Introduction

During the ovine oestrous cycle, prolactin secretion increases throughout the follicular phase, starting after the onset of luteal regression and reaching maximum concentrations at the time of the preovulatory LH surge (Kann and Denamur, 1974; Reeves et al., 1970; Cahill et al., 1981). Whether this increase in prolactin secretion is involved in the patterns of growth and regression of follicle populations remains unknown. Endocrine requirements for growth of different follicular populations have been investigated, but these studies have been restricted mainly to FSH and LH (Scaramuzzi et al., 1993; Cain et al., 1995; Campbell et al., 1995). Prolactin is involved in ovarian function, and has different effects depending on the ovarian compartment, species and physiological status. Most of the established effects of prolactin on ovarian physiology arise from physiological or pathological hyperprolactinaemic states. Prolactin downregulates the synthesis of the LH receptor in ovarian follicles of lactating rabbits (Kermabon et al., 1994), and hyperprolactinaemia interferes with the mechanism of ovulation (Tsai-Morris et al., 1983; Yoshimura et al., 1989) by affecting plasmin generation (Yoshimura et al., 1992, 1994) and decreasing the activity of local proteases involved in tissue remodelling during the periovulatory period (Lin et al., 1988; Murray et al., 1996). Administration of prolactin to prepubertal rats advances the onset of puberty and accelerates follicular growth (Advis et al., 1981; Kawagoe et al., 1989; Peluso, 1989). Prolactin also has profound effects on steroidogenesis and inhibits basal (Jonassen et al., 1991) and stimulated (McNeilly et al., 1991) progesterone production. These effects are mediated by prolactin receptors (CRH-P) which are abundant in the ovarian follicles and corpora lutea. Prolactin enhances the expression of the LH receptor (Berrone et al., 2000) and the expression of genes involved in follicular development, such as the genes for proopiomelanocortin and FSH receptor (Berrone et al., 2000). Prolactin also affects the activity of enzymes involved in steroidogenesis, such as aromatase and 17beta-hydroxysteroid dehydrogenase (Berrone et al., 2000). Prolactin also affects the activity of enzymes involved in steroidogenesis, such as aromatase and 17beta-hydroxysteroid dehydrogenase (Berrone et al., 2000). Prolactin also affects the activity of enzymes involved in steroidogenesis, such as aromatase and 17beta-hydroxysteroid dehydrogenase (Berrone et al., 2000). Prolactin also affects the activity of enzymes involved in steroidogenesis, such as aromatase and 17beta-hydroxysteroid dehydrogenase (Berrone et al., 2000). Prolactin also affects the activity of enzymes involved in steroidogenesis, such as aromatase and 17beta-hydroxysteroid dehydrogenase (Berrone et al., 2000).
et al., 1982; Tsai-Morris et al., 1983) secretion of oestrogens. Administration of bromocriptine to pregnant gilts to suppress prolactin secretion resulted in an increase in the secretion of oestradiol (Szafranska and Ziecik, 1990). Evidence from studies in vitro indicates that prolactin may modulate granulosa cell differentiation in a development-related manner (Veldhuis et al., 1980; Jones et al., 1983). Prolactin, either alone or in combination with hCG, stimulates synthesis of progesterone by increasing the activity of 3β-hydroxysteroid dehydrogenase and 20α-hydroxysteroid dehydrogenase (Yoshida et al., 1987); prolactin also stimulates granulosa cell proliferation when given in combination with FSH (Roy and Greenwald, 1988). However, at high doses, prolactin inhibits progesterone secretion in human (Soto et al., 1985) and rabbit (Lin et al., 1988) granulosa cells. Evidence for a direct effect of prolactin on ovarian follicles requires the presence of specific receptors in granulosa or theca cells. Two forms of prolactin receptor mRNA have been found in rat ovarian granulosa cells, the long and short prolactin receptor. Changes in the localization and relative concentrations of these receptors throughout the oestrous cycle (Clarke et al., 1993; Nagano and Kelly, 1994) indicates that the effects of prolactin on ovarian follicles may differ in relation to the stage of development of the follicle. These two forms of the receptor mRNA have also been detected and quantified in adult sheep ovaries (Anthony et al., 1995); however, to date, cellular localization and development-related expression of prolactin receptors in ewes throughout the oestrous cycle have not been studied. In red deer, prolactin receptor mRNA has been localized in the theca compartment throughout the oestrous cycle and pregnancy (Clarke et al., 1997), indicating that prolactin has a modulatory role in androgen biosynthesis or LH receptor expression in this species. Whether prolactin secretion during the follicular phase of the oestrous cycle is involved in ovarian follicular dynamics has not been studied to date. The use of ultrasonography in sheep has enabled detailed studies of follicular dynamics during the oestrous cycle (Schrick et al., 1990; Souza et al., 1996; and Rawlings et al., 1996). The aim of present study was to determine whether prolactin modulates the patterns of growth and regression of different follicle populations during the follicular phase of the sheep oestrous cycle, by attempting to suppress the secretion of prolactin with 2-bromo-α-ergocryptine, a dopamine D2 receptor agonist, and monitoring follicular dynamics by transrectal ultrasonography. Plasma concentrations of FSH and LH were also determined by frequent sampling to assess the effects of the treatments on FSH and LH secretion.

