# Hormonal and ovarian responses in Romney ewe hoggets after synchronisation and superovulation treatment

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## ABSTRACT

Twelve, 10-month old ewe hoggets (Av LW 32.5 kg) were placed in metabolism crates under controlled lighting (11 h L:13 h D) for a 9 d adaptation period and were fed for maintenance. They were synchronized by vaginal insertion of CIDR and treated with gonadotrophins for superovulation. Of the 12 hoggets 9 had two or more successive plasma samples with > 0.9 ng/ml progesterone ($P_4$) indicating they had ovarian activity prior to treatment. Mean $P_4$ concentrations prior to treatment (d -3) were 0.69 ± 0.18 ng/ml and had risen to 1.50 (± 0.38), 1.72 (± 0.35), 1.36 (± 0.11) and 1.31 (± 0.10) ng/ml for d 0 to d 3 of treatment. On d 9, 10 and 11, prior to CIDR removal, $P_4$ levels were back to base line levels (0.60, 0.46 and 0.57 (± 0.06) ng/ml respectively). The mean number of follicles and corpora lutea were 5.0 (± 0.95) and 1.8 (± 0.53), respectively. Total CL had a negative correlation ($r = -0.64$, $P < 0.05$) with the $P_4$ concentration on the day of CIDR removal. Six of the 12 ewes had an LH peak within 25 hours after CIDR removal. In addition, those ewes treated during late cycle tended ($P > 0.10$) to have a better ovarian response than those treated at early or mid cycle. These results suggest it may be necessary to use two CIDR to maintain a sufficiently long period of $P_4$ elevation. In addition, the day of the cycle that the CIDR is inserted may be important to achieve maximum response from ovulation enhancement treatment in ewe hoggets.

**Keywords:** Ewe lamb; LH; Progesterone; Ovarian response; CIDR; sheep; multiple ovulation.

## INTRODUCTION

Use of a juvenile multiple ovulation and embryo transfer (MOET) scheme has potential to greatly increase genetic gains in sheep (Smith, 1986). Rangel-Santos (1991) has proposed if the number of ovulations reaches 10 in juvenile ewes, then MOET can be a feasible means to reduce the generation interval. However, these numbers of ova seem to be elusive (Rangel-Santos, *et al.*, 1991:Campbell, *et al.*, 1994).

Hare and Bryant, (1985) produced data in which neither breed nor the occurrence of puberty affected ovulation rate in young ewes. Fernie, *et al.*, (1993) reported large variation in ovarian responses to superovulation and they stated the variations were due to individual animal variations rather than season, breed or age. Campbell *et al.*, (1994) reported tremendous variability in ovarian response of prepubertal and mature ewes treated with like MOET schemes. However, in that study the authors reported an age difference in ovarian response to the superovulation treatments. To help explain this variation the present trial was designed to better characterize the hormonal and ovarian response in ewe hoggets after an ovulation rate enhancing treatment.

## MATERIALS AND METHODS

Twelve, 10-month old Romney ewe hoggets (AV LW 32.5 kg) were placed in metabolism crates under controlled lighting (11 h L:13 h D) for a 9 d adaptation period on May 31, 1993. Ewes were fed for maintenance a mixture (50:50) of lucerne chaff and a pelleted sheep diet (16 % protein). After the adaptation period each ewe was treated (d 0) with an EAZI-breed CIDR® type G (Carter-Holt-Harvey, Hamilton, New Zealand). On d 12 ewes were given (s.c.) 1200 IU PMSG (Folligon; Intervet, Auckland, New Zealand) followed by an 100 μg GnRH (Fertagyl; Intervet, Auckland, New Zealand) injection (i.m.) 48 h later at CIDR removal. This regime had previously given better results than treatments based on purer forms of FSH in Romney ewe lambs at Massey University (Rangel-Santos, 1991). Ovaries were excised via laparotomy 5 d after CIDR removal for visual appraisal of ovarian response. The number of corpora lutea (CL) and follicles (>5mm) were recorded.

