG–48 ewes which also had the highest mean ovulation rate (5.3). However, total progesterone (sum of daily values throughout the cycle) following sponge removal increased as a result of an increase in ovulation rate but did not follow a linear fashion (Table II).

TABLE II. OESTRUS PARAMETERS[a] AND OVULATION RATES OF PROGESTAGEN-TREATED YANKASA EWES GIVEN PMSG AND HCG

Group No.	Treatment group	Interval from sponge withdrawal and PMSG treatment to onset of oestrus (h)	Mean duration of oestrus (h)	Mean no. of mounts per oestrus period	Ovulation rate (Mean No. of corpora lutea)	Oestrous cycle length (d)	Total progesterone concentration (ng/mL)[b]
1	Control	53.3 ± 9.21	25.0 ± 0.3	18.0 ± 3.0	1.0 ± 0.0	17.0 ± 0.6	23.5 ± 5.7
2	PMSG only	36.3 ± 6.1	38.8 ± 9.3	26.5 ± 8.2	1.7 ± 0.2	16.3 ± 1.2	59.6 ± 2.84
3	PMSG + HCG 24 h later	26.0 ± 3.8	51.0 ± 3.6	29.0 ± 3.8	2.0 ± 0.4	17.8 ± 0.5	43.85 ± 6.8
4	PMSG + HCG 48 h later	27.6 ± 2.2	50.0 ± 7.6	27.0 ± 5.1	5.3 ± 0.7	22.3 ± 0.5	107.99 ± 4.7
5	PMSG + HCG 72 h later	27.6 ± 3.1	59.2 ± 6.7	27.5 ± 0.4	3.0 ± 0.0	20.3 ± 0.5	105.7 ± 5.5

[a] Mean ± SEM.
[b] Sum of the daily values throughout the oestrous cycle.

3.5. Time of GnRH treatment and ovulation rates

Mean ovulation rates were 1.0 ± 0.0, 2.0 ± 6, 2.7 ± 3 and 1.3 ± 0.3 while durations of oestrus were 20.4 ± 1.8, 16.0 ± 3.1, 34.0 ± 1.8 and 30.5 ± 4.5 hours for control ewes and those treated with GnRH at 0, 24 or 48 hours following withdrawal of progestagen pessaries respectively (Table III).

Plasma progesterone also showed significant treatment effects (Fig. 7).

3.6. Determination of time of ovulation

In Yankasa ewes, ovulation occurred between 30–32 hours after onset of oestrus (Table IV).

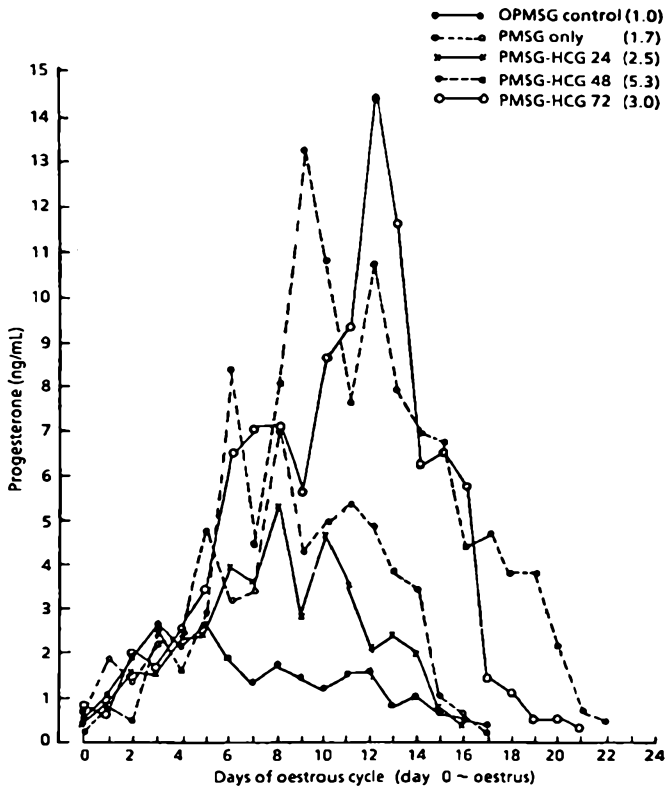

FIG. 6. Mean plasma progesterone concentrations during the oestrous cycle for ewes treated with HCG at different intervals from PMSG injection (mean ovulation rates are in brackets).