**Materials and Methods**

**Animals**

Eighteen adult cyclic Spanish Merino ewes aged 5–6 years from an experimental herd (INIA, Madrid) (40°N, 25°), were used. Sheep were kept outside under natural lighting conditions with free access to indoor facilities. Sheep were fed with hay, grass pellets, commercial feed and water ad libitum. The experiments were conducted during the breeding season (November–January) and experimental procedures followed the European Community Council Directive 86/669/CEE (18 February 1986) which regulates the protection of animals used in experimentation. Before the experiments, sheep were weighed to adjust the dose of treatments to the mean weight of the animals (42.3 ± 1.1 kg).

**Drugs**

Cloprostenol (Estrumate™, Pitman-Moore, Friesoythe) and bromocriptine mesylate (D-102, Biomol Research Labs, Inc., Plymouth Meeting, PA) were used. Bromocriptine was weighed out the day before each experiment, solubilized with absolute ethanol at 5 mg ml⁻¹ and diluted further with distilled water to a final concentration of 1 mg ml⁻¹. Aliquots were prepared and stored at −20°C until use.

**Experiment 1**

Eighteen cyclic ewes were used in January. Oestrus was synchronized with two 125 μg cloprostenol injections 10 days apart. Concurrent with the second cloprostenol injection (time 0), ewes were allocated randomly to one of three groups (n = 6 per group) and given one of three treatments. Group 1 (control): ewes received one i.m. injection of vehicle (20% ethanol in distilled water) every 12 h from time 0 to 72 h; group 2 (0.03 mg bromocriptine per kg per day): ewes received one i.m. injection of 0.62 mg bromocriptine mesylate every 12 h from time 0 to 72 h; and group 3 (0.06 mg bromocriptine per kg per day): ewes were given 1.25 mg bromocriptine mesylate every 12 h from time 0 to 72 h. After the second cloprostenol injection, oestrus was detected using vasectomized rams fitted with coloured harnesses. Blood samples (2.5 ml per sample) were collected by jugular venepuncture into heparinized tubes (Vacutainer, Systems Europe, Beckton Dickinson, Cedex) every 3 h from time 0 until 72 h, when the experiment concluded. The first blood sample (time 0) and each sample coincident with the time of treatment was collected immediately before the treatment injection. Samples were collected at 20 min intervals from 38 to 52 h after time 0 to assess the possible effects of the treatments on LH pulse frequency. Blood samples were centrifuged immediately (1200 g at 4°C for 20 min) and plasma was separated and stored at -20°C until hormone analyses.

**Experiment 2**

Eighteen cyclic ewes were used at the end of January. The same schedule of treatments as described in Expt 1 was performed. Blood samples were collected at 4 h intervals from time 0 until 72 h to assess the consistency of the endocrine responses between both experiments. Transrectal ultrasonography was carried out at 12 h intervals starting at time 0 (first scan immediately before administration of the
Follicular dynamics in bromocriptine-treated ewes 179

first injection) using the procedure detailed below. Oestrus was detected every 12 h using vasectomized rams with coloured harnesses, such that each ewe was scanned until oestrus (last scan coincided with oestrus detection). Ovulation rates were estimated by laparoscopy on day 4 of the oestrous cycle.

