Abstract. During late gestation in the ewe, the pituitary content of LH is reduced by about 95%, presumably due to the presence of high concentrations of ovarian steroids. The aim of this study was to determine whether the pituitary content of LH in the ewe can increase after long-term administration of ovarian steroids, when only estradiol (E) is removed or if both E and progesterone (P) must be withdrawn to allow synthesis of LH to occur. Ten ovariectomized ewes were treated with implants containing E and P. After 3 weeks of treatment, the E implants were removed from 5 ewes (−E+P) and both steroid implants were removed from the remaining 5 ewes (−E−P). Five ovariectomized ewes received P implants at the beginning of the experiment and these implants were left in place for the duration of the study; 5 ovariectomized ewes served as controls (C). All animals were injected with 100 μg GnRH iv 3, 6 and 9 weeks after the initiation of treatment. The area under the LH-response curve was used as an indication of the pituitary content of LH. All steroid treatments markedly reduced basal levels of LH. LH levels increased only in −E−P ewes, beginning 6 weeks after initiation of the study. After 3 weeks, −E+P and −E−P ewes released less LH (P < 0.05) in response to GnRH than did C ewes, whereas P animals did not differ from controls. LH release in response to GnRH in −E+P and −E−P groups had increased by 6 and 9 weeks and was not different from that of C ewes. After 9 weeks, LH release in P ewes was reduced (P < 0.05) compared with C ewes. These data suggest that, after the pituitary content of LH has been suppressed by ovarian steroids, the presence of P alone does not inhibit replenishment of the pituitary content of LH.

Current evidence indicates that the concentration of LH in the pituitary is strictly related to the reproductive status and is modulated by the concentrations of ovarian steroids in serum (Debeljuk et al. 1974; Pelletier & Thimonier 1975; Haresign & Lamming 1978). The high concentrations of these hormones during late gestation, for example, are likely responsible for the marked reduction in the pituitary LH content of ewes observed at this time (Crowder et al. 1982). It has also been shown that chronic treatment of ovariectomized ewes with progesterone (P) and/or estradiol (E) can mimic the endocrine conditions of late pregnancy and can provide a suitable model to investigate the effects of these steroids on gonadotropin secretion (Moss et al. 1981); in addition, it is possible to estimate pituitary content of LH by determining release of LH in response to a challenge with GnRH (Crowder et al. 1982).

Prolonged treatment of ovariectomized ewes with E or E+P depresses release of LH in response to GnRH (Goodman & Karsch 1980; Tamanini et al. 1986). Removal of these steroids allows the replenishment of pituitary stores of LH in 3 to 4 weeks (Tamanini et al. 1986). However, from the data available to date the exact role of P in the inhibition and recovery of pituitary content of LH has not been determined since ewes given P in conjunction with E do not respond differently from ewes treated with E alone (Diekman & Malven 1973; Moss et al. 1981; Tamanini et al.
Materials and Methods

Silastic capsules containing E or P were prepared as described by Karsch et al. (1987) and implanted either subcutaneously (E) or intraperitoneally (P). The experiment was performed during the anestrous season using 20 crossbred ewes which had been ovariectomized for at least 3 months. Five ewes were not treated and served as controls (C), 5 received only P implants and 10 were given both E and P implants. After 3 weeks of treatment, the 10 ewes with both E and P implants, were randomly divided into two groups: E implants were removed from 5 animals (−E+P) and both E and P implants were removed from the remaining 5 ewes (−E−P). The experimental design is depicted in Fig. 1. Three, 6 and 9 weeks after the beginning of the treatment (placement of implants), all the ewes were moved to indoor pens, fitted with jugular cannulas and injected with 100 μg GnRH. Blood samples were collected at 30-min intervals for 6 h beginning 1 h before the GnRH challenge. At the end of sampling, cannulas were removed and the ewes were returned to their outdoor pens. All blood samples were allowed to clot at 4°C overnight and serum was harvested and stored at −20°C until concentrations of LH were measured.