TABLE III. EFFECT OF TIME OF GnRH INJECTION ON OESTRUS PARAMETERS AND OVULATION RATES OF YANKASA EWES*

Treatment	Time from pessary withdrawal to onset of oestrus (h)	Duration oestrus (h)	Ovulation rates	Post-synchronization oestrous cycle length (d)
Control	52.4 ± 6.4	20.4 ± 1.8	1.0 ± 0.0	16.8 ± 0.8
GnRH at 0 h	26.8 ± 2.6	16.0 ± 3.1	2.0 ± 0.6	17.7 ± 0.3
GnRH at 24 h	32.4 ± 4.9	34.0 ± 0.8	2.7 ± 0.3	18.0 ± 0.6
GnRH at 48 h	50.6 ± 1.2	30.5 ± 4.5	1.3 ± 0.3	19.7 ± 0.3

* Mean ± SEM.

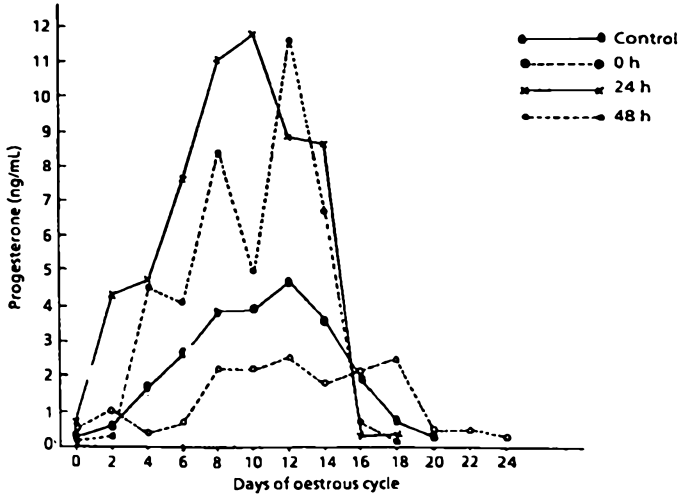

FIG. 7. Mean plasma progesterone concentrations during the oestrous cycle for ewes treated with GnRH at different intervals from withdrawal of progesterone.

TABLE IV. TIMING OF OVULATION IN YANKASA SHEEP

Animal No.	Time of ovarian examination from onset of oestrus (h)	Status
1	6	−ve
2	6	−ve
3	12	−ve
4	12	−ve
5	18	−ve
6	18	−ve
7	24	−ve
8	24	−ve
9	30	+ve
10*	30*	−ve
11	32	+ve
12	36	+ve
13	36	+ve
14	42	+ve

−ve = no ovulation.
+ve = ovulation.
* Examination of animal that had not ovulated was repeated at 32 h.

TABLE V. OESTRUS PARAMETERS, OVULATION RATES AND LITTER SIZE IN PMSG TREATED vs UNTREATED YANKASA EWES

Parameters*	PMSG treated	Untreated
Duration of oestrus	35.5 ± 3.5	20.5 ± 3.5
Ovulation rate	2.1 ± 0.8	1.5 ± 0.2
Litter size	1.8 ± 0.2	1.2 ± 0.2

* Mean ± SEM.