Ultrasonography

An Aloka 500 SSD ultrasound scanner (Ecotron, Madrid) fitted with a transrectal linear array transducer of 30 cm and 7.5 MHz was used. Scanning was carried out every 12 h according to the procedure described by Schrick et al. (1993). This procedure was validated in this laboratory for Merino ewes by histomorphological methods (González de Bulnes et al., 1994), which gave percentages of detection of 68.7%, 93%, 92.3% and 100% for 2, 3, 4 and > 4 mm follicles, respectively, and was subsequently used to describe follicular dynamics during the oestrous cycle in sheep (López Sebastián et al., 1997) and goats (G. de Bulnes et al., 1999) and during treatment with FSH in ewes (López Sebastián et al., 1999). Each follicle ≥ 2 mm in diameter was recorded, measured and localized topographically on a map-graph of both ovaries. This information provided an individual follow-up of the changes in size of each detectable follicle and enabled identification of newly detected follicles and follicles that had decreased in size or had regressed.

Hormone assays

Prolactin. Plasma samples were analysed in duplicate tubes by radioimmunoassay. Ovine prolactin-I-I iod (AFP-4328C, NHPP, NIDDK, Baltimore, MD) was iodinated using the lactoperoxidase method. The ovine prolactin antiserum (anti-o-prolactin-2, AFP-C358106) was raised in rabbits and used in the assay at a final dilution of 1:2 10^6. The second antibody had been raised in sheep against rabbit immunoglobulins and was added at a final dilution of 1:90. The sensitivity of the assay was 0.1 ng ml^-1, and the intra- and interassay coefficients of variation were < 10%.

LH. Plasma samples were analysed in duplicate tubes by radioimmunoassay using ovine LH-I-2 iod (AFP 7071B, NHPP, NIDDK) iodinated using the lactoperoxidase method. Ovine LH (AFP 7071B, NHPP, NIDDK) was used as a reference standard and an anti-ovine LH-I antiserum (AFP-192279) was raised in rabbits and used at a final dilution of 1:2 10^5. The second antibody was raised in sheep against rabbit immunoglobulins and used at a final dilution of 1:90. The sensitivity of the assay was 0.1 ng ml^-1, and the intra- and interassay coefficients of variation were < 10%.

FSH. FSH concentrations were measured in plasma samples in duplicate tubes using a radioimmunoassay. Ovine FSH-I-SIAFP-21 iod (AFP-7571A, NIDDK, NHPP) was iodinated using the lactoperoxidase method. Ovine FSH-RP-1 (AFP-5679C) was used as a reference preparation, and anti-ovine FSH-I antiserum (AFP-C5288113) was raised in rabbits and used at a final dilution of 1:160 000. The second antibody was raised in sheep against rabbit immunoglobulins and added to the tubes at a final dilution of 1:90. The sensitivity of the assay was 18.5 pg per tube, and the intra- and interassay coefficients of variation were < 10%.

Statistical analyses

Plasma concentrations of prolactin, LH and FSH were analysed by full repeated samples two-way ANOVA to assess the effects of treatment, time and interactions between both factors in each experiment. The time of onset of the preovulatory LH surge, the time of maximum LH secretion in the peak, the duration of the preovulatory LH surge and the maximum LH concentration in the peak were compared by one-way ANOVA to assess the effect of the treatments. A pulse of LH was considered as an increase of greater than 50% over the basal concentration of LH followed by a significant decrease to basal concentrations. Differences among treatments in LH pulse frequency were assessed by ANOVA. Maximum concentrations of LH detected during the period of pulsatile secretion, mean concentrations of LH determined from cloprostenol injection to the onset of the preovulatory LH surge, time of maximum FSH concentrations and maximum FSH concentrations were compared by ANOVA for the effect of treatment.