LH assay

Serum concentration of LH was measured by radioimmunoassay (Niswender et al. 1969). All samples from an individual group of ewes were assayed together. The intra-assay coefficient of variation was < 15%. Serum concentrations of LH are expressed in terms of NIH-LH-21.

Analysis of data

The area under the curve of the GnRH-induced release of LH was determined using a Zeiss Videoplan computerized planimeter. The coefficient of variation associated with the measurement was < 10%. Analysis of variance was performed on data previously subjected to log transformation. Differences between means of treatment groups and controls were evaluated using Duncan's new multiple range test.

Fig. 1.

Diagram showing the experimental design. Horizontal bars indicate the length of the hormonal treatments for each group of ewes (N = 5).
Results

The basal levels of LH observed before the injection of GnRH are depicted in Fig. 2. In control ewes, serum LH concentrations averaged 2.8 µg/l; in P-treated ewes they were markedly lower throughout the experiment. In -E+P ewes, basal serum LH concentrations were always very low (about 0.1 µg/l); -E-P animals had low serum LH concentrations 3 weeks after the beginning of the experiment and higher values thereafter with maximum values occurring at the end of the experiment (1.5 ± 0.51 µg/l).

Administration of 100 µg GnRH induced release of LH in all the ewes (Fig. 3). Three weeks after the beginning of treatment, control ewes released more LH than ewes in the -E-P and -E+P groups (241 ± 49.07 relative units vs 42.6 ± 14.9 and 65.4 ± 13.5, respectively, \( P < 0.05 \)). No significant differences were observed between control and P-treated ewes (364.2 ± 91.8). Six weeks after initiation of treatment, GnRH-induced release of LH in -E-P and -E+P ewes was markedly higher than that observed 3 weeks before and was not different from the release observed in control ewes or in ewes treated only with P. Further, it appeared that at 6 weeks after initiation of the experiment, the release of LH in response to GnRH challenge increased more rapidly in -E+P ewes than in -E-P ewes (239.8 µg/l vs 113.2 µg/l, \( P < 0.05 \)).

**Fig. 2.**

Effects of chronic administration of ovarian steroids on basal levels of LH at different times after initiation of treatment as compared with controls (animals without any kind of treatment). Each bar represents mean ± SEM of five ewes. Top panel, ewes treated with P till the end of the experiment; middle panel, ewes given E and P for 3 weeks, then only P for the remaining 6 weeks; bottom panel, ewes treated with E and P only for 3 weeks. In the bottom panel, bars with a double asterisk are different (\( P < 0.05 \)) from the bar with a single asterisk.
± 59.3 vs 115.1 ± 29.3, respectively). At the end of the experiment, LH release from −E−P and −E+P animals (136.4 ± 18.8 and 198.7 ± 26.7 relative units, respectively) was not different from the LH output observed in the same ewes 3 weeks before or in controls. GnRH-induced release of LH (85.4 ± 12.5) in ewes treated only with P for 9 weeks was significantly lower (P < 0.05) than that in either the same group at the beginning of the experiment or in control ewes.

Discussion

High circulating levels of P and E dramatically reduced the content of LH in the pituitary of ovariectomized ewes as estimated by the basal levels of LH and release of LH induced by challenge with GnRH. These results are in agreement with previous data from this laboratory (Moss et al. 1981; Crowder et al. 1982; Tamanini et al. 1986) and other laboratories (Chamley et al. 1974a,b; Jenkin et al. 1977). On the contrary, treatment with P alone for 3 weeks, even though it reduces basal secretion of LH, does not influence pituitary responsiveness to GnRH stimulation (i.e. GnRH-induced release of LH in P-treated ewes is not different from release of LH observed in controls). Therefore, it seems likely that E is responsible for the inhibition of LH secretion and GnRH-induced release of LH after 3 weeks of treatment. Moss et al. (1981) observed that treat-

![Fig. 3. Effects of chronic administration of ovarian steroids on GnRH-induced release of LH in ovariectomized ewes at different times after initiation of treatment as compared with controls (animals without any kind of treatment). Each bar represents mean ± SEM of five ewes. Top panel, ewes treated with P till the end of the experiment; middle panel, ewes given E and P for 3 weeks, then only P for the remaining 6 weeks; bottom panel, ewes treated with E and P only for 3 weeks. Bars with an asterisk are different from control (P < 0.05).](image-url)
ment with P alone for 3 weeks did not change the pituitary content of LH even though circulating levels of this hormone were significantly lowered. The effects of removing either E alone or both E and P are quite similar: in both cases, GnRH-induced release of LH increases and no significant differences are observed in these ewes compared with controls, either 6 or 9 weeks after the beginning of treatment (i.e. 3 or 6 weeks after steroid removal). In -E+P ewes, however, the amount of LH in the pituitary that can be secreted in response to GnRH seems to be restored earlier than in -E−P animals; we presume that the progressive increase in basal levels of LH observed in -E−P ewes after E removal delays the replenishment of LH stores in the pituitary and, consequently, GnRH-induced LH release is reduced (i.e. during this period, LH in the -E−P ewes is released as soon as it is synthesized, thus reducing the rate of accumulation in the anterior pituitary).

The persistence of high circulating levels of P in the -E+P group does not inhibit secretion of LH in response to GnRH and pituitary stores are replenished as early as 3 weeks after withdrawal of E. Unfortunately, the design of the present experiment (particularly regarding the schedule of the challenges with GnRH) does not allow us to establish the minimal interval between removal of E and replenishment of pituitary content of LH, i.e. the time necessary to allow the synthesis of LH to occur after E has been removed. To do this, we should have challenged ewes with GnRH more frequently, but this probably would have overstressed the pituitary and masked the changes observed in this study.

Administration of P alone for more than 6 weeks seems to reduce the ability of the pituitary to secrete LH. In previous studies (Moss et al. 1981; Tamanini et al. 1986), we observed that treatment (3 weeks) with P did not decrease the pituitary content of gonadotrophin. In those experiments, treatment with P started immediately after ovariectomy; in the present study, ewes were ovariectomized at least 3 months before the beginning of the experiment. It is possible that the longer period after ovariectomy could have modified the ability of the pituitary to secrete LH and/or the responsiveness of the pituitary to GnRH challenge. It is more likely, however, that these differences are due to the different period of P administration (3 vs 9 weeks). Normally, the luteal phase of the estrous cycle of the ewe is about 2 weeks. During this period, the pituitary content of LH increases (Roche et al. 1970), similar to that observed during the first 3 weeks after removal of E in the -E+P group, presumably to replace the LH released during the preceding ovulatory surge.

In previous studies, we did not examine the effect of P alone for periods longer than 3 weeks. It seems possible that the prolonged administration of P in this study may have reduced the frequency of GnRH pulses to the point where there was insufficient stimulation of the gonadotrophs for synthesis of LH (Karsch et al. 1987). We cannot explain why treatment with E+P for 3 weeks followed by P alone for 6 weeks did not also result in decreased release of LH after challenge with GnRH at the end of the study. One possibility is that E caused the GnRH-pulse generator to become refractory to P. If this were the case, then these ewes would have been influenced by P for only 6 weeks, a period that did not affect GnRH-induced secretion of LH in ewes treated only with P. A second explanation may be that after a prolonged treatment with P, the pituitary becomes less responsive to GnRH. Indeed, in rats, P has been shown to reduce the number of receptors for GnRH (Clayton & Catt 1981). E, even in the presence of P, can increase the number of receptors for GnRH in the pituitary gland of ewes (Moss et al. 1981; Crowder et al. 1982). Therefore, the pituitary gland of the ewes treated with E and P may have been more responsive to endogenous GnRH (required to increase the content of LH) and to the exogenous challenge we administered to assess pituitary content of LH. In contrast, in those ewes treated only with P, the response of the pituitary to both endogenous and exogenous GnRH would have been less owing to the presence of fewer receptors for GnRH.

We conclude that, after prolonged administration of P and E, 1) basal levels of LH are reduced and increase only if both steroids are removed, and 2) the removal of E allows the synthesis of LH to occur even in the presence of P. Furthermore, treatment with P alone for longer than 6 weeks reduces the pituitary responsiveness to GnRH challenge.

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