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Timing of the LH peak and ovulations in superovulated Coopworth ewes synchronised with progesterone-containing CIDR® devices

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INTRODUCTION

The Controlled Internal Drug Releasing device, or CIDR® (type G, Carter Holt Harvey, Hamilton, New Zealand) containing progesterone (9% w/w, 0.3g) has been shown to effectively synchronise the oestrous cycle of unstimulated (Maxwell and Burnes, 1986; Smith et al., 1991) or superovulated (Thompson and Smith, 1988, Thompson et al., 1990) ewes. However, comparable superovulatory responses between ewes synchronised with either the CIDR® device or progesterone-impregnated sponge was only observed when the CIDR® device was replaced with a new device prior to gonadotrophin administration (Thompson et al., 1990). Even so, time from pessary withdrawal to onset of oestrus was reduced in superovulated ewes synchronised with 2 devices compared to those synchronised with sponges (Smith and Tervit, 1988, Thompson et al., 1990). This suggests that other temporally related events such as the LH surge and ovulation may differ in timing from those reported for progesterone-sponge synchronised ewes. Information on the time to LH peak and ovulation is required if the 2-CIDR® synchronisation regime is to be applied to commercial multiple ovulation-embryo transfer (MOET) programmes, where the use of intrauterine insemination is performed routinely (Tervit, 1989).

MATERIALS AND METHODS

Experiment 1

Hormone treatments

Matute Coopworth ewes were allocated to a 3 x 2 factorial design (N=36, n=6), investigating the ovarian and endocrine responses to 3 gonadotrophin treatments and 2 synchronisation treatments. CIDR® (type G) devices and gonadotrophins were administered according to protocols described elsewhere (Thompson et al., 1990). Briefly, half the ewes were synchronised with a single 12-day CIDR® device (1-CIDR), and the other half with a sequential CIDR® device regime (2-CIDR), whereby the original CIDR® device is replaced with a new device on Day 9 (Smith and Thompson, 1988; Thompson et al., 1990). Ewes were then randomly allocated into 3 gonadotrophin treatment groups; 0, 800 ui pregnant mare serum gonadotrophin (PMSG, "Pregneyol", Heriot Agencies, FernTree Gully, Australia) plus 12 mg follicle stimulating hormone (FSH, "FSH-P", Burns-Biotec, Omaha, USA) given as a decreasing dosage over three days: 800 ui PMSG plus 18 mg FSH. Gonadotrophin treatments commenced on Day 10 of CIDR® device insertion. CIDR® devices were removed at the last FSH injection, at which time vasectomised rams, fitted with crayon harnesses, were introduced for oestrous detection.

Blood sampling

Plasma samples were obtained by jugular venepuncture with 10 ml heparinised "Vacutainers" (Becton Dickinson, Rutherford, USA) and subsequent centrifugation at 1000 g for 30 min. Samples were stored at -20°C prior to radioimmunoassay (RIA). Daily sampling began 2 days before initial CIDR® device insertion. On Days 0 and 9 following CIDR® insertion, ewes were sampled twice, once immediately before CIDR device insertion and again approximately 6 h following insertion. Ewes treated with a single CIDR® device were handled the same as ewes treated with two devices. Intensive (2 hourly) sampling was initiated 12 h before final CIDR® device withdrawal and continued until 24 h.
after an observed oestrus or 66 h after CIDR\textsuperscript{a} device withdrawal for non-oestrous ewes.

**Oestrous and ovarian observations**

Ewes were examined during the intensive sampling period and mating marks recorded. The ovaries of each ewe were examined by laparoscopy approximately 6 days following the onset of oestrus. Numbers of healthy corpora lutea, cystic or luteinised follicles were recorded.

**Experiment 2**

**Timing of ovulations**

Mature Coopworth ewes (N=72) were synchronised with the 2-CIDR regimen and superovulated with 800 IU PMSG plus 12 mg FSH as described above. They were allocated at random to one of 6 groups (n=12), each group representing a set time interval from device withdrawal to ovariarian examination: 34 h, 38 h, 42 h, 46 h, 50 h and 54 h. Vasectomised rams were introduced with ewes at CIDR\textsuperscript{a} device removal. Oestrous detection was performed once, at approximately 36 h after device withdrawal. The ovaries of each ewe were examined by laparoscopy and the data recorded included the number of ovulation points and unruptured pre-ovulatory follicles (approximately 4-6 mm in diameter) visible at time of examination.

**Blood sampling**

Blood samples were taken at 4 hourly intervals by jugular venepuncture (as described above) from a further 6 ewes treated as above commencing the day before CIDR\textsuperscript{a} device withdrawal and finishing 60 h after withdrawal. Samples were assayed for LH.