Measurements of individual follicles were transferred from ovarian graphs to a datasheet, and two different calculations were performed. (i) Total size-class follicular population means: mean number of follicles detected for each individual size class (2–3 mm, 4–5 mm and > 5 mm) at each time point, and mean number of newly detected follicles (≥ 4 mm). (ii) Means of changes in size of individual follicles (≥ 4 mm): means of follicles increasing in size, decreasing in size or remaining stable at each time point in each ewe. These means were calculated for follicular populations detected at time 0 and newly detected follicles 12 h later. The percentage of regressed follicles was calculated as the ratio between the number of regressed follicles and the number of stable and growing follicles for each follicle size class at each time point, and thereafter expressed as a percentage. Follicles with a diameter below the limits of detection of the technique (< 2 mm) were considered to be regressed. The growth and regression rates of individual follicles were calculated as the mean increase or decrease in size (mm) per day, respectively. Data from follicular dynamics were analysed by two-way ANOVA for the effects of time and treatment. Contingency tables and the chi-squared test were used to analyse the effects of treatment and time on the indices of follicular regression. The time of appearance of the ovulatory follicle was also determined in each ewe.

Results

Prolactin

In both experiments, plasma prolactin concentrations were significantly reduced (P < 0.01) throughout the follicular phase, after treatment with bromocriptine. In Expt 1, mean plasma concentrations of prolactin were 14.2 ± 0.3
Differences in prolactin concentrations and LH and FSH levels were analyzed using repeated measures ANOVA. The significance level was set at 0.05. Differences among treatments in the mean time of the preovulatory LH surge were not significant in either of the experiments. In Expt 1, maximum LH concentrations were reached at 53.6 ± 4.3, 57.2 ± 4.2 and 63.8 ± 7.3 h after the second cloprostenol injection in controls and in ewes treated with 0.03 or 0.06 mg bromocriptine per kg per day, respectively. In Expt 2, maximum LH concentrations occurred at 61.6 ± 4.6, 60.0 ± 3.8 and 67.2 ± 2.9 h after time 0 in controls and in ewes treated with 0.03 or 0.06 mg bromocriptine per kg per day, respectively. Mean duration of the preovulatory LH surge was not affected by treatments (Expt 1: 10.1 ± 0.5, 9.9 ± 0.9 and 10.5 ± 1.4 h in controls and in ewes treated with 0.03 mg or 0.06 mg bromocriptine per kg per day, respectively), nor were there differences in the maximum LH concentration in the preovulatory surge (Expt 1: 26.7 ± 4.3, 15.6 ± 0.9 and 19.2 ± 3.1 ng ml⁻¹ in controls and in ewes treated with 0.03 mg or 0.06 mg bromocriptine per kg per day, respectively). In relation to the analysis of LH pulse frequency before the preovulatory LH surge, one ewe treated with 0.03 mg bromocriptine and one ewe treated with 0.06 mg bromocriptine were excluded because they had an LH surge during the period of frequent sampling. Mean pulse frequency was 0.93 ± 0.2 pulses per h in controls, 0.15 ± 0.08 in ewes treated with 0.03 mg bromocriptine (P < 0.05) and 0.3 ± 0.1 in ewes treated with 0.06 mg bromocriptine (P < 0.05) (Fig. 2). Differences between the two groups treated with bromocriptine were not significant. Maximum LH concentrations determined during the period of pulsatile secretion were not affected by treatments (0.51 ± 0.1, 0.40 ± 0.1, 0.54 ± 0.09 ng ml⁻¹ in controls and in ewes treated with 0.03 mg or 0.06 mg bromocriptine per kg per day, respectively). Mean plasma concentrations of LH after the cloprostenol injection to the onset of the preovulatory LH surge were significantly reduced (Expt 1: 26.7 ± 4.3, 15.6 ± 0.9 and 19.2 ± 3.1 ng ml⁻¹ in controls and in ewes treated with 0.03 mg or 0.06 mg bromocriptine per kg per day, respectively) (Fig. 3). Differences between the two groups treated with bromocriptine were not significant. Maximum LH concentrations in Expt 1 (54.0 ± 3.6, 60.5 ± 2.6 and 66 ± 2.4 ng ml⁻¹ in controls and in ewes treated with 0.03 or 0.06 mg bromocriptine per kg per day, respectively) and Expt 2 (60.8 ± 4.4, 60.6 ± 3.6 and 67.2 ± 2.9 ng ml⁻¹ in controls and in ewes treated with 0.03 or 0.06 mg bromocriptine per kg per day, respectively).
Follicular dynamics in bromocriptine-treated ewes