Blood samples were collected via jugular venipuncture from each ewe every three days, for 60 d, prior to CIDR treatment. Additional blood samples were taken 3 d prior to, and daily for 3 d after CIDR insertion and for 3 d before CIDR removal. These samples were analyzed for progesterone ($P_4$) concentration by radioimmunoassay (RIA) (Kirkwood *et al.*, 1984) to determine cyclic activity. To determine the hormonal response to the superovulation treatments, an intensive blood sampling was conducted. Blood samples were taken via jugular catheterization beginning 17 h after CIDR removal and continuing for 24 h at 15 min intervals. These samples were analyzed for luteinizing hormone (LH) concentration using RIA (Xu *et al.*, 1991) to characterize the LH response to superovulation treatment. The plasma was separated immediately after blood collection by centrifugation and stored in plastic vials at -20 °C until analysis. The inter- and intra-assay coefficients of variation for the $P_4$ and LH assays were 11.9%, 9.9% and 13.4%, 5.6%, respectively. The assay sensitivities were 0.056 and 0.123 ng/ml for $P_4$ and LH respectively.

To determine the day of the oestrous cycle in which superovulation treatments began, each ewe's progesterone profile was individually graphed. Based on these graphs the hoggets were classified as to their stage of the oestrous cycle

when the CIDR were inserted: early (d 1-6), mid (d 7-11) and late (d 12-17) cycle.

Statistical analysis was conducted using the general linear model of SAS (1985) after log 10 (Y + 1) transformation of ovarian response data. The data are presented as means with their standard errors.

## RESULTS AND DISCUSSION

The ewe hoggets had an average live weight (LW) of 32.5 kg at the time they were placed in the metabolism crates. After 21 d in the metabolism crates the LW was 33.6 kg and at the end of the trial the LW was 30.3 kg. However, this final weight is a little misleading as it was taken after laparotomy and the animals had not had sufficient time to recover from being off feed for 24 h.

Pre-treatment progesterone ($P_4$) levels sustained above 0.9 ng/ml of plasma suggest that 9 of the 12 hoggets had cycled in mid-May with the remaining three in the beginning stages of their first cycle just prior to CIDR insertion.

There were no differences ($P > 0.10$) between groups starting treatment in the early , mid or late stages of the cycle for the number of CL, large follicles or total ovarian response (TOVA) calculated from the number of CL plus the total number of large follicles (Table 1). In contrast McMillan and Hall (1991) reported ewes treated at mid cycle only had two thirds the number of follicles of early or late cycle ewes. The differences in follicle numbers between the two trials could be attributed to the size of follicles counted, the earlier counting of follicles by McMillan and Hall or the age of the ewes. However, these data tend to support work of McMillan and Hall in that ewes treated in early and late cycle appear to have better ovulation responses than the mid cycle ewes. Over the three groups the mean number of CL and follicles were 1.8 (± 0.53) and 5.0 (± 0.95).

**TABLE 1:** Mean number of corpora lutea, follicles, and total ovarian response for the tree stages of the cycle at which superovulation treatment began.

| Time of CIDR insertion | n | Corpora Lutea | Large Follicles | TOVA[1] |
|---|---|---|---|---|
| Early cycle | (5) | 2.2 | 4.0 | 6.2 |
| Mid cycle | (3) | 0.7 | 4.7 | 5.3 |
| Late cycle | (4) | 2.3 | 6.8 | 9.0 |
| SEM | | 1.09 | 1.94 | 2.52 |

[1]TOVA = Total ovarian reponse (total CL + total follicles).

The correlation of the total number of large follicles with the stage of the cycle at which treatment commenced was positive but non significant (r = 0.37). There was a negative correlation between the total number of CL and the $P_4$ concentration on the day of CIDR insertion and on the day of CIDR removal (r = -0.40, $P > 0.1$; and r = -0.64, $P < 0.05$, respectively). In contrast, Sharma *et al.*, (1993) reported a positive correlation (r = 0.64) between $P_4$ on the day of superovulation treatment and the number of transferable class embryos. Embryos were not collected in the present study but the negative relationship between $P_4$ on the day of CIDR insertion and total CL suggest embryo numbers would be down in ewes with higher $P_4$ on the day of super–ovulation treatment.