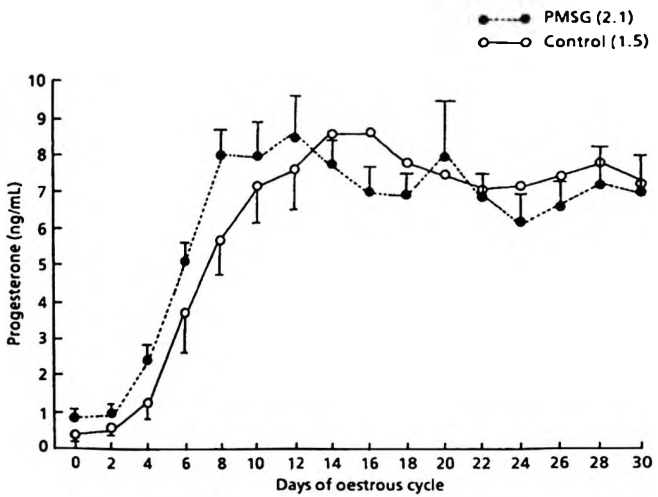

FIG. 8. Mean plasma progesterone concentrations during early pregnancy in PMSG-treated and untreated ewes.

3.7. Effect of PMSG on ovulation rate and litter size

Mean ovulation rates and litter size for PMSG treated and untreated Yankasa ewes were 2.1 versus 1.8 and 1.5 versus 1.2 respectively (Table V). Plasma progesterone profiles of the two groups up to day 30 post service confirmed pregnancy but were not influenced either by the observed ovulation rates or litter size (Fig. 8).

4. DISCUSSION

This project has helped to elucidate pubertal traits by the application of hormone measurements and maps out, for the first time, progesterone profiles during normal oestrous cycles in Yankasa sheep. It also demonstrates the occurrence of short oestrous cycles in the breed [15].

It was also necessary to conduct preliminary investigations to correlate actual ovulation rates and observed litter size in the field using abbattoir specimens. The results of the study [2] indicated a direct relationship between the two traits, further confirming the need to seek ways to improve litter size through an increase in

ovulation rate. Furthermore, it was significant to observe that the probability of embryo survival was very high (0.82) for twin ovulations. To this end studies to improve ovulation rate/litter size through genetic and non-genetic means have been designed, with the latter being the focus of the present report.

Generally, the gonadotrophins used caused an increase in ovulation rate. A dose–response relationship was demonstrated between PMSG dosage and ovulation rate similar to that observed by Kelly et al. [16] and Ainsworth et al. [9], indicating that the ovaries of Yankasa ewes are sensitive to PMSG. However, ovulations in the latter were accompanied by the presence of persistent mature follicles. This may be indicative of inadequate endogenous luteinizing hormone (LH) to cope with increased follicular development, though a consistent association between preovulatory LP peak and ovulation rate was not observed [17, 18].

Treatment with HCG appeared to have an additive effect in terms of increasing ovulation rate. The effect of time of HCG injection was also significant; the optimum time for increasing the number of ovulations was 48 hours after PMSG injection at sponge withdrawal, possibly due to a potentiation of endogenous LH. In a report by Bindon et al. [19], the intervals between oestrus and LH peak appeared to be better correlated with ovulation rate. In the present study, time of GnRH injection also influenced ovulation rate.

Following treatment with gonadotrophins, the interval from sponge withdrawal to onset of oestrus was shorter, while duration of oestrus was longer than for control ewes. Similar observations were made by Jeffcoate et al. [20]. Gonadotrophin treatment also prolonged the length of the oestrous cycle in line with observations by Karsch et al. [21]. Generally, there was a positive association between the number of corpora lutea and plasma progesterone. This is consistent with previous reports [16, 22, 23].

The results of the fertility trial showed that increase of litter size was made possible in Yankasa ewes by gonadotrophin administration. The present results, however, suggest that wider testing is necessary in view of the variability in ovulation rates following gonadotrophin treatment. For purposes of application of artificial insemination, recommendations on time of insemination can be based on time of ovulation observed in the study relative to onset of oestrus or withdrawal of the progestagen pessary. It is therefore recommended that 500 IU PMSG should be used to cause superovulation for increasing litter size in Yankasa ewes. A combination of this treatment with HCG may also improve synchrony of ovulation for the effective application of artificial insemination. The injection of GnRH at sponge withdrawal may also be a suitable alternative; it is recommended that artificial insemination should be done between 30 and 34 hours from the onset of oestrus in Yankasa sheep.

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