Administration of bromocriptine (0.06 mg per kg per day) reduced ($P < 0.01$) the mean number of 2–3 mm follicles throughout the follicular phase compared with controls and ewes treated with 0.03 mg bromocriptine per kg per day, but there was no significant difference between control ewes and ewes treated with 0.03 mg bromocriptine per kg per day (Fig. 4a). The mean number of 4–5 mm follicles was similar among treatments throughout the follicular phase (Fig. 4b), and there were no differences in the mean number of follicles > 5 mm among treatments. The treatments did not affect the mean number of newly detected follicles, or follicles that were increasing or decreasing in size in the 4–5 mm size class (Table 1) at any stage during the follicular phase, nor did the treatment affect the growth rates (mm per day) of these follicles (Table 2). The overall percentages of regression of 4–5 mm follicles detected at time 0 did not differ among treatments (58.8 ± 11.9, 56.2 ± 12.4 and 75.0 ± 12.5 in controls and in ewes treated with 0.03 or 0.06 mg bromocriptine per kg per day, respectively). However, bromocriptine treatment tended to decrease the percentage of regressed follicles of

Fig. 2. Plasma LH concentrations (ng ml$^{-1}$) in representative ewes from (a) the control groups and from groups treated with (b) 0.03 or (c) 0.06 mg bromocriptine per kg per day during the period of LH pulsatile secretion (A) and during the preovulatory LH surge (B) in Expt 1. Note different scales for A (left) and B (right).
4-5 mm at 12 and 24 h of treatment (Fig. 5). In control ewes, two of four follicles > 5 mm regressed at 36 h after time 0, and the remaining two became ovulatory. Five of the ewes treated with 0.03 mg bromocriptine had a follicle of > 5 mm at time 0. Two of these follicles became ovulatory at 48 h and two at 60 h; the remaining follicle regressed at 60 h. Two of the ewes treated with 0.06 mg bromocriptine had a follicle > 5 mm, and these became ovulatory 60 h after time 0. Ovulation rates did not differ among treatments, and were 1.060.0, 1.160.2, and 1.060.2 in controls and in ewes treated with 0.03 or 0.06 mg bromocriptine per kg per day, respectively.

Discussion

Administration of bromocriptine, a dopamine D2 receptor agonist, during the follicular phase of the ovine oestrous cycle markedly reduced prolactin secretion in a dose-dependent manner. These results are in agreement with studies in which CB-154 was administered to ewes during the oestrous cycle (Niswender, 1974) or in seasonal anoestrus (McNeilly and Land, 1979; Land et al., 1980) and to rams (Barenton et al., 1982; Ravault et al., 1982; Regisford and Katz, 1993). In sheep, dopamine controls prolactin secretion via specific D1 receptors located in the ventromedial hypothalamus (Curlewis et al., 1995; Colthorpe and Curlewis, 1996) and D2 receptors on the pituitary lactotrophs (Curlewis et al., 1993). Therefore, it is assumed that bromocriptine acts primarily on the pituitary gland to inhibit prolactin secretion.

In the present study, neither dose of bromocriptine modified plasma FSH concentrations compared with the control group. These concentrations are similar to those reported by Souza et al. (1997) in Finn-Merino ewes with an ovary autotransplanted to the neck during the follicular phase of the oestrous cycle. Studies in sheep showed no effects of the agonist on FSH plasma concentrations when given to seasonal anoestrous ewes to suppress prolactin (Land et al., 1980) or to Dorset rams in the spring and autumn (Regisford et al., 1995; Colthorpe and Curlewis, 1996).
The results from the present study are in contrast with observations in lactating women (Seki et al., 1974) and in Ile de France rams in the summer (Barenton et al., 1982) and winter (Ravault et al., 1982) in which bromocriptine caused an increase in FSH plasma concentrations. Increased plasma FSH concentrations might be expected as a result of the inhibitory effects of the agonist on LH pulsatile secretion, which would decrease oestradiol secretion leading to augmented plasma FSH concentrations. In contrast, in the present study, bromocriptine did not interfere with the mechanisms by which FSH secretion is decreased during the follicular phase, despite the effects of the agonist on LH pulse frequency. A possible explanation is that in bromocriptine-treated ewes, the reported inhibitory effect of prolactin on oestradiol synthesis (McNeilly et al., 1982; Jonassen et al., 1991) is attenuated as plasma prolactin concentrations are reduced, possibly resulting in oestradiol and FSH secretion similar to that in control ewes.

