Seasonal variations in the cyclic luteal ovarian activity in the Tadmit ewe in Algeria

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Summary. Seasonal variations of the cyclic luteal activity were assessed by measurements of progesterone concentrations in peripheral plasma collected daily or every 4 days, throughout 15 months, in 16 Tadmit ewes, in Algeria. One female was cyclic at all times of the year; 15 ewes showed a period of cyclic luteal inactivity of <53 days in 10 animals and ranging between 2.5 and 3.5 months for the 5 others (mean duration 52 days). The onset of ovarian inactivity took place from the beginning of February to the end of April (mean date 19 March). The recovery of luteal function was much more definite; it occurred in May for 13 ewes (mean date for the 16 animals 10 May) when daylength was still increasing.

These data suggest that at this latitude (36°30'N) and for this particular breed of sheep (Tadmit) declining daylength is not the major environmental stimulus for the resumption of breeding activity.

Introduction

Seasonal variations in reproductive performance of the ewe have often been reported and the hypothesis had arisen that photoperiod is the major factor controlling the seasonality of breeding. Ewes are generally regarded as being ‘short day’ breeding animals. However, although it has been suggested that the seasonality depends upon the breed and the geographic location (Hafez, 1952), most previous experiments were concerned with breeds inhabiting temperate zones in the northern hemisphere, mainly in Great Britain and in France (Ortavant, Mauléon & Thibault, 1964; Thimonier & Mauléon, 1969; Land, Pelletier, Thimonier & Mauléon, 1973; Wheeler, 1973; Wheeler & Land, 1977). In these earlier experiments ovarian activity was assessed by detection of behavioural oestrus and/or by determination of the ovulation rate by laparoscopy.

Therefore, the purpose of the present study was to investigate annual changes in cyclic luteal activity, determined by measurement of the plasma concentrations of progesterone, in Tadmit ewes, native to Algeria.

Materials and Methods

Animals

The 30 Tadmit ewes, aged 3–5 years and multiparous, were penned together throughout the experimental period, in the absence of males, in a shed (10 × 15 m) where they were exposed to

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the external seasonal climatic conditions, in the Algiers area (36°39'N; 2°59'W). Text-figure 1 gives the temperature and photoperiod changes during the year the sheep were studied. A concentrate diet was fed, and hay and water were always available. All ewes were shorn in March 1976.

![Text-figure 1. Annual variations in air temperature and photoperiod in the Algiers area (36°39'N; 2°59'W) between May 1975 and July 1976 when the sheep were studied.](image)

**Blood collection**

Blood was collected by venepuncture from the jugular vein, from the beginning of May 1975 to the end of July 1976, between 09:00 and 12:00 h. Blood collections were carried out daily (10 ml) from 20 October to 30 November 1975, 15 January to 27 February 1976, 7 April to 19 June 1976 and from 11 to 31 July 1976; during the other periods of the year blood was collected every 4 days (30 ml). Blood was centrifuged immediately and stored at −25°C before assay.

**Progesterone assay**

[1,2-3H]Progesterone (sp. act. 47.8 Ci/mmol), purchased from the New England Nuclear Corporation (Boston, U.S.A.), was purified twice a month by thin-layer chromatography (two successive migrations in the same direction in a hexane–ethyl acetate (1:1, v/v) solvent system on silica gel GF254 (Merck, Darmstadt, West Germany) and stored at +4°C in benzene–ethanol (9:1, v/v). Progesterone for use as standards was obtained from Sigma (St Louis, U.S.A.). All solvents were analytical grade (Merck).

For each blood collection, an aliquot of 0.25 ml plasma (+ 1000 c.p.m. labelled progesterone for recovery) was extracted once with 10 volumes of diethyl ether. Progesterone was measured in duplicate by a radioimmunoassay using 4000 c.p.m. [3H]progesterone (0.1 ml), a progesterone-11α-hemisuccinate–BSA antibody (purchased from Bio-organics, Inc., Wellesley Hills, U.S.A.) at a dilution of 1:10 000 (0.1ml), plasma extracts (0.1ml) or standard steroids (0–500 pg/0.1 ml), and 1 ml cold charcoal-coated dextran solution (250 mg Norit A (Sigma) + 25 mg Dextran T 70 (Pharmacia, Uppsala, Sweden) in 100 ml 0.1 M-phosphate buffer, pH 7.4) to separate free and bound fractions, after 3 h incubation at +4°C. All final dilutions were prepared in the 0.1 m-phosphate buffer, pH 7.4 (9 g sodium chloride + 1 g sodium azide + 1 g gelatin in 1000 ml NaH2PO4 + Na2HPO4 solution).

Liquid scintillation counting was carried out in a Packard Tricarb 3380 counter, at least 2 h after addition of 10 ml scintillation fluid (4.0 g 2,5-diphenyl-oxazole + 0.1 g p-bis-2-(5-phenyl-oxazolyl)-benzene and 20 g naphthalene in 700 ml diovan and 300 ml toluene).

After corrections for recovery, dilutions and water blank, results were expressed as ng progesterone/ml plasma.
The method used was specific (% steroid cross-reactions against the progesterone antibody were: 17α-hydroxyprogesterone, 1.6; 20α-dihydroprogesterone, 2.5; 20β-dihydroprogesterone, 3.0; oestrogens, 0.0; cortisol, 0.0), sensitive (after Logit-log transformation, standard curves were linear between 5 and 100 pg; blank values were 9.0 pg/tube), precise (within-assay and between-assay variances were, respectively, 9.1 and 9.2%) and accurate (the correlation coefficient between observed and expected values was 0.999).

Statistical analysis

Student's t test was used to determine levels of significance.

Results

Text-figures 2 and 3 give a few representative individual profiles of annual variations of concentrations of plasma progesterone.

**Text-fig. 2.** Three representative individual profiles of annual variations of plasma concentrations of progesterone among 9 Tadmit ewes studied daily or every 4 days between May 1975 and July 1976, in Algeria.

**Text-fig. 3.** Two representative individual profiles of annual variations of plasma concentrations of progesterone among 7 Tadmit ewes studied every 4 days between January and July 1976, in Algeria.

Cyclic changes in the mean plasma progesterone concentrations

The hormone profile was established from the 10 ewes from which daily blood collections were taken during October and November 1975 when ovarian cyclicity was regular in all the animals; in the absence of detection of the behavioural oestrus, Day 0 was considered to be the first day, following the raised levels, when plasma concentrations of progesterone were below 0.5 ng/ml.
The duration of the ovarian cycle in the Tadmit ewe varied, between sheep, from 15 to 19 days (17.5 ± 1.3 days); this result is in agreement with our observations in another flock of ewes of the same breed assessed by detection of behavioural oestrus. In all animals, the cyclic pattern of plasma progesterone concentrations showed the 4 classical successive phases: (i) during the follicular phase, for 4–7 days (4.1 ± 2.1 days) it remained below 0.5 ng/ml, mostly around 0.2 ng/ml; (ii) it increased after ovulation, during the luteinization of the follicle; (iii) from Days 8–9, the plasma progesterone level presented an elevated and more or less long and regular plateau; (iv) then, at the end of the cycle, it declined rapidly; the luteal phase lasted generally between 10 and 13 days (12.6 ± 2.3 days), plasma progesterone concentrations reaching about 2–4 ng/ml (2.6 ± 0.2 ng/ml).

Number of ovarian cycles throughout the year

The pattern of plasma concentrations of progesterone during the ovarian cycle demonstrated clearly that, when blood samples were collected every 4 days from Tadmit ewes, even if only one value in each 20-day period was about or below 0.5 ng/ml then it was possible to conclude that ovulation had occurred and therefore that the ewe was cyclic. If the 5 values were above 0.5 ng/ml, the ewe was considered pregnant; if all were below, the female was in anoestrus (Thimonier, 1973; Ammar-Khodja et al., 1976).

Table 1 gives the number of observed cycles over the full period of study. For each ewe, the mean length of one cycle was established by the ratio between the duration of the cyclic ovarian activity period and the number of well delimited observed cycles; it ranged from 17.1 days (Ewe 640) to 20.3 days (Ewe 35) but was mostly between 17.5 and 18.5 days.

Date of onset and duration of the period of cyclic luteal inactivity

For all the animals luteal phases were absent at the same time of the year, i.e. ‘the cyclic ovarian inactivity period’. However, the onset, duration and end of this period varied between animals (Table 1). Onset took place from the beginning of February to the end of April (in

### Table 1. Characteristics and seasonal variations of the cycle of luteal activity in the Tadmit ewe in Algeria

<table>
<thead>
<tr>
<th>Ewe</th>
<th>No. of observed cycles</th>
<th>Mean length of ovarian cycle (days)</th>
<th>Duration of the ovarian inactivity period (days)</th>
<th>No. of luteal phases lacking before or after the ovarian inactivity period</th>
</tr>
</thead>
<tbody>
<tr>
<td>690*</td>
<td>24</td>
<td>18.0</td>
<td>25 (14 April–9 May)</td>
<td>2</td>
</tr>
<tr>
<td>642*</td>
<td>23</td>
<td>18.5</td>
<td>34 (20 March–23 April)</td>
<td>0</td>
</tr>
<tr>
<td>626*</td>
<td>20</td>
<td>18.0</td>
<td>44 (1 April–14 May)</td>
<td>2</td>
</tr>
<tr>
<td>221*</td>
<td>22</td>
<td>18.2</td>
<td>35 (1 April–5 May)</td>
<td>2</td>
</tr>
<tr>
<td>240*</td>
<td>17</td>
<td>19.5</td>
<td>45 (1 April–15 May)</td>
<td>1</td>
</tr>
<tr>
<td>203*</td>
<td>21</td>
<td>18.1</td>
<td>52 (15 March–6 May)</td>
<td>2</td>
</tr>
<tr>
<td>640*</td>
<td>18</td>
<td>17.1</td>
<td>77 (1 March–17 May)</td>
<td>1</td>
</tr>
<tr>
<td>211*</td>
<td>17</td>
<td>17.5</td>
<td>114 (1 February–25 May)</td>
<td>1</td>
</tr>
<tr>
<td>217*</td>
<td>18</td>
<td>17.6</td>
<td>104 (1 February–15 May)</td>
<td>1</td>
</tr>
<tr>
<td>659†</td>
<td>9</td>
<td>19.3</td>
<td>27 (7 April–3 May)</td>
<td>1</td>
</tr>
<tr>
<td>31†</td>
<td>9</td>
<td>18.6</td>
<td>24 (27 April–20 May)</td>
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</tr>
<tr>
<td>224†</td>
<td>5</td>
<td>20.0</td>
<td>93 (10 March–12 June)</td>
<td>0</td>
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<tr>
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<tr>
<td>201†</td>
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<td>18.7</td>
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<td>0</td>
</tr>
<tr>
<td>207†</td>
<td>6</td>
<td>—</td>
<td>63 (13 March–13 May)</td>
<td>1</td>
</tr>
<tr>
<td>35†</td>
<td>11</td>
<td>20.3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Ewes studied between May 1975 and July 1976.
† Ewes studied between January 1976 and July 1976.
February for 2 ewes; in March for 6 ewes and in April for 7 others; mean date 19 March). However, the time of recovery of the luteal function was much more precise: except for 2 ewes (just after 15 April) and 1 ewe (beginning of June) it took place in May (mean date 10 May). Consequently, the duration of the luteal inactivity period varied, being 35 days or less, i.e. approximately 2 cycles, in 5 ewes, 43–53 days in 5 ewes and 2.5–3.5 months in the 5 others. Ewe 35 showed cyclic ovarian activity at all times of the year. The mean duration for the 15 ewes was 52 days.

However, this period of luteal inactivity was not always absolutely clearly defined; although a hormone profile typical of a cycle did not occur, there were some plasma concentrations of progesterone greater than 0.5 ng/ml in some ewes (Nos 626, 203, 640 and 211). There were also some perturbations in the regular cyclic pattern before or after the inactivity period. Table 1 shows that for all 16 ewes, from January to July there were 13 and 9 missing luteal phases, respectively.

**Discussion**

The mean plasma progesterone concentrations and the pattern of progesterone production throughout the ovarian cycle in the Tadmit ewe are in agreement with other reports obtained, for various breeds, with similar assay techniques (Stabenfeldt, Holt & Ewing, 1969; Thorburn, Bassett & Smith, 1969; Obst & Seamark, 1970; Lemon & Thimonier, 1973; McNatty, Revfeim & Young, 1973; Baird, Land, Scaramuzzi & Wheeler, 1973; Ammar-Khodja & Brudieux, 1978).

Our study shows that the measurement of progesterone in blood samples collected daily, or at least every 4 days, is a good means of following luteal function and so assessing variations in ovarian activity.

Our results demonstrate that 15 Tadmit ewes out of 16 showed a period of cyclic ovarian inactivity with between-sheep variations in duration. Nevertheless, this period would be compared not to an anoestrous period, because we could not preclude the possibility of silent ovulations (Thimonier & Mauléon, 1969), but rather to the anovulation period described for other ewes.

For several breeds in temperate zones (Ortavant et al., 1964), it has been established that the sheep is a ‘short day’ breeding animal (Hammond, 1944) and the natural annual variations in daylength are the major factor controlling the seasonality of breeding in ewes (Yeates, 1949; Hafez, 1952; Thwaites, 1965). It is generally agreed that breeding activity is stimulated by declining daylength or a particular low number of hours of light per day and is supressed by increasing daylength or a high number of hours of light per day.

Considering the northern hemisphere only, and assessment of seasonal breeding activity by determination of dates of onset and cessation of the behavioural oestrus, this concept is confirmed for several breeds, despite large individual differences: e.g. dates of the first and last oestrus and the duration of anoestrus were respectively: in Iceland, at Hvanneyri (64°34'N), November, May and 219 days for the native Iceland breed (Dyrmundsson, 1978); in Scotland, at Roslin (Midlothian, about 56°N), October, May and 166 days for Finnish Landrace, September, February and 181 days for Tasmanian Merino, October, February and 235 days for Scottish Blackface (Wheeler & Land, 1977); in France, at Nouzilly (47°30'N), August, February and 164 days for Romanov, August, December and 210 days for Solognote (Land et al., 1973); in France, at Jouy-en-Josas (48°30'N), August, January and 179 days for Ile-de-France, July, March and 114 days for Préalpes du Sud (Thimonier & Mauléon, 1969). However, there are, in the literature, data that do not lead to the same conclusion: Hafez (1954) and Schafer (1964) listed a number of breeds which reached the peak of their reproductive activity during the longest days, and Thimonier & Mauléon (1969) observed a short period of ovarian activity at the end of April or at the beginning of May and the first ovarian cycles in
June, i.e. when daylength is increasing, in Préalpes du Sud and Ile-de France ewes. In the same way, in Israel (31°15'N) 80% of Mutton Merino ewes conceived in the months of lengthening daylight (Goot, 1969). Likewise, in south Carolina (33°N) (U.S.A.) the Western Whiteface ewe became anoestrous by early February (Rawlings, Kennedy, Chang, Hill & Henricks, 1977) and commenced cyclic activity in early May (Rawlings et al., 1977) or in early June (Godley, Wilson & Hurst, 1966). Further, according to Hulet, Shelton, Gallagher & Price (1974) the ovulation rate in Rambouillet ewes both increased and declined earlier in Texas (about 30°N) (minimum from March to May) than in Idaho (about 45°N) (minimum from April–May to July). Likewise, our own observations showed that in Algeria (36°39'N) the seasonal luteal ovarian activity of the Tadmit ewe started on average during May, when daylength is still increasing.

We would therefore argue that the short-day photoperiodic theory is not applicable to all breeds of sheep or to all environmental conditions. The above bibliographic review shows that this photoperiodic mechanism is not applicable to ewes at low altitudes; the duration of the seasonal anovulation period is shortened and the ewes begin breeding earlier in the year in sheep living nearer the equator. In Ouled Djellal rams in the Algiers area, the production rate of testosterone was lowest in autumn and early winter months, increased in February–March and reached highest values in summer, before decreasing in autumn (Darbeida & Brudieux, 1980).

Without excluding the possibility of between-breed differences in duration of the seasonal anoestrous period and in the dates of onset and cessation of ovarian activity, it is, however, possible that, at low latitudes, photoperiod may not be the principal environmental cue which times the reproductive endocrine cycle in sheep.

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