Duration of oestrus, ovulation rate, time of ovulation and plasma LH, total oestrogen and progesterone in Galway adult ewes and ewe lambs

J. F. Quirke, J. P. Hanrahan and J. P. Gosling†

The Agricultural Institute, Animal Husbandry Department, Ballinrobe, Co. Mayo and †The Biochemistry Department, University College, Galway, Ireland

Summary. The mean duration of oestrus, ovulation rate, duration of the preovulatory LH discharge, time interval between sponge removal and beginning of the LH discharge, total LH discharged, maximum LH value observed and the concentration of progesterone in the peripheral plasma during the luteal phase of the oestrous cycle was similar in Galway adult ewes and 8-month-old ewe lambs after treatment with intravaginal sponges containing 30 mg cronolone for 12 days and injection of 500 i.u. PMSG. The interval between sponge removal and the onset of oestrus was shorter for adults than for ewe lambs; the interval between the onset of oestrus and the beginning of the LH discharge was longer in adults. During the period 12–36 h after sponge removal the mean plasma total oestrogen concentration was significantly higher in lambs than in adults. In a separate study of the time of ovulation in Galway ewe lambs given the same progestagen-PMSG treatment, ovulation did not occur in any lamb before 17 h after the onset of oestrus and the majority ovulated close to the end of oestrus.

Introduction

The lambing rate in ewe lambs after mating at a single oestrus is generally low, although there is great variation both within and between breeds, and 20–40% of mated ewe lambs commonly fail to lamb (Dyrmundsson, 1973; Keane, 1974a, b; Forrest & Bichard, 1974; Bichard, Younis, Forrest & Cumberland, 1974; Edey, Kilgour & Bremner, 1978). The lambing rate following mating at a single oestrus is also low in Galway ewe lambs, with values ranging from 27 to 38% at a spontaneously occurring oestrus and from 17 to 37% when oestrus is hormonally induced (Quirke, 1978, 1979a, b). It is apparent that these values are lower than those normally observed with adult ewes and the reasons for this are not fully understood. The fertilization rate appears to be reasonably high and similar in Galway ewe lambs and adult ewes following progestagen–PMSG treatment (Quirke & Hanrahan, 1977), but there is evidence of a very high wastage rate of fertilized eggs (63%) in ewe lambs of this breed (Quirke, 1979a). There is also evidence, from an egg transfer study (Quirke & Hanrahan, 1977), of a difference in egg quality between ewe lambs and adults. However, other studies (Quirke, Hanrahan & Gosling, 1978) have shown no difference between the proportions of adult and lamb recipients which subsequently lambed after transfer of embryos collected from adult ewes. These findings, together with the well known differences between ewe lambs and adults in the duration of oestrus, indicate an unfavourable hormonal milieu around the time of oestrus and, perhaps, to unfavourable relationships between the timing of ovulation and behavioural oestrus as factors in the poor fertility of ewe lambs.

0022-4251/81/020265-08$02.00/0
© 1981 Journals of Reproduction & Fertility Ltd
The present experiments were undertaken to provide information on hormone characteristics of the oestrous cycle for Galway adult ewes and ewe lambs and to determine the time of ovulation relative to the end of oestrus in Galway ewe lambs after a standard progestagen—PMSG treatment during the breeding season.

**Materials and Methods**

*Experiment 1*

The 23 mature Galway ewes (3–5 years) and 27 Galway ewe lambs used were assembled into a single flock during early November 1977. The lambs were born during the previous spring and were about 8 months old at the start of the experiment. All females were treated with intravaginal sponges containing 30 mg cronolone (Laboratories Searle, Perisud, Montrouge, France) for 14 days beginning on 23 November and received 500 i.u. PMSG (Intervet Ltd, Cambridge, U.K.) intramuscularly at the time of sponge removal when the animals were weighed.