Table 2 presents the mean $P_4$ concentrations calculated for each of the 8 days of blood sampling and shows there were significant differences due to stage of the cycle when treatment was started ($P < 0.01$) and the times within each cycle ($P < 0.001$). The mean $P_4$ concentration at 3 d prior to CIDR insertion was 0.12, 1.28 and 0.96 ±0.20 ng/ml for early, mid and late cycle treatments respectively ($P < 0.001$). These values reflect the stage of the cycle the ewes were determined to be in at the start of treatment. There were no other differences for $P_4$ concentrations between the three stages except at d 12 after treatment. However, within the three stages of treatment, there was a lower $P_4$ value at 3 d prior to treatment and the day of treatment ($P < 0.001$) as compared to 24 h after CIDR insertion for the early cycle ewes (0.12, 0.78 and 1.25 ± 0.16 ng/ml respectively). On d 12, 13 and 14 of CIDR treatment, the $P_4$ concentration was lower ($P < 0.001$) than d 1, 2 and 3 for early cycle ewes. The mid cycle group started out with an elevated $P_4$ concentration 3 d prior to CIDR treatment of 1.28 ng/ml. This higher value should be expected as these animals were in the mid-luteal phase of the cycle when endogenous $P_4$ would be secreted at high levels. In addition, this group had a higher than expected mean $P_4$ level (2.85 ng/ml) on the day of CIDR insertion, largely caused by one animal (4.72 ng/ml). There were no differences ($P > 0.1$) in any of the $P_4$ concentrations at any of the different times of blood collection for the late cycle ewes.

The $P_4$ concentrations 24 h after insertion were lower than those reported by Smith *et al.*, (1993) of 3.7 ± 0.28 ng/ml in ovariectomized (ovx) ewes. However, by 4 d after CIDR treatment, $P_4$ in their ewes decreased to 1.7 ± 0.2 ng/ml. A similar trend was seen in the current study. One might expect the $P_4$ values of the entire ewes in the present trial to be as elevated as those of ovx ewes. The $P_4$ concentrations are in agreement with those reported by Smith *et al.*, (1993) of

**TABLE 2:** Mean progesterone concentrations (ng/ml) in ewes treated with a CIDR® device relative to stage of oestrous cycle.

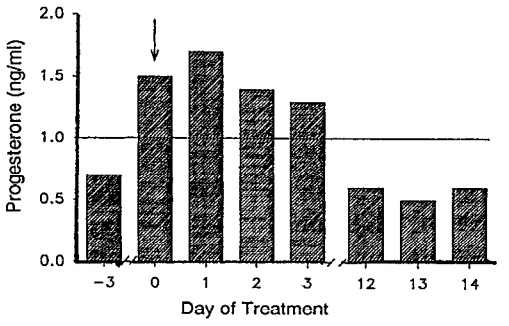
| | n | -3 | 0[1] | 1 | 2 | 3 | 12 | 13 | 14 | SEM | Significance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Early cycle | 5 | $0.12^{a}$ | $0.78^{b}$ | $1.25^{c}$ | $1.39^{c}$ | 1.35c | $0.44^{a,b}$ | $0.46^{a,b}$ | $0.52^{a,b}$ | 0.16 | *** |
| Mid cycle | 3 | $1.28^{a,b}$ | $2.85^{c}$ | $1.93^{a,c}$ | $1.24^{a,b}$ | $1.27^{a,b}$ | $0.72^{b}$ | $0.48^{b}$ | $0.70^{b}$ | 0.44 | ** |
| Late cycle | 4 | 0.96 | 1.40 | 2.17 | 1.40 | 1.29 | 0.72 | 0.52 | 0.54 | 0.41 | NS |
| SEM | | 0.20 | 0.63 | 0.73 | 0.25 | 0.21 | 0.08 | 0.11 | 0.11 | | |
| Significance | | ** | NS | NS | NS | NS | * | NS | NS | | |

[1]Day 0 = CIDR Insertion. Blood sample taken prior to treatment.
[a,b,c] Within rows, means with different superscripts are significantly different.

0.90 ng/ml on d 11 and those of Thompson *et al.*, (1992) of 0.8 ng/ml on d 9 after a single CIDR treatment.

Across stages, blood taken 3 d prior to CIDR insertion showed the mean basal $P_4$ concentration was 0.69 (± 0.18) ng/ml (Figure 1.). On the day of treatment and for 3 d after treatment $P_4$ concentrations had risen significantly ($P<0.001$) to 1.50 (± 0.38), 1.72 (± 0.35), 1.36 (± 0.11) and 1.31 (± 0.10) ng/ml respectively. However on d 12, 13 and 14 prior to CIDR removal, $P_4$ concentrations were back to lower levels (0.60, 0.48 and 0.57 (± 0.02) ng/ml). The $P_4$ concentrations did not follow the profiles reported by Carlson *et al.*, (1980). In their data, ovx sheep treated with a CIDR (type-S) had 2.8 ng/ml and entire ewes had 4.5 ng/ml of $P_4$ 3 d after CIDR treatment as compared with 1.31 ng/ml in the current trial. They found $P_4$ to be sufficiently elevated (2.5 ng/ml) to block the first oestrus after CIDR insertion. Lack of elevated $P_4$ throughout the treatment period may be a cause of poor ovarian response in our trials with young animals.

**FIGURE 1:** Mean progesteron concentrations (ng/ml) in ewes (n=12) at -3, 0, 1, 2, 3, 12, 13 and 14 days of CIDR treatment (↓ = CIDR insertion).




The data from the intensive blood sampling, indicated 6 of the 12 hoggets had a peak LH response during the period of 17 to 41 h after CIDR removal. Three of the six were in the late stages of a LH peak at the first sampling period and the last peak was detected 25 h after CIDR removal and three of the 6 animals that did not have a peak LH response did not ovulate either. Those that failed to ovulate were treated in early (2 animals) and mid (1 animal) cycle. Low $P_4$ concentrations may have led to the other three having an LH peak before the sampling period began. The timing for our 24 h sampling period was based on results cited by several authors. Quirke *et al.*, (1981) suggested the time from sponge removal to LH discharge was 40 h for ewe lambs and Knight *et al.*, (1992) reported that 85% of PMSG-treated Romney ewes exhibited oestrus by 28 h after CIDR removal. In addition Thompson *et al.*, (1990) had earlier reported 80% of single CIDR-treated Perendale ewes showed oestrus 14-30 h after pessary removal. Further evidence to support our choice for the sampling window for LH peak was reported by Smith and Parr (1992) where 75% of ewes treated with a single CIDR for 14 d had a mean oestrus 32 h after CIDR withdrawal. Likewise, data from our own (unpublished) trial suggest the time from sponge removal to oestrus to be between 30 - 36 h in young ewes. However, Thompson *et al.*, (1992) reported PMSG-treated Coopworth ewes given a single CIDR had a LH peak 11 ± 8 h after CIDR removal. It should be noted these ewes were treated with 12 mg FSH which would probably advance the time of ovulation.

## CONCLUSIONS

Trends in this trial suggest there is a possible link between a young ewe's stage of the cycle at the beginning of a MOET treatment and the ovarian response. Those treated in late cycle may have a better response. Progesterone concentrations were not maintained at levels above 1 ng/ml throughout the 14 d period CIDR were present. It appears that, to obtain an adequate synchrony and multiple ovulation in young ewes, the CIDR would need to be replaced with a new CIDR as suggested by Thompson *et al.*, (1990; 1992). It is evident further research is needed to better understand the endogenous mechanisms controlling the oestrous cycle and superovulation in ewe hoggets before juvenile MOET can be a feasible option to use for increasing the rate of genetic gain.

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