Data from the current study indicate that bromocriptine has no effect on the preovulatory LH surge, which is consistent with results from Louw et al. (1974) and Niswender et al. (1974). However, bromocriptine reduced LH pulse frequency from 0.9 pulses per h in controls to 0.1 and 0.2 pulses per h in groups treated with 0.03 and 0.06 mg bromocriptine per kg per day, respectively, but did not affect the maximum LH concentrations during the period of pulsatile secretion. Land et al. (1980) found a non-significant reduction in LH pulse frequency, from one pulse every 5 h to one pulse every 8 h after treating seasonally anoestrous ewes with bromocriptine (0.06 mg per kg per day, CB-154).

Table 1. Mean number of newly detected follicles increasing or decreasing in size in the 4–5 mm size class after luteolysis in control and bromocriptine-treated ewes as detected by ultrasonography in Experiment 2

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Newly detected follicles</th>
<th>Follicles increasing in size</th>
<th>Follicles decreasing in size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>B1</td>
<td>B2</td>
</tr>
<tr>
<td>0</td>
<td>2.8 ± 0.3</td>
<td>2.8 ± 0.4</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>12</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.5</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>24</td>
<td>0.6 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>36</td>
<td>0.5 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>48</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>60</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>72</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Time after the second cloprostenol injection.
C: control; B1: 0.03 mg bromocriptine per kg per day; B2: 0.06 mg bromocriptine per kg per day.
Each value represents the mean ± SEM of follicles of each category from the populations detected at time 0 and newly detected 12 h after cloprostenol (*).

Table 2. Mean growth rate and regression rate of follicles (4–5 mm) detected in serial ultrasound scans in ewes in Experiment 2

<table>
<thead>
<tr>
<th>Time after luteolysis (days)</th>
<th>Growth rate (mm per day)</th>
<th>Regression rate (mm per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>B1</td>
</tr>
<tr>
<td>1</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>1.2 ± 0.2</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
</tr>
</tbody>
</table>

Follicle populations detected at time 0 (A) and 12 h later (B).
C: control; B1: 0.03 mg bromocriptine per kg per day; B2: 0.06 mg bromocriptine per kg per day.

and Katz, 1993). The results from the present study are in contrast with observations in lactating women (Seki et al., 1974) and in Ile de France rams in the summer (Barenton et al., 1982) and winter (Ravault et al., 1982) in which bromocriptine caused an increase in FSH plasma concentrations. Increased plasma FSH concentrations might be expected as a result of the inhibitory effects of the agonist on LH pulsatile secretion, which would decrease oestradiol secretion leading to augmented plasma FSH concentrations. In contrast, in the present study, bromocriptine did not interfere with the mechanisms by which FSH secretion is decreased during the follicular phase, despite the effects of the agonist on LH pulse frequency. A possible explanation is that in bromocriptine-treated ewes, the reported inhibitory effect of prolactin on oestradiol synthesis (McNeilly et al., 1982; Jonassen et al., 1991) is attenuated as plasma prolactin concentrations are reduced, possibly resulting in oestradiol and FSH secretion similar to that in control ewes.

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reported a dose-dependent decrease in LH pulse frequency in cyclic ewes infused with dopamine, but there was no effect on the preovulatory LH surge. The effects of the D2 receptor agonist on pulsatile LH secretion may be primarily by direct action on the hypothalamic GnRH pulse generator. With regard to this hypothesis, in sheep, there is evidence of synapses between dopamine and LHRH-containing neurones (Kuljis and Advis, 1989), although it is not known whether sheep LHRH cells express the D2 receptor, or how specific bromocriptine is for this receptor subtype. Experiments using GnRH cell lines reveal that these cells only respond to dopamine via the D1 receptor, and that bromocriptine does not stimulate these receptors (Martinez de la Escalera et al., 1992). Alternatively, bromocriptine might act primarily on the D2 receptors in the pituitary lactotrophs to inhibit prolactin, and the reduction in pulsatile LH secretion occurs as a result. This hypothesis indicates that during the follicular phase, prolactin may modulate GnRH-induced LH secretion from the gonadotrophs in a positive manner. This process requires a pituitary cell–cell contact between gonadotrophs and lactotrophs as has been described by Soji et al. (1992) and the presence of prolactin receptors in gonadotrophs as reported in rats (Morel et al., 1994) and sheep (Tortonese et al., 1998). In pituitary cell cultures from other species, prolactin has been shown to increase GnRH-induced LH secretion (Oguchi et al., 1997). The potential role of prolactin as modulator of GnRH pulse frequency should not be excluded, as prolactin receptor is expressed in sheep hypothalamic areas (Tortonese et al., 1996), which are involved in the control of GnRH secretion (Thiery et al., 1989).

Results of follicular dynamics should be interpreted with caution. Firstly, in ewes treated with 0.06 mg bromocriptine per kg per day, some residual prolactin secretion remained (0.7–1.7 ng ml\(^{-1}\)) which may have exerted a physiological effect. Secondly, bromocriptine modified both prolactin and pulsatile LH secretion. However, the results indicate a direct effect of decreased plasma prolactin concentrations on the population of 2–3 mm follicles. The mean number of follicles showed a bromocriptine dose-dependent reduction compared with control values, which appears to be correlated with the dose-dependent decrease of prolactin secretion, rather than with the similar changes in LH secretion induced by both doses of bromocriptine. If the effects of the agonist on number of 2–3 mm follicles were due to the altered LH pulsatile secretion, these effects should be similar in ewes treated with 0.06 and 0.03 mg bromocriptine per kg per day. In addition, it is not known whether FSH and LH are essential for continued development of these follicles (Scaramuzzi et al., 1993). A positive relationship has been shown between the circannual rhythm of prolactin secretion in ewes and the number of 2–3 mm follicles (McNatty et al., 1984), which increases during seasonal anoestrus and decreases in the transition from anoestrus to the breeding season, as revealed by ultrasonography (Ravindra and Rawlings, 1997). The number and growth patterns of gonadotrophin-dependent (4–5 mm) and ovulatory (> 5 mm) follicles were similar in control and bromocriptine-treated ewes. These follicles showed normal growth and regression patterns, coincident with a reduced pulsatile LH secretion, decreased prolactin concentrations and FSH concentrations characteristic of the follicular phase. Growth and regression profiles of these follicles were unaffected by the absence of normal pulsatile LH secretion. Pulsatile LH exposure is not essential for gonadotrophin-dependent follicles (Picton et al., 1990; McNeill et al., 1992; Scaramuzzi et al., 1993); however, these follicles are dependent on FSH, although their precise requirements are unknown (Scaramuzzi et al., 1993).

In conclusion, prolactin secretion during the follicular phase of the sheep oestrous cycle may participate in maintaining the viability of gonadotrophin-responsive follicles, which are to be recruited into the pool of gonadotrophin-dependent follicles, and may also modulate pulsatile LH secretion. Experiments involving the simultaneous administration of bromocriptine and prolactin to ewes during the follicular phase of the oestrous cycle, and monitoring LH secretion and follicular dynamics, will confirm whether the reported effects are the result of prolactin deprivation.

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Fig. 5. Time course profile of regression from 0 to 72 h after induction of luteolysis of 4–5 mm follicles detected at time 0 in Expt 2. Ewes were treated with 0.03 (●) or 0.06 (○) mg bromocriptine per kg per day; ○, control ewes. Each value represents the mean ± SEM of percentages of regressed follicles in each ewe (n = 6 per treatment) at each time point. Half error bar removed for clarity.
Follicle dynamics in bromocriptine-treated ewes

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