**Blood sampling and detection of oestrus**

Blood samples were taken from all ewes at 12 h after sponge removal and at 4 h intervals thereafter until oestrus was detected. The ewes were tested individually for oestrus, using Finnish Landrace rams, 1 h after each sample was taken. After the first positive test for oestrus the frequency of blood sampling was increased to every 2 h for a total of 10 samples (i.e. until 21 h after the onset of oestrus). The onset of oestrus was assumed to have occurred 2 h before the first positive test and the duration of oestrus was determined by checking the ewes individually every 2 h until two consecutive tests confirmed that the ewe would not accept the ram. All samples were assayed for LH and, in addition, the total oestrogen content was measured in samples taken at 12, 16, 20, 24, 28, 32 and 36 h after sponge removal.

Blood samples, for progesterone assay, were taken between 08:00 and 10:00 h on the 10th and 11th days of the cycle (day of onset of synchronized oestrus = Day 0). All blood samples were taken by jugular venepuncture into evacuated tubes containing EDTA. Samples were chilled in iced water after collection and the plasma obtained following centrifugation was stored at −20°C until assay. The animals were fed hay and concentrates throughout the experiment and showed no obvious signs of stress during the intensive blood sampling period.

The ovarian status of all of the ewe lambs and of the majority of the adult ewes was determined, by endoscopy (Boyd & Ducker, 1973), on the day of sponge insertion. Ovulation rate was assessed in all animals at either endoscopy or slaughter on the 14th day after sponge removal.

**Hormone assays**

*LH* concentrations were determined in duplicate plasma samples by means of a liquid phase double-antibody radioimmunoassay which employed a rabbit anti ovine LH antiserum (Hanrahan, Quirke & Gosling, 1977). For iodination, by a lactoperoxidase method, LER 1374A ovine LH was used, and the results are expressed with reference to the NIH-LH-S18 standard. The sensitivity of the assay when assaying 100 µl aliquots was 0.43 ± 0.09 ng/ml (mean ± s.e.m., n = 7), and was calculated from the error in the zero and the slope for each standard curve. The assay of 250, 1000 and 5000 pg quantities of standard added to a plasma sample with a low concentration of LH gave mean recoveries of 96, 109 and 92% respectively. The intra- and inter-assay coefficients of variation for levels of 3.6, 12.5 and 47.9 ng LH/ml were
10·2, 12·1, 9·0 and 15·6, 10·2, 14·2% respectively. The mean LH concentration in samples taken between 12 and 24 h after sponge removal was 8·0 ng/ml (s.d. = 4·0) and the preovulatory LH discharge was assumed to have started when the LH concentration exceeded 20 ng/ml.

**Progesterone.** Duplicate plasma samples were used for progesterone measurement by a conventional radioimmunoassay procedure as described previously (Quirke, Hanrahan & Gosling, 1979a). Addition of 2·5 or 6·0 ng progesterone/ml to plasma with a low progesterone content gave mean recoveries of 100 and 93·7% respectively. The intra- and inter-assay coefficients of variation for levels of 2·7 and 5·8 ng progesterone/ml were 2·6, 1·9 and 16·0, 7·7% respectively. The sensitivity, calculated as for the LH assay, was 0·2 ng/ml.

**Total oestrogen.** The method used for 1-ml aliquots of plasma was that of Henricks, Dickey, Hill & Johnston (1972). The radioimmunoassay employed an antiserum raised in rabbits against oestradiol-17β, 17-hemisuccinate-bovine serum albumin which showed almost 100% cross-reactivity with oestradiol-17β, oestradiol-17α and oestrone, as found also by Hoffman (1972). The efficiency of extraction with 6 volumes of diethyl ether was 86–94% and the results were corrected for procedural losses. The sensitivity of the assay was about 3 pg/ml. Samples were assayed in triplicate and the within-replicate variation was within 3%. The results were expressed as pg total oestrogen/ml plasma. The inter-assay coefficient of variation for the determination of a sample containing 30 pg total oestrogen/ml was 3·0% (13 assays).

**Experiment 2**

Galway ewe lambs were treated with intravaginal sponges containing 30 mg cronolone (Searle) for 12 days beginning on 16 November 1977. The 104 lambs were weighed and received 500 i.u. PMSG (Intervet) intramuscularly at the time of sponge removal. The time of onset and duration of oestrus were determined by checking the lambs individually for oestrus, using intact Finnish Landrace rams, every 3 h beginning 12 h after sponge removal. As the animals came into oestrus they were assigned at random to one of 5 groups for ovarian examination by endoscopy at 17, 22, 27, 32 and 37 h after the onset of oestrus to determine whether ovulation had occurred. The groups examined at 17 and 22 h were also examined again at 27 and 32 h respectively after the onset of oestrus. All of the lambs were slaughtered 14 days after sponge removal. The reproductive organs were removed and the numbers of corpora lutea in the ovaries were recorded.

The data were analysed by χ² and Student's t tests.

**Results**

**Experiment 1**

On the day of insertion of the intravaginal progestagen sponges, 17 lambs had one or more corpora lutea; there was no evidence of any luteal tissue in the ovaries of the remaining 10 lambs and these had apparently not attained puberty. Three animals, 1 adult ewe and 2 ewe lambs, failed to show oestrus and did not appear to have an LH peak within 80 h of sponge removal. At endoscopy, however, only one of these animals, a ewe lamb, did not have a corpus luteum in its ovaries. The concentration of total oestrogens in the plasma in all three was within the ranges observed for oestrous ewes and lambs. Data from these three animals were excluded from the results. One adult ewe which came into oestrus did not appear to have an LH peak during the period 19 h before to 21 h after the onset of oestrus; a single corpus luteum and 2 apparently luteinized follicles were observed in the ovaries of this ewe at endoscopy. This animal was excluded from the results relating to the preovulatory LH discharge but was included elsewhere.
The liveweight of the ewe lambs was 64% that of the adult ewes, but there were no differences for the mean duration of oestrus, ovulation rate, duration of the LH discharge, time interval from sponge removal to the beginning of the LH discharge, total LH released (sum of the values observed during the LH discharge) and plasma progesterone levels (Table 1). The time interval between sponge removal and the onset of oestrus was shorter ($P < 0.05$) for adults than for ewe lambs while the time between the onset of oestrus and the beginning of the preovulatory LH discharge was longer in adults ($P < 0.01$). The plasma oestrogen concentrations were significantly higher in ewe lambs than in adult ewes on each of the 7 sampling occasions (Text-fig. 1). The within-age class correlation between the duration of oestrus and the average (taken over the 7 sampling occasions) oestrogen concentration was 0.23 ($P > 0.05$). The corresponding correlations for individual sampling occasions ranged from 0.18 to 0.29. All aspects of the results were similar for lambs which had apparently attained puberty before the start of the experiment and those which failed to do so (as judged by the presence or absence of CL in their ovaries at endoscopy before sponge insertion).

### Table 1. Responses (mean ± s.e.m.) of Galway adult ewes and ewe lambs to progestagen–PMSG treatment during the breeding season

<table>
<thead>
<tr>
<th></th>
<th>Adult ewes</th>
<th>Ewe lambs</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>72.9 ± 1.5</td>
<td>46.6 ± 1.6**</td>
</tr>
<tr>
<td>Interval from sponge removal to onset of oestrus (h)</td>
<td>31.2 ± 1.5</td>
<td>36.9 ± 1.6**</td>
</tr>
<tr>
<td>Duration of oestrus (h)</td>
<td>27.0 ± 1.6</td>
<td>31.8 ± 1.9</td>
</tr>
<tr>
<td>No. of CL (ovulation rate)</td>
<td>1.91 ± 0.19</td>
<td>1.76 ± 0.22</td>
</tr>
<tr>
<td>Interval from onset of oestrus to LH discharge (h)</td>
<td>6.4 ± 0.87</td>
<td>3.8 ± 0.68*</td>
</tr>
<tr>
<td>Duration of LH discharge (h)</td>
<td>8.1 ± 0.49</td>
<td>8.0 ± 0.38</td>
</tr>
<tr>
<td>Interval from sponge removal to LH discharge (h)</td>
<td>37.6 ± 1.19</td>
<td>40.7 ± 1.49</td>
</tr>
<tr>
<td>Maximum plasma LH conc. observed (ng/ml)</td>
<td>100.0 ± 10.0</td>
<td>93.4 ± 6.9</td>
</tr>
<tr>
<td>Total LH discharged (ng/ml)$\dagger$</td>
<td>259.5 ± 30.3</td>
<td>240.0 ± 19.2</td>
</tr>
<tr>
<td>Plasma progesterone (ng/ml)</td>
<td>5.4 ± 0.5</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Day 10 of the oestrous cycle</td>
<td>5.3 ± 0.5</td>
<td>5.6 ± 0.7</td>
</tr>
</tbody>
</table>

$\dagger$ Sum of the LH values observed during the period of discharge.

Values significantly different from those for adult ewes: *$P < 0.05$, **$P < 0.01$.  

![Text-fig. 1. Sequential patterns of mean total oestrogen in the peripheral plasma of Galway adult ewes (●) and ewe lambs (○) in relation to sponge removal and injection of PMSG (0 h). The vertical bars represent the s.e.m.](image-url)
Experiment 2

Of the 104 lambs treated, 17 failed to exhibit oestrus by 89 h after sponge removal; 8 of these animals appeared to have ovulated in response to the hormone treatment because CL were present at slaughter. Two lambs that came into oestrus failed to ovulate and it was not possible to view the ovaries of another lamb at laparoscopy. These 20 animals have been excluded and the results relate to the remaining 84 lambs which came into oestrus and also ovulated in response to the progestagen–PMSG treatment. The mean (±s.e.m.) values for liveweight, interval from sponge removal to the onset of oestrus, duration of oestrus and ovulation rate were 45.5 ± 0.5 kg, 39.6 ± 1.03 h, 29.9 ± 1.23 h and 1.64 ± 0.08, respectively. Ovulation did not occur in any lamb before 17 h after the onset of oestrus and the majority ovulated close to the end of oestrus (Table 2).

Table 2. Time of ovulation in Galway ewe lambs treated with progestagen–PMSG

<table>
<thead>
<tr>
<th>Time after onset of oestrus (h)</th>
<th>No. of lambs examined</th>
<th>No. with CL</th>
<th>Percentage ovulating</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 h</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22 h</td>
<td>18</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>27 h (a)</td>
<td>16</td>
<td>8</td>
<td>50</td>
</tr>
<tr>
<td>(b)*</td>
<td>17</td>
<td>12</td>
<td>71</td>
</tr>
<tr>
<td>32 h (c)</td>
<td>17</td>
<td>16</td>
<td>94</td>
</tr>
<tr>
<td>(d)†</td>
<td>18</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>37 h</td>
<td>15</td>
<td>15</td>
<td>100</td>
</tr>
</tbody>
</table>

* Previously examined at 17 h.
† Previously examined at 22 h.

Discussion

The average duration of oestrus in lambs in the present work was similar to that of adult ewes and was also close to the value previously reported for mature Galway ewes following the same progestagen–PMSG treatment (Quirke, Jennings, Hanrahan & Gosling, 1979b). The concentration of total oestrogens in jugular plasma, however, was more than 100% greater in lambs. These results contrast with findings for untreated lambs in which the duration of oestrus is considerably shorter than in adults (Hafez, 1952; Hanrahan & Quirke, 1975; Edey et al., 1978) and plasma levels of oestradiol during the 48 h preceding oestrus in lambs and adults are similar (Smith, Drost, Fairclough, Peterson & Tervit, 1977). The higher concentration of oestrogens in jugular venous plasma of lambs in the present work is, however, consistent with the results of Trounson, Willadsen & Moor (1977), which showed that follicles obtained from young lambs 24 h after stimulation with PMSG had a 46% greater capacity to synthesize oestrogens during their first day of culture in vitro than did those from similarly treated adult ewes. The apparent increase in the duration of oestrus in lambs following progestagen-PMSG treatment may, therefore, be a reflection of the high levels of oestrogen which accompany this treatment in lambs. Such an interpretation is consistent with observations that in adult ovariectomized ewes there is a positive association between dose of exogenous oestrogen and duration of oestrus (Scaramuzzi, Lindsay & Shelton, 1971; Fletcher & Lindsay, 1971; Land, Thompson & Baird, 1972). We must, however, set this against the evidence of lack of association between endogenous oestrogen levels and duration of oestrus in both the present work and in other studies (Smith et al., 1977; Bindon, Blanc, Pelletier, Terqui & Thimonier,
1979). There is also the consideration that the interval from sponge removal to oestrus is longer rather than shorter in lambs compared with adults (Quirke & Hanrahan, 1977), despite the higher endogenous oestrogen levels in lambs and the evidence that increasing the dose of exogenous oestrogen reduces the interval to oestrus in ovariectomized adults (Scaramuzzi et al., 1971). These apparent contradictions make it clear that variation, within physiological limits, in plasma oestrogen levels are not related in a simple fashion with the timing of onset or duration of oestrus. Adult ewes and ewe lambs clearly provide ideal material for studying this problem without violating physiological conditions.

Most aspects of the preovulatory LH discharge were similar in the two age groups and the total LH released and maximum levels observed were close to those previously reported for adult Galway ewes at the second oestrus following progestagen treatment (Quirke, Hanrahan & Gosling, 1979a). The difference between the two groups in the timing of the start of the LH peak relative to the onset of oestrus, although statistically significant, was still fairly small, only 3 h, and is unlikely to account for the difference in fertility usually observed between ewe lambs and adults. Indeed, the interval between the onset of oestrus and the beginning of the preovulatory LH discharge is known to vary widely according to breed and number of ova shed and to depend on the dose of progestagen when oestrus is synchronized with progestagens (Thimonier & Pelletier, 1971; Mauer et al., 1972; Land, Pelletier, Thimonier & Mauléon, 1973; Lewis, Bolt & Inskeep, 1974; Bindon et al., 1979; Quirke et al., 1979a). In mature ewes the interval between the start of the preovulatory LH discharge and ovulation is relatively constant (21–26 h) and ovulation is generally held to occur around the end of oestrus (Robinson, 1959; Parsons, Hunter & Rayner, 1967; Holst & Braden, 1972; Cumming et al., 1973). It would appear from the results of Exps 1 and 2 that these relationships are normal in progestagen-PMSG treated Galway ewe lambs.

It has been shown (Foote, Gooch, Pope & Casida, 1957; Moore & Rowson, 1959; Bindon, 1971) that progesterone is essential in ewes for continued development of the preimplantation embryos and the maintenance of pregnancy. The results of Exp. 1 and those of Smith et al. (1977) indicate that luteal function is similar in ewe lambs and adult ewes, and inadequate progesterone is therefore unlikely to be the underlying cause of reduced fertility in ewe lambs compared with adults. This is further supported by the finding that ewe lamb and adult ewe uteri are equally capable of supporting embryo survival (Quirke et al., 1978). The present experiments, together with the results of Smith et al. (1977), have failed to find differences between ewe lambs and adult ewes in various characteristics of the oestrous cycle which could account for the problem of reduced fertility in ewe lambs. Egg transfer experiments have shown that the uterine environment of ewe lambs and adults are equivalent when fertilized eggs are transferred around Day 4 of the cycle (Quirke et al., 1978), although there is a significant difference in the quality of cleaved eggs from ewe lamb and adult ewe donors when transferred to a common adult uterus (Quirke & Hanrahan, 1977). These findings, the results of the present work and the evidence of a different pattern of oestrogen secretion by lamb follicles in vitro (Trounson et al., 1977) all suggest that conditions in the developing follicle or in the reproductive tract between ovulation and before the 8–16-cell cleavage stage are related to the reduced fertility of ewe lambs.

We thank Miss Greta Tisdall for performing the total oestrogen assays; Assumpta Glynn, Martina Lee, W. Loughnane, T. Lally and G. McLoughlin for technical assistance; NIAMDD, Bethesda, Maryland, U.S.A., for purified ovine LH; Dr G. Jenkin and Dr B. Heap, A.R.C., Babraham, U.K., for rabbit anti-ovine LH (DBS 3/5); Dr B. Hoffman, Institut fur Physiologie, Technische Universität München, Germany, for rabbit antiserum to oestradiol; and Dr B. Cook, Glasgow Royal Infirmary, U.K., for sheep antiserum to progesterone (Y 20/5).
References


Received 17 January 1980