**Hormone Analysis**

Plasma LH concentrations were determined in duplicate using a homologous RIA procedure described for ovine plasma by Scaramuzzi et al., (1970). The intra-assay coefficients of variation were 34\% for the low control (mean concentration = 0.43 ng/ml) and 18\% for the high control (12.4 ng/ml). The interassay coefficients of variation were 39\% and 13\% (n = 18), respectively for the two control samples. The assay sensitivity, defined as the first point on the standard curve that was different from 0, was 0.03 ng (0.30 ng/ml).

Plasma progesterone concentrations were determined in duplicate by RIA following plasma extraction with diethyl ether (Fairclough et al., 1975). The intra-assay coefficients of variation were 14\% for the low control (mean concentration = 0.77 ng/ml) and 12\% for the high control (4.02 ng/ml). The interassay coefficients of variation were 24\% and 17\% (n = 8), respectively for the two control samples. The assay sensitivity was 0.03 ng (0.15 ng/ml).

**Statistical Analysis**

Ovulation rate (OR) was determined from numbers of corpora lutea and total stimulations (TS) from corpora lutea and other recorded follicular structures. Between treatment differences were compared by analysis of variance, using the GENSTAT statistical package (Rothamstead Experimental Station, Rothamstead, UK). Furthermore, OR as a percentage of TS (%OR) was compared between treatments by analysis of variance following logit transformation. For Experiment 2, this included predicting %OR against time after fitting a quadratic function on the logit scale. In Experiment 1, the intervals from CIDR\textsuperscript{a} device withdrawal to the onset of oestrus (OO) or to the LH peak (PLH), and daily plasma progesterone concentrations were compared by analysis of variance following log transformations.

**RESULTS**

**Experiment 1**

**Progesterone profiles**

The progesterone profiles for 1- or 2-CIDR ewes are presented in Figure 1. There were no differences between gonadotrophin groups in mean plasma progesterone concentrations during CIDR\textsuperscript{a} device insertion, therefore the data were pooled within CIDR\textsuperscript{a} treatments. Plasma progesterone concentrations increased in all ewes following CIDR\textsuperscript{a} device insertion. Values were highest 6 h after insertion, being -4 ng/ml (range 2.5-8 ng/ml). However, concentrations declined with time to -0.8 ng/ml (range 0.4-1.5 ng/ml) by Day 9 following insertion. In ewes which received a second CIDR\textsuperscript{a} device, progesterone concentrations increased immediately following reinsertion and were significantly (P<0.001) higher than for ewes retaining their original device. Peak concentrations in 2-CIDR ewes were -2.8 ng/ml (range 1.7-8.6 ng/ml) and occurred at 6 h from insertion. Although these levels also declined with time, they remained significantly higher (P<0.001) until after CIDR\textsuperscript{a} device withdrawal.

**FIGURE 1** Profiles of mean plasma progesterone concentrations of 1-CIDR and 2-CIDR ewes during CIDR\textsuperscript{a} device insertion. (Arrows indicated time of device insertion or withdrawal)

**Ovarian response**

The response of ewes treated in Experiment 1 is presented in Table 1. There was no significant difference in OR and TS between 1-CIDR and 2-CIDR treated ewes. However, %OR was significantly higher (P<0.05) in 2-CIDR ewes (91 ± 2\%) than 1-CIDR ewes (84 ± 3\%). As expected, a highly significant (P<0.001) difference was observed for OR and TS between gonadotrophin-treated and control ewes, but no differences were observed between the two levels of FSH treatment. The interval to OO (P<0.05) and PLH (P<0.001) were both significantly extended in 2-CIDR ewes compared with 1-CIDR ewes. A linear response was observed in the interval to OO (P<0.001), with

TABLE 1 Ovarian response and interval (h) to onset of oestrus and LH peak from CIDR<sup>R</sup> device withdrawal in ewes synchronised with either 1- or 2-CIDR regimes (Expt. 1)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>CIDR Regime</th>
<th>N</th>
<th>Mean Ovulation Rate (±SEM)</th>
<th>Mean Total Stimulation Rate (±SEM)</th>
<th>Mean Interval (h) to Oestrus Onset (±SEM)</th>
<th>Mean Interval (h) to LH Peak (±SEM)</th>
</tr>
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<td></td>
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<td></td>
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<tr>
<td>0</td>
<td>1</td>
<td>5</td>
<td>1.6 ± 0.2</td>
<td>1.8 ± 0.3</td>
<td>32 ± 6</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>800 +12</td>
<td>2</td>
<td>6</td>
<td>1.7 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>38 ± 2</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>800 +18</td>
<td>2</td>
<td>6</td>
<td>9.0 ± 2.2</td>
<td>12.0 ± 2.0</td>
<td>18 ± 2</td>
<td>11 ± 8</td>
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800+18 < 800+12 < 0, whereas a significant shortening of PLH was observed between control and gonadotrophin-treated ewes (P<0.001). No difference was, however, observed between the 2 gonadotrophin treatments for PLH.

Experiment 2

Timing of ovulation

All but 5 ewes exhibited oestrus within 36 h from CIDR<sup>R</sup> device withdrawal. Only 3 ewes had a total stimulation response of 2 or less as determined by ovarian examination. A summary of the ovarian responses observed at fixed intervals from CIDR<sup>R</sup> device withdrawal is presented in Table 2 and the predicted %OR from CIDR<sup>R</sup> device withdrawal is presented in Figure 2. No difference was found between the 6 groups for TS (mean ± s.e.m. = 6.6 ± 0.4). However, %OR increased significantly over time in both a linear (P<0.001) and quadratic (P<0.01) fashion. Little ovulatory activity was observed at 34 h after CIDR<sup>R</sup> withdrawal. However this changed dramatically for ewes examined 38 h after withdrawal, with 7/12 ewes having commenced ovulating. Furthermore, 30% of the total stimulations were recorded as ovulation points. By 45 h following CIDR<sup>R</sup> device withdrawal, 75% of follicles were estimated to have ovulated and the predicted %OR had plateaued by 51 h after withdrawal. The pre-ovulatory LH peak in the further group of 6 ewes occurred at 22 ± 1 h after CIDR<sup>R</sup> withdrawal. This is similar to that observed in ewes treated with 800iu PMSG and 12 mg FSH and synchronised with 2-CIDR devices in Experiment 1 (20 ± 1 h).

**DISCUSSION**

The results from Experiment 1 confirm that superovulated ewes synchronised with a single CIDR<sup>R</sup> device are likely to behave differently to those synchronised with the 2-CIDR regime, as previously described (Thompson et al., 1990). However, differences in the ovarian response reported here were not as apparent as in the previous report. This maybe due to the much smaller sample size in the present study. Nevertheless, there are clear endocrinological differences between 1-and 2-CIDR ewes. Ewes treated with two devices had significantly higher levels of plasma progesterone during follicular development and significantly extended intervals from device withdrawal to oestrus onset and peak LH (even in the absence of supplemental gonadotrophins). Thus the data obtained here support the hypothesis proposed by Thompson et al., (1990) that progesterone concentration (or prostaglandin activity) during the follicular phase plays an important role in the subsequent ovarian response. The precise mechanism by which this occurs is not clear at this stage, but probably relates to progesterone control of the rate of follicular maturation, whereby insufficient blood concentration of progesterone leads to premature follicular maturation and steroidogenesis.

One disturbing aspect of this study was the marked level of asynchrony between the onset of oestrus and the pre-ovulatory LH peak in 1 CIDR® ewes. This was particularly evident in the group which did not receive gonadotrophins. This is in contrast to the data of Shackell (1991), in which there was a close temporal relationship between oestrus onset (30 h) and peak LH (31 h) in ewes synchronised with an 11-day CIDR<sup>R</sup> device.
treatment. We have no explanation for the differences between the two studies.

It would appear that the time to onset of oestrus and peak LH following device withdrawal in 2-CIDR, unstimulated ewes was between that of 1-CIDR ewes and those synchronised with MAP or FGA sponges (Shackell, 1991). Furthermore, in contrast to 1-CIDR ewes, a very close association was observed between oestrus onset and peak LH (38 h, respectively) with very little variability for 2-CIDR ewes. Perhaps a 2-CIDR regime may improve the performance of artificially inseminated, unstimulated or low-moderate dose gonadotrophin-stimulated 1-CIDR treated ewes.

We have routinely superovulated Coopworth ewes in season with 800 iu PMSG and 12 mg FSH (Burns-Biotec equivalents) following a 2-CIDR synchronisation regime. This regime has proven to be extremely reliable. Under these conditions, oestrus onset will occur around 20 h following CIDR device withdrawal, with the corresponding LH peak closely associated with oestrus onset. From the results obtained in Experiment 2, 75% of all follicles had ovulated by 45 h following device withdrawal and no real increase was estimated after 51 h following device withdrawal. Thus timing of artificial insemination and embryo recovery can now be matched in these events.

REFERENCES