Effects of bromocriptine-induced hypoprolactinaemia on gonadotrophin secretion and testicular function in rams (Ovis aries) during two seasons

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The influence of low circulating concentrations of prolactin on gonadotrophin and testosterone secretion, sperm production and testicular growth was investigated in rams during two different seasons. Treatment of Dorset rams (n = 23) with bromocriptine (4 mg day\(^{-1}\)) during the spring (n = 11) and autumn (n = 12) caused a significant decrease in basal, mean and total serum prolactin concentrations (P < 0.01). In spring, serum prolactin concentrations returned to pretreatment values, one week after the termination of treatment. Basal, mean and total serum concentrations of LH were significantly higher in treated rams than in controls during the treatment period in autumn (P < 0.05). Secretion of LH was not affected by bromocriptine treatment during spring. There were no differences in the secretion of FSH between treated and control rams in either season. Serum concentrations of testosterone were significantly lower in treated rams than in control rams during the treatment period in autumn (P < 0.05) but not during spring. Semen volume from treated rams was significantly lower during the period after treatment in autumn (P < 0.05). Scrotal circumference decreased during both seasons in treated animals, but this change in size was significant only during spring (P < 0.05). Conversely, there was an increase in scrotal circumference in control rams during both seasons. It is concluded that prolactin may (i) affect LH secretion and, (ii) influence testicular function in rams, by directly affecting testosterone and semen production during autumn, and retarding testicular growth in spring.

Introduction

Sheep are seasonal breeders and the annual cycle of alternating periods of reproductive activity and inactivity is controlled by photoperiod (Lincoln and Short, 1980). Decreasing daylength or short days are accompanied by increased release of pituitary gonadotrophins and marked testicular recrudescence, leading to enhanced testosterone production, spermatogenesis and pronounced testicular growth in rams (Ovis aries). Alternatively, as daylength becomes progressively longer, there is a decrease in gonadotrophin and testosterone secretion, sperm production and scrotal size, and subsequently testicular atrophy (Lincoln and Davidson, 1977; Mickelson et al., 1982; Karsch et al., 1984; Langford et al., 1987). The secretion of prolactin from the anterior pituitary also undergoes seasonal variation in rams. Long photoperiods are marked by high circulating prolactin concentrations, while low prolactin concentrations characterize short photoperiods (Pelletier 1973; Lincoln et al., 1978; Howles et al., 1980; Langford et al., 1987).

The regulation of testicular activity by gonadotrophins has been well documented in rams (Sanford et al., 1977). The two basic functions of the testes, steroidogenesis and the production of spermatozoa, are controlled by LH and FSH, respectively (Aman and Scharbacher, 1983). The increased secretion of LH from the pituitary gland stimulates the secretion of testosterone from the Leydig cells of the testes. Spermatogenesis in the seminiferous tubules is augmented by the stimulatory action of FSH on the Sertoli cells, resulting in increased testicular size. These two processes in the testes are, however, closely related. Increases in serum testosterone concentrations are correlated with increases in LH pulse frequency (Sanford et al., 1978) and mean FSH concentrations (Langford et al., 1987) during short days. Furthermore, sperm production and testicular size were also reported to increase at the onset of the breeding season when gonadotrophins and testosterone concentrations were high (Langford et al., 1987). Pituitary prolactin secretion is mainly controlled by hypothalamic factors. Dopamine, an ergot alkaloid, is a prolactin-secreting inhibitory factor (Weiner and Betha, 1981). Evidence suggests that prolactin may influence changes in gonadotrophin secretion and testicular function in rams. Ravault et al. (1982) reported that treatment of hyperprolactinaemic rams with bromocriptine, a dopamine D-2 receptor agonist, resulted in a decrease in prolactin concentrations, and decreased LH and testosterone mean pulse frequency. Increases in circulating concentrations of FSH occurred concomitantly with a decrease in circulating prolactin concentrations during natural photoperiods in summer (Barenton and Pelletier, 1982) and winter (Ravault et al., 1982). Furthermore, induced hypoprolactinaemia during the long photoperiod caused a delay in testicular redevelopment in rams during the subsequent short photoperiod (Barenton and Pelletier, 1980; Sanford and Dickson, 1981).

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1980), suggesting that the increase in prolactin concentration during long photoperiods is required to prime the testes for redevelopment during short days (Sanford and Dickson, 1980; Howles et al., 1982; Sanford et al., 1984a,b). However, dopamine can affect GnRH secretion and consequently LH and FSH secretion (Koike et al., 1991). The secretion of β-endorphins (Seewannyan and Lincoln, 1990) and α-melanocyte stimulating hormone (α-MSH) (Pan et al., 1992) are also regulated via dopaminergic neurones. Furthermore, bromocriptine was reported to inhibit effectively the expression of pro-opiomelanocortin (POMC) in a human small cell lung cancer cell line, that carries the POMC gene (Farrell et al., 1992). These factors, regulated by dopamine, may directly or indirectly affect reproduction in rams via effects on prolactin secretion or activity.

Although prolactin may play an important role in the maintenance of reproduction in the ram, this role has not been fully elucidated. Other studies have focused on reducing circulating prolactin concentrations in hyperprolactinaemic, sexually inactive rams in short term treatments (Barenton et al., 1982; Ravault et al., 1982) or in rams over a long period (Sanford and Dickson, 1980). This study was therefore performed to determine the effects of bromocriptine-induced hypoprolactinaemia on gonadotrophin secretion, the exocrine and endocrine functions of the testes, and on testicular growth in rams, during autumn, when prolactin concentrations are low and sexual activity is at its peak, and during spring, when prolactin concentrations are increasing to values normally observed during summer, the nadir of the sexual cycle. It is established that seasonal changes in prolactin secretion, in response to changes in photoperiod, are accompanied by reproductive physiological adaptations. In some breeds of sheep, for example the Dorset breed, the seasonal change in prolactin secretion is evident, but there is a lesser degree of seasonality in reproductive activity (D’Occhio et al., 1984). Determination of the effects of hypoprolactinaemia on reproduction in this breed, which is relatively insensitive to seasonal changes, may identify a role for prolactin in more seasonally responsive breeds and in non-seasonal animals or those with clinical disorders of prolactin secretion.

### Materials and Methods

**Animals and experimental procedure**

Sexually mature (aged 6–11 months), Dorset rams (n = 23) were maintained under natural light and temperature conditions as daylength became increasingly longer (February–March 1991, spring; n = 11) and as daylength became increasingly shorter (August–October, 1991, autumn; n = 12). Rams were kept together throughout the study, at the Experimental Station Farm in New Brunswick, NJ (40°28’ latitude). The rams weighed 80–110 kg and were fed a 14% protein diet consisting of ground corn/soybean meal mix. The diet was supplemented with vitamins and minerals and water was available *ad libitum*. Rams were involved in reproductive behaviour studies performed with oestrous ewes once a week. The initiation of treatment occurred exactly two months after the winter and summer solstices, respectively, when rams were assigned to either a treatment or control group. During 30 days, the treated group of rams (n = 6 per season) was given s.c. injections of 4 mg 2-bromo-α-ergocriptine (Bromocriptine: Sandoz Pharmaceuticals, East Hanover, NJ; 2 mg twice a day) dissolved in ethanol and 0.9% NaCl (60:40 v/v) as described by Ravault et al. (1977). Control rams (n = 5 in spring; n = 6 in autumn) received vehicle only.

Rams were subjected to intensive blood sampling once a week before (pretreatment: 1 week), during (treatment: 4 weeks) and after (post-treatment: 1 week) the initiation of treatment. Polyethylene catheters (Intramedic, i.d. 0.58 mm × o.d. 0.965 mm) pretreated with heparin complex (7% w/w; TDMAC, Warrington, PA) were inserted into the jugular vein of each ram. During the sampling period, 5 ml of blood was collected at 15 min intervals for 6 h beginning at 09:00 h. Blood samples were allowed to clot overnight at 4°C and then centrifuged at 1800 g for 20 min at 4°C on the following day. Serum was then harvested and stored at −20°C for later hormone analyses.

Scrotal circumference was measured by palpating the testes to the bottom of the scrotum and measuring the greatest circumference with a flexible nylon tape. Semen samples were collected from rams by electroejaculation once a week before (pretreatment: 2 weeks), during (treatment: 4 weeks) and after (post-treatment: 6 weeks) treatment. The accessory glands were stimulated with an electroejaculator (Bailey Western Instrument Company, 4950 York Street, Denver, CO 80216) every 3 s for 2 min, while a container equipped with a piece of latex rubber, was held over the penis. The samples were then placed in a waterbath kept at a constant temperature (37°C) until they were returned to the laboratory. In the laboratory, semen samples were diluted in 2.9% sodium citrate dihydrate solution (J. T. Baker, Chemical Co., Phillipsburg, NJ). The concentration of spermatozoa was measured with a spectrophotometer (Beckman DU-64) at 550 nm and the percentage of transmittance measured (Salisbury et al., 1943). Values were calculated by extrapolation to a standard curve, by plotting log spermatozoa versus percentage of transmittance.

**Hormone assays**

Serum concentrations of ovine prolactin, oLH and oFSH were measured by homologous double-antibody radioimmunoassays. The values for o-prolactin, oLH and oFSH were expressed in terms of NIDDK-oPRL-1-2, NIADDK-oLH-1-3 and NIADDK-oFSH-1, respectively. The aforementioned antigens were iodinated with Na125I by the chloramine T method of Greenwood and Hunter (1963). The antibodies against these pituitary hormones were raised in rabbits (NIDDK-anti-oPRL-2; NIADDK-anti-oLH-1 and NIDDK-anti-oFSH-1) and used at a final tube dilution of 1:600 000 for prolactin, 1:700 000 for LH and 1:100 000 for FSH. Bound antigen was separated from free antigen by adding sheep anti-rabbit gamma-globulin at a dilution of 1:20 with 5% (w/v) polyethylene glycol (molecular mass 8 kDa).

**Prolactin.** NIDDK-oPRL-2 (AFP-7150B) was used as a reference standard. Assay sensitivity (calculated as 95% confidence limit of buffer control tubes) was 0.50 ± 0.01 ng ml⁻¹. The intra-assay coefficients of variation (CV) were 8 and 7.5%.
and the interassay CVs were 10 and 8.5% at mean prolactin concentrations of 254 and 18 ng ml⁻¹, respectively.

**Luteinizing hormone.** The reference standard was NIADDK-oLH-I-3 (AFP-9598B) and the assay sensitivity was 0.9 ± 0.1 ng ml⁻¹. The intra-assay CVs were 6 and 7% and interassay CVs were 10 and 9% at mean LH concentrations of 5.8 and 0.43 ng ml⁻¹, respectively.

**Follicle-stimulating hormone.** NIADDK-oFSH-1 (AFP5679C) was used as a reference standard. The assay sensitivity was 1.00 ± 0.01 ng ml⁻¹. Intra-assay CVs were 11 and 7.3% and interassay CVs were 7.3 and 6.8% at mean FSH concentrations of 1.24 and 0.83 ng ml⁻¹, respectively.

**Testosterone.** Serum concentrations of testosterone were measured by radioimmunoassay following extraction with 10 volumes of methylene chloride (J. T. Baker Inc., Phillipsburg, NJ). Recovery of the steroid was 80%. Antiserum raised in rabbits was provided by A. L. Johnson (The University of Notre Dame, IN) and used at an initial dilution of 1:18,000. Crossreactivity with 5α-dihydrotestosterone (DHT) was 50% but was less than 1% for other steroid hormones. Bound and unbound testosterone were separated by dextran-coated charcoal (10% w/w) and bound testosterone was measured by determining the amount of radioactivity using a scintillation counter. The assay sensitivity was 50 ± 0.01 pg ml⁻¹. Intra-assay CVs were 9 and 10% and interassay CVs were 10 and 10.2% at testosterone concentrations of 63 and 581 pg ml⁻¹, respectively.

**Data and statistical analyses**

Mean hormone concentrations were obtained by averaging the values for all 25 samples taken during the 6 h sampling period for each animal. Total hormone secretion was determined by triangulation of the area under the response curve. A peak was determined as a point that was the mean ± 2 SD higher than the previous point, followed by two or more declining values (Sanford et al., 1984b). Hormone peak frequency was expressed as the total number of peaks that occurred during the 6 h sampling period. Peak amplitude was determined by the highest hormone concentration associated with a peak minus the hormone concentration at the onset of the peak. Basal hormone concentrations were determined by averaging the concentration of the samples that were not characterized as a peak (Xu et al., 1992).

Measures of serum prolactin, LH, FSH and testosterone concentrations and semen characteristics were averaged for experimental periods: pretreatment, before the initiation of bromocriptine administration; treatment, during bromocriptine administration; and post-treatment, after the termination of bromocriptine treatment. The means were subjected to analysis, using general linear model procedures (SAS, 1988). The main effects of the model were treatment (bromocriptine-treated and control), season (spring and autumn), and experimental period (pretreatment, treatment and post-treatment). Differences owing to season were detected; and data were therefore further analysed within each season. Specific differences in scrotal circumference between treatment groups were determined by paired comparison t test (SAS, 1988) within each season.

**Results**

**Spring**

**Prolactin.** Bromocriptine treatment caused a significant decrease in mean (62.1 ± 15.0 μg ml⁻¹ in 6 h) and total (168.9 ± 10.2 ng ml⁻¹ in 6 h) serum concentrations of prolactin to treatment values of 1.3 ± 0.01 ng ml⁻¹ and 0.5 ± 0.01 μg ml⁻¹ in 6 h, respectively (P < 0.01). One week after the termination of treatment, mean and total concentrations of prolactin in serum returned to pretreatment values, and did not differ from values in control rams (Fig. 1a, b). Basal prolactin concentrations and peak amplitude values were significantly lower in treated rams than in control rams during treatment (P < 0.01). There was no difference in basal prolactin
Table 1. Basal hormone concentrations, peak frequency and peak amplitude interval in bromocriptine-treated and control rams during pretreatment, treatment and post-treatment experimental periods in spring

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Treatment</th>
<th>Experimental period</th>
<th>Basal concentration (ng ml⁻¹)</th>
<th>Peak frequency (peaks in 6 h)</th>
<th>Peak amplitude (ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin</