Increased ovulation rate in androstenedione-immune ewes is not due to elevated plasma concentrations of FSH

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Summary. Two experiments were undertaken to determine the hormonal response of Merino ewes to immunization against androstenedione (Fecundin®). In Exp. 1 peripheral concentrations of LH, FSH and progesterone were monitored in spontaneously cycling ewes (20 immunized and 21 controls). In Exp. 2 (10 immunized and 10 controls) the same hormones were measured in ewes before and after prostaglandin (PG)-induced luteolysis and, in addition, the pattern of pulsatile LH secretion was determined during the luteal (PG + 12 days), early follicular (PG + 24 h) and late follicular (PG + 40 h) phase of the oestrous cycle. Ovulation rates were measured in both experiments.

The results of these experiments indicate that androstenedione-immune animals have elevated ovulation rates (0.6–0.7 greater than control animals; P < 0.05) associated with elevated plasma concentrations of LH and progesterone. The magnitude of the increase in plasma progesterone was correlated with androstenedione antibody titre (r = 0.6, P < 0.001). LH pulse frequency of androstenedione-immune ewes tended to be higher at all stages of the oestrous cycle, but this difference was only significant (P < 0.05) during the luteal phase.

Mean plasma concentrations of FSH did not differ significantly between immunized and control ewes at any stage of the cycle. Analysis of periodic fluctuations in FSH during the luteal phase revealed that androstenedione-immune animals had a similar number of fluctuations of a similar amplitude to those of control animals, but the nadir of these fluctuations was lower (P < 0.05) in immunized animals. A significant (P < 0.05) negative correlation existed between androstenedione antibody titre and the interval between FSH peaks (r = −0.49) and androstenedione antibody titre and FSH nadir concentrations (r = −0.46).

It is concluded that plasma FSH concentrations are not a determinant of ovulation rate in androstenedione-immune ewes and that increased LH concentrations, or perturbation of normal intraovarian mechanisms, may be responsible for the increase in ovulation rate observed in ewes immunized against androstenedione.

Keywords: androstenedione-immunity; ovulation rate; LH; FSH; progesterone; sheep

Introduction

Immunization against androstenedione leads to increases in ovulation rate in sheep (Scaramuzzi & Hoskinson, 1984; Webb et al., 1984). Despite the practical application of this phenomenon, in the form of a commercial product Fecundin® (Scaramuzzi et al., 1983; Philipon & Driancourt, 1987) to increase twinning rates in sheep, the mechanisms responsible for this increase in ovulation rate are unclear. Although the immediate cause is an increase in the number of large oestrogenic follicles (i.e. follicles >3.5 mm in diameter and having an intrafollicular oestradiol concentration >100 ng/
ml) during both the luteal and follicular phase of the oestrous cycle (Scaramuzzi & Hoskinson, 1984; Campbell, 1988), the endocrinological basis for this increase in ovulation rate remains uncertain. Studies designed to examine the endocrinological response of ewes to androstenedione-immunity, conducted during the late 1970s on small numbers of highly stimulated animals, concluded that androstenedione-immune animals have elevated concentrations of plasma LH (Martensz et al., 1976; Martensz & Scaramuzzi, 1979), while FSH concentrations were either unchanged or depressed during the luteal phase of the oestrous cycle (Martensz et al., 1979; Martensz & Scaramuzzi, 1979). Similar results have also been reported for ewes passively immunized against androstenedione (Pathiraja et al., 1984). Analysis of the pattern of LH secretion (review: Scaramuzzi & Hoskinson, 1984) has shown that immunization against androstenedione results in increases in pulse frequency during anoestrous and the luteal phase, but not during the follicular phase of the oestrous cycle.

The endocrinological response of the androstenedione-immune ewe is at odds with one of the current theories of the control of ovulation rate, namely that the concentration of FSH in plasma is a major determinant of ovulation rate (Baird, 1983; McNatty et al., 1985, 1987). McNatty et al. (1988) have reported elevated FSH concentrations in androstenedione-immune ewes during the luteal phase of the oestrous cycle. As this observation contradicts all previous published reports, the effect of steroid immunization on FSH concentrations requires further clarification, particularly in the light of recent studies which have shown that immunization against androstenedione has no overall effect on ovarian oestradiol secretion (Campbell et al., 1990a) and results in an increase in ovarian inhibin secretion (Campbell et al., 1990b) during the luteal phase of the oestrous cycle.

The experiments described in this paper examine the effect of controlled immunization against androstenedione on the concentration of gonadotrophins in the peripheral circulation of relatively large numbers of ewes. The first experiment examines these parameters in spontaneously cycling ewes, in which the cycle was synchronized before the start of blood sampling. The second experiment examines these parameters following prostaglandin-induced luteolysis. The pattern of pulsatile LH secretion at three different stages of the oestrous cycle was also examined.

Materials and Methods

Experiment 1

Animals. This experiment, conducted during the breeding season, utilized 48 4–5-year-old Merino ewes with a mean body weight of 40.8 ± 0.7 kg. The immunized ewes, which had been previously immunized 6–8 months before the start of the experiment, received booster immunization injections of Fecundin® (Glaxo Animal Health, Boronia, NSW, Australia; 2 ml s.c. containing 2 mg androstenedione-7a-carboxyethylthioether: human serum albumin with a DEAE-Dextran immunoadjuvant) twice, the first time 4 weeks before the start of blood sampling and the second time 9 days after the first observed period of oestrus.

Experimental treatments. Oestrus was synchronized in the 48 ewes, 24 controls and 24 androstenedione-immune, by using intravaginal progestagen-impregnated sponges (Repromap®, Upjohn Pty Ltd, Rydalmere, NSW, Australia) inserted for 12 days. Each treatment group was further divided into sub-groups of 10 cannulated and 14 non-cannulated ewes. The cannulated group had indwelling jugular polyvinyl cannulae (1.0 mm i.d., 1.5 mm o.d.; Dural Plastics & Engineering, Dural, NSW, Australia) inserted 1 day before the start of blood sampling. Whilst in the pens the ewes had ad-libitum access to a diet of oaten chaff and grain, which maintained their body weight. The non-cannulated ewes were maintained under paddock conditions.

Time of blood sampling. Blood sampling began 15 days after sponge withdrawal. In the cannulated ewes blood samples were taken at 2-h intervals for 7 days. In the non-cannulated ewes, jugular blood samples were taken by venepuncture at 4-h intervals for a similar period of time. At the end of the first period of intensive bleeding the cannulae were removed and the ewes were placed outside with the other ewes for 12 days. During this period all ewes were bled twice daily (at 06:00 and 18:00 h) by venepuncture. At the end of this period the same ewes were recannulated and a 6-day period of intensive bleeding, as used earlier, was undertaken in all ewes. Plasma was separated by centrifugation at 2000–3000 g. The plasma was then stored frozen until assayed.
**Ovulation rate.** Ovulation rate was determined by endoscopy, by the mid-ventral route (Evans & Maxwell, 1987) in conscious ewes under local anaesthesia (Xylocaine, Astra Pharmaceutical Pty Ltd, North Ryde, NSW, Australia, 2 ml, s.c.), at around Day 6 of each cycle.

**Experiment 2**

**Animals.** The animals used in this experiment were 4–5-year-old Merino ewes (N = 20) with a mean body weight of 42.6 ± 0.6 kg. The experiment was conducted during the breeding season (April).

**Experimental treatments.** The ewes were placed into single pens and fed a maintenance diet of pelleted lucerne:oats (60:40 w/w), containing 17 MJ gross energy/kg and 14% protein. Oestrous was synchronized in 10 control and 10 androstenedione-immune ewes by two intramuscular injections of prostaglandin (PG: 125 µg Estrumate®, Coopers Animal Health, North Ryde, NSW, Australia) given 10 days apart. At 12 days after the second PG injection (i.e. mid-luteal) a third injection of PG was given. The androstenedione-immune ewes, which had been previously immunized 6–8 months before the start of the experiment, were given a booster injection of immunogen (Fecundin®, 2 ml, s.c.) 8 days before the third PG injection.

**Time of blood sampling.** All ewes had jugular catheters inserted the day before intensive blood sampling. Blood samples were taken at 3 stages of the oestrous cycle: (i) the mid-luteal phase for 8 h at 15-min intervals (i.e. 12 days after the second PG injection); (ii) the early follicular phase for 6 h at 10-min intervals (i.e. 21–27 h after the third PG injection); and (iii) the late follicular phase for 6 h at 10-min intervals (i.e. 37–43 h after the third PG injection).

In addition, blood samples were taken every 8 h for 2 days before the first period of intensive blood sampling and every 2–4 h from the time of third PG injection for 90 h.

**Ovulation rate.** Ovulation rate was determined by endoscopy 6 days after the end of blood sampling.

**Hormone assays**

Jugular venous plasma LH (Scaramuzzi et al., 1970), FSH (Radford et al., 1987) and progesterone (Scaramuzzi et al., 1975) concentrations were determined by established radioimmunoassays. The sensitivity of the assays for LH, FSH and progesterone were 0.18, 0.06 and 0.13 ng/ml respectively. The intra- and inter-assay coefficients of variation (at 50% displacement of tracer) for LH, FSH and progesterone were respectively 4.3 and 5.4%, 1.7 and 2.8%, 6.5 and 14.9%.

Antibody titres to androstenedione in plasma from immunized ewes were also determined (Abraham, 1974). Briefly, serial dilutions of plasma from immunized ewes were incubated overnight with tritiated androstenedione ([1,2,6,7-3H]androstenedione, sp. act. 284 mCi/mg; 5000 c.p.m. added per tube). Bound and free ligand were separated by Dextran-coated charcoal (20 mg Norit A/ml plus 2 mg Dextran T70/ml; Pharmacia, Uppsala, Sweden) and the titre expressed as the final plasma dilution which bound 50% of added label. In Exp. 1 antibody titres were determined on blood samples taken during the respective follicular phases and represent an interval from time of booster injection to time of blood sampling of 4 weeks for Cycle 1 and 8–9 days for Cycle 2. In Exp. 2 antibody titres were determined 7 days after boosting.

**Statistical analysis**

**Experiment 1.** Data from each ewe for each hormone were centred around the peak of each preovulatory LH surge. Hormonal data from ewes sampled by venepuncture or by cannula did not differ and were pooled. Due to the variation between animals in the interval between the successive LH surges, the scale was interrupted and adjusted midway between the mean interval of the two surges at Day 9 to form two time periods. This also coincided with the time of the second booster immunization. For LH and FSH these two periods were then divided into a number of time periods of physiological interest (the zero point being the LH surge), as follows: (i) Follicular phase 1, Days −4.75 to −0.66; (ii) Preovulatory Peak 1, Days −0.5 to 0.5; (iii) Postovulatory Peak, Days 0.57 to 5.5; (iv) Luteal Phase, Days 6 to 9 and −8.5 to −6; (v) Follicular Phase 2, Days −5.5 to −0.66 and (vi) Preovulatory Peak 2, Days −0.5 to 0.5.

For progesterone, data were analysed in two phases only; from Days −6 to 9 (about LH Surge 1) and from Days −9 to 3 (about LH Surge 2). Before analysis LH and progesterone values were transformed to logarithms (ln x). FSH data were analysed untransformed. Within each phase, analysis of variance was used (of the ‘split plot’ type that allow for sheep and sheep × time contributions to the random variability). Additionally, the ‘time’ factor was partitioned into linear, quadratic and remainder terms in order to determine the nature of the response.

In addition to the above analysis, it was noted that individual animals exhibited fluctuations in FSH concentrations throughout the oestrous cycle, as has been noted by other workers (Miller et al., 1981; Bister & Paquay, 1983). The nature of these fluctuations was determined using the Munro (Elsevier; Biosoft, Cambridge, UK) pulse analysis program for the Apple Macintosh computer. Only fluctuations during the luteal phase were submitted to analysis as the preovulatory and Day 1 peaks were considered to be of a different nature. Differences between treatments were compared with unpaired t tests.
Experiment 2. The characteristics of pulsatile LH secretion were determined using the Munro (Elsevier: Biosoft) pulse analysis program. Statistical tests of these pulse characteristics were conducted using analysis of variance (ANOVA) in a split-plot design.

To compare peripheral LH, FSH and progesterone concentrations, the data were centred about the peak of the preovulatory LH surge and divided into 4 time periods that corresponded to the luteal phase (−96 to −56 h), follicular phase (−52 to −10 h), ovulatory LH surge (−10 to +10 h) and post-ovulatory surge (+10 to +36 h). After log transformation (ln x) of LH and progesterone values these data were analysed using a split plot in time ANOVA. FSH values were analysed untransformed. As different bleeding frequencies were used over the peripheral monitoring period, there were occasions when estimates of hormone concentrations at a particular time were not available for all animals. Therefore, in order to be included in the data set, >70% of the ewes had to contribute to the estimate of a hormone concentration at a particular time.

Differences in ovulation rate were compared by χ² test. Differences in antibody titre between immunized groups were compared using the Wilcoxon signed-rank test.

Results

Experiment 1

Cyclicity, detection of oestrus, ovulation rate and antibody titre. Of the 48 ewes, data for 4 androstenedione-immune and 3 control ewes were removed from the experimental analysis: 3 immunized and 1 control ewe were not cycling and had continuous elevated progesterone concentrations, presumably due to persistent corpora lutea. The other 3 ewes not used were cycling but were not synchronized with the rest of the flock and as a result the preovulatory LH surge was not detected.

Androstenedione-immune ewes all had antibody titres to androstenedione and there was a significant response (P < 0·001) to the mid-cycle booster immunization (reciprocal antibody titres (median [range]) for the first cycle, 533 [100–6481], and second cycle, 745 [100–31 155]). Immunization resulted in a significant (P < 0·05) elevation in ovulation rate (mean ± s.e.m.) in both cycles (Cycle 1: controls 1·2 ± 0·1, immunized 1·8 ± 0·1; Cycle 2: controls 1·2 ± 0·1, immunized 1·9 ± 0·1). Immunized ewes had mainly double ovulations with some single and triple ovulations, while control ewes had mainly single ovulations.

The length of the oestrous cycle (mean ± s.e.m.), defined as the period between successive LH surges, was longer (P < 0·05) in immunized (18·2 ± 0·2 days) than in control (17·7 ± 0·2 days) ewes.

Progesterone. The mean overall progesterone profiles, centred about the two LH surges, are shown in Fig. 1. Sheep in the immunized and control groups had similar patterns of progesterone secretion. This pattern consisted of high luteal phase concentrations followed by a rapid fall at the time of luteolysis to very low concentrations over the follicular phase. Progesterone concentrations were higher (P < 0·001) in androstenedione-immune ewes during the luteal phase of the oestrous cycle but not during the follicular phase. After ovulation peripheral progesterone concentrations increased much more quickly in immunized ewes when compared to control ewes. Following the mid-cycle booster injection, progesterone concentrations in immunized ewes increased further to a new plateau concentration. One of the striking features of progesterone concentrations in androstenedione-immune ewes was the large variation between animals during the luteal phase (range 5–50 ng/ml). Some of this variation can be attributed to differences in antibody titre and peripheral mid-luteal progesterone concentrations (r = 0·6; P < 0·001).

Luteinizing hormone. The pattern of mean LH concentrations, centred about the two LH surges, is shown in Fig. 1 for both treatment groups. The androstenedione-immune and control ewes had similar patterns of LH. This consisted of low basal concentrations throughout the luteal phase with no significant time effect and a period of linearly increasing concentrations after luteal regression. After the preovulatory surge LH concentrations fell briefly to luteal-phase levels and then increased to form a broad peak which was largely quadratic in nature, and occurred 2–3 days
Fig. 1. Jugular venous concentrations of (a) progesterone, (b) LH and (c) FSH in spontaneously cycling untreated (N = 21; hatched area represents a band of mean ± 1 s.e.m. wide) and androstenedione-immune (N = 20; ○; mean ± s.e.m.) in ewes in Exp. 1. Data have been aligned with respect to consecutive LH surges. Note the broken scale on the horizontal axis and the log-scale on the vertical axis of the LH plot.

after the LH surge. Peripheral LH concentrations were higher (P < 0.01) in androstenedione-immune ewes at all of the 6 stages of the oestrous cycle used in this study. Although treatment by time interactions were present in the data they are not readily summarized as they tended to be irregular.

**Follicle-stimulating hormone.** The mean overall FSH profiles, centred about the two LH surges, are also shown in Fig. 1. Ewes in the immunized and control groups had similar patterns of FSH secretion. This pattern consisted of a marked, largely linear, depression during the follicular phase which was followed by a peak coincident with the LH surge, followed by another peak 1–2 days later. After the second FSH peak plasma concentrations declined in both groups and then
increased to a broader peak around Day 6. After Day 6 mean FSH values stayed relatively high and constant until the time of luteolysis. Mean FSH concentrations, at virtually all times during the cycle, were lower in androstenedione-immune ewes, but these differences were not statistically significant (Fig. 1).

Although mean luteal-phase FSH concentrations were relatively constant, FSH values in individual animals displayed regular fluctuations. Animals in both treatment groups showed regular fluctuations in FSH with a period of 3–6 days although with considerable variation between animals (Fig. 2). Comparison of the characteristics of these fluctuations during the luteal phase showed that numbers and amplitudes of fluctuations were similar in the two groups (Table 1). The nadir, however, of these fluctuations was lower \( (P < 0.05) \) in immunized animals than in control ewes. A significant \( (P < 0.05) \) negative correlation existed between androstenedione antibody titre and the interval between FSH peaks \( (r = -0.49) \) and androstenedione antibody titre and FSH nadir concentrations \( (r = -0.46) \).

**Table 1. Characteristics of luteal phase FSH fluctuations in control and androstenedione-immune (A4-Immune) ewes**

<table>
<thead>
<tr>
<th></th>
<th>No. of fluctuations in luteal phase</th>
<th>Interval (days)</th>
<th>Amplitude (ng/ml)</th>
<th>Nadir (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 21)</td>
<td>2.71 ± 0.16</td>
<td>4.89 ± 0.19</td>
<td>1.28 ± 0.09</td>
<td>1.01 ± 0.07</td>
</tr>
<tr>
<td>A4-Immune (N = 20)</td>
<td>2.35 ± 0.17</td>
<td>5.37 ± 0.33</td>
<td>1.48 ± 0.11</td>
<td>0.79 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m.

*Significantly different from control ewes, \( P < 0.05 \).

**Fig. 2.** Concentration of FSH in jugular venous plasma in (a) spontaneously cycling untreated ewes and (b) androstenedione-immune ewes with a low or high \( (1:1385 \text{ and } 1:13,335) \) titre of antibody in plasma. Time zero is the time blood sampling began. The arrows represent the time of the preovulatory LH surges.
Fig. 3. Jugular venous concentrations of (a) progesterone, (b) LH and (c) FSH in untreated (N = 10; hatched area represents a band of mean ± 1 s.e.m.) and androstenedione-immune (N = 10; ○; mean ± s.e.m.) ewes in Exp. 2. Data have been aligned with respect to the time of the preovulatory LH surge. Note the log-scale on the vertical axis of the LH and progesterone plot.

Experiment 2

Cyclicity, ovulation rate and antibody titre. The ewes all had normal oestrous cycles, as verified by peripheral plasma progesterone concentrations, and all responded to the third PG injection. The mean (± s.e.m.) time from PG injection to the LH surge was not significantly different between treatment groups (54·5 ± 3·4 h in controls and 56·8 ± 4·2 h in immunized). Ovulation rates (mean ± s.e.m.) were elevated (P < 0·05) in androstenedione-immune ewes, with these animals having mainly twin ovulations (1·6 ± 0·2 compared with 1·1 ± 0·1 in controls). Immunized ewes...
had antibody titres against androstenedione of 1:2332 (median) with a great deal of variation between animals (range 1:235 to 1:58 210).

**Progesterone.** Mid-luteal progesterone concentrations were around 10 times higher in androstenedione-immune ewes than in control ewes ($P < 0.001$: Fig. 3). After PG injection, progesterone concentrations in both treatment groups exhibited a depression ($P < 0.001$) with time. Plasma progesterone concentrations were higher ($P < 0.01$) in androstenedione-immune ewes than in control ewes during the follicular phase. During the preovulatory surge or post-surge periods progesterone concentrations in both treatment groups were very low and were not significantly different.

**Luteinizing hormone.** While both groups had similar patterns of secretion (Fig. 3), LH concentrations were elevated in androstenedione-immune ewes during the luteal and follicular phase ($P < 0.05$). There were no significant differences in plasma LH concentrations between treatments during or after the LH surge.

Overall means for parameters of pulsatile LH secretion are presented in Table 2. Both treatment groups had a similar pattern of LH secretion with an increase in pulse interval and nadir from the luteal to the follicular phase ($P < 0.01$). There was no effect of stage of cycle on pulse amplitude. Pulses occurred more frequently in androstenedione-immune ewes at all stages of the cycle, but this difference in pulse interval was only significant ($P < 0.01$) during the luteal phase. There were no significant differences in either pulse amplitude or nadir between treatment groups within stages of the oestrous cycle.

**Follicle-stimulating hormone.** Control ewes had a depression in FSH concentrations ($P < 0.01$) from the luteal to the follicular phase, a preovulatory peak coincident with the LH surge, and a second peak 24 h later. In androstenedione-immune ewes, the pattern of secretion was very similar, with the exception that there was no depression in plasma FSH concentrations over the transition from the luteal to the follicular phase (Fig. 3). At no stage of the cycle was there a significant difference in plasma FSH concentrations between treatment groups.

**Discussion**

The androstenedione-immune ewes in this experiment all responded to immunization with detectable antibody titres and an increase in ovulation rate. While the immunization treatment given to ewes in Exps 1 and 2 were similar and efforts were made to standardize the time of year and breed of sheep, the response to immunization, in terms of antibody titre, was greater in Exp. 2 ($P < 0.05$). The reason for this difference in response is unclear, although strain of ewe and

<table>
<thead>
<tr>
<th>Stage of cycle</th>
<th>Treatment</th>
<th>Pulse interval (min)</th>
<th>Pulse amplitude (ng/ml)</th>
<th>Nadir (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-luteal</td>
<td>Control</td>
<td>$115.8 \pm 12.7^\dagger$</td>
<td>$1.1 \pm 0.1$</td>
<td>$0.3 \pm 0.1^\dagger$</td>
</tr>
<tr>
<td></td>
<td>Immunized</td>
<td>$87.9 \pm 4.0^{*\dagger}$</td>
<td>$1.4 \pm 0.4$</td>
<td>$0.6 \pm 0.2^\dagger$</td>
</tr>
<tr>
<td>Early follicular</td>
<td>Control</td>
<td>$58.3 \pm 6.0$</td>
<td>$1.2 \pm 0.3$</td>
<td>$1.0 \pm 0.2$</td>
</tr>
<tr>
<td>(PG + 24 h)</td>
<td>Immunized</td>
<td>$46.8 \pm 1.7$</td>
<td>$1.7 \pm 0.3$</td>
<td>$1.4 \pm 0.1$</td>
</tr>
<tr>
<td>Late follicular</td>
<td>Control</td>
<td>$63.1 \pm 4.9$</td>
<td>$0.8 \pm 0.1$</td>
<td>$0.9 \pm 0.1$</td>
</tr>
<tr>
<td>(PG + 40 h)</td>
<td>Immunized</td>
<td>$55.6 \pm 3.5$</td>
<td>$1.0 \pm 0.2$</td>
<td>$1.1 \pm 0.1$</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m.
*Significantly different from control ewes within a stage of the oestrous cycle, $P < 0.05$.
†Comparison within treatment groups, significantly different from both stages of the follicular phase, $P < 0.05$. 

Discussion

The androstenedione-immune ewes in this experiment all responded to immunization with detectable antibody titres and an increase in ovulation rate. While the immunization treatment given to ewes in Exps 1 and 2 were similar and efforts were made to standardize the time of year and breed of sheep, the response to immunization, in terms of antibody titre, was greater in Exp. 2 ($P < 0.05$). The reason for this difference in response is unclear, although strain of ewe and

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previous immunization history may be important factors. This difference, however, is great enough to make direct comparison of the two experiments hazardous. The results do confirm, however, that hormonal profiles of progesterone, LH and FSH during the follicular phase are very similar in spontaneously cycling ewes and in ewes in which luteolysis has been induced by prostaglandins.

A major finding of the experiments reported in this paper is that peripheral FSH concentrations are not positively correlated with ovulation rate in androstenedione-immune ewes. This finding confirms earlier reports for both active and passive immunization (Scaramuzzi & Hoskinson, 1984; Pathiraja et al., 1984) but is at variance with the report by McNatty et al. (1988) of elevated FSH concentrations in ewes actively immunized against androstenedione. The reason for this difference is uncertain, but could be due to differences in immunogen, level of antibody titre or breed. As McNatty et al. (1988) used the same homologous NIAMDD oFSH assay as was used in this study, it is unlikely that assay variation is the source of the difference. The observation that the ovulation rate responses of ewes treated with a combination of Fecundin® and PMSG are additive over a wide range of doses (Scaramuzzi et al., 1987) also supports the idea that increased FSH activity is not a determinant of ovulation rate in androstenedione-immune ewes. Immunized sheep may, however, secrete FSH of increased bioactivity and this possibility is worth further examination.

Although we observed no significant effect of androstenedione-immunity on the concentration of FSH in the peripheral circulation, it is likely that relative changes in the pattern of FSH secretion may play a key role in follicle selection during the normal cycle (Baird, 1983; McNeilly et al., 1991). Of particular relevance is the depression in FSH that occurs following luteolysis as the FSH theory states that this depression, caused by increased secretion of oestradiol and inhibin from the dominant follicle (Baird et al., 1991), results in the atresia of non-ovulatory follicles (Baird, 1983). The magnitude of this depression was much less marked in androstenedione-immune ewes than in controls in Exp. 2 and during the second cycle of Exp. 1 when antibody titres were relatively high. The significance of this observation is open to question, however, as during the first cycle of Exp. 1 the magnitude of the follicular phase depression in FSH was similar to that in controls, yet the ovulation rates of immunized ewes were elevated. The fluctuations in peripheral FSH concentrations observed in this and other studies (Miller et al., 1981; Bister & Paquay, 1983) probably reflect fluctuations in ovarian oestradiol and inhibin secretion associated with sequential development and regression of oestrogenic follicles (Sirois & Fortune, 1988; Campbell et al., 1990c). The period between these fluctuations was around 4–6 days, although with a great deal of variation between animals. The synchronization of the FSH trough on Day 3 and the peak on Day 6 is probably due to the induction of atresia in large non-ovulatory follicles by the LH surge (McNatty et al., 1984), with the result that folliculogenesis is well synchronized early in the cycle. Later in the cycle, however, the fluctuations in FSH become less synchronized, presumably reflecting differences in follicle growth rates between animals (Sirois & Fortune, 1988) resulting in a stable mean FSH concentration (Fig. 1). The overall lack of effect of immunization on the luteal-phase fluctuations in FSH would suggest that the normal pattern of follicular growth is maintained in androstenedione-immune ewes despite the development of a greater number of large oestrogenic follicles in the luteal phase (Scaramuzzi & Hoskinson, 1984; Campbell, 1988). However, the negative correlation between antibody titre and the period of the fluctuations suggest that this pattern may be disrupted as the level of immunity increases.

The endocrine effects of immunization against androstenedione are complex and on first examination are difficult to reconcile with our current concepts on the control of LH (Karsch et al., 1984; Martin, 1984) and FSH (Martin et al., 1988; Baird, 1991) secretion by the pituitary. Both the current experiments confirmed our earlier observations that immunization against androstenedione results in increases in peripheral concentrations of progesterone and LH, but not FSH (review: Scaramuzzi & Hoskinson, 1984). It is generally accepted that LH secretion during the luteal phase is controlled by the synergistic action of oestradiol and progesterone (Karsch et al., 1984; Martin, 1984), yet the concentration of progesterone in jugular venous plasma is greatly elevated in androstenedione-immune ewes (Figs 1 and 3). This increase in progesterone concentrations is probably a
consequence of both increased ovarian secretion and decreased metabolic clearance (Campbell et al., 1990a).

Despite the presence of a greater number of large oestrogenic follicles (Scaramuzzi & Hoskinson, 1984; Campbell, 1988) and increased stimulation by LH (Scaramuzzi & Hoskinson, 1984; Campbell et al., 1990a; Figs 1 and 3), immunization against androstenedione does not lead to an increase in ovarian oestradiol secretion, presumably due to immuno-neutralization of androgen precursors for aromatase (Campbell et al., 1990a). We have previously proposed that the apparent discrepancy between circulating steroid concentrations and the level of pituitary LH secretion, in ewes actively immunized against androstenedione, could be explained by interference with negative feedback by cross-reacting antibodies which result in an increase in binding activity for plasma oestradiol and progesterone (Campbell et al., 1990a). In contrast to oestradiol, the secretion of inhibin reflects the increase in the number of follicles, resulting in an elevation in peripheral inhibin concentrations in immunized ewes (Campbell et al., 1990b). This elevation in inhibin concentrations may counteract any interference with negative feedback on FSH, but not LH, caused by immunization, thus explaining the existence of high plasma concentrations of LH and unchanged FSH concentrations in androstenedione-immune ewes. The increase in inhibin secretion in immunized ewes is the probable cause of the significant depression in nadir FSH concentrations seen during the luteal phase in Exp. 1 and the lack of a depression in FSH following luteolysis seen in Exp. 2.

As the concentration of LH in jugular venous plasma is elevated in ewes immunized against androstenedione, it is possible that this increase in LH is responsible for the elevation in ovulation rate. The effect of LH on ovulation rate is uncertain although it is well established that LH plays an important role in the maturation of the preovulatory follicle (Baird & McNeilly, 1981). Attempts to increase ovulation rate by exogenous injection of purified LH have produced equivocal results (Scaramuzzi & Radford, 1983). Generally, prolific breeds of sheep do not have elevated LH concentrations (Cahill et al., 1981; Scaramuzzi & Radford, 1983; Bindon, 1984; Lah lou-Kassi et al., 1984) although increased LH pulse frequency (Thomas et al., 1984) and increased LH pulse amplitude (McNatty et al., 1987) in prolific sheep have been reported, albeit with associated elevated FSH concentrations. Studies of ewes treated with a GnRH agonist have shown no effect of LH alone on follicle populations (Picton et al., 1990; McNeilly et al., 1991). Treatment of such ewes with a combination of FSH and large amplitude LH pulses can, however, result in an inhibition of the stimulatory effects of FSH on follicle growth (Picton, 1989; McNeilly et al., 1991). Treatment of ewes in which follicular development has been suppressed by GnRH-agonist, with antiserum to LH blocks the stimulatory action of FSH on follicle development (Picton, 1989). These data therefore suggest that the role of LH in follicle selection is to modulate the action of FSH and it is unlikely, from the available evidence, that increased LH stimulation per se can lead to increases in ovulation rate.

Over recent years evidence has been accumulating on intraovarian mechanisms that may influence follicle selection (reviews Hsueh et al., 1984; Ireland, 1987). Of particular relevance are the autocrine and paracrine roles of steroid hormones and their interaction with gonadotrophins and growth factors (Hsueh et al., 1984; Scaramuzzi & Campbell, 1990). Immunization against androstenedione alters steroid concentrations in antral fluid (Campbell, 1988) and it is therefore not unreasonable to propose that such immunization may increase ovulation rate through intraovarian mechanisms. This is a possibility worthy of further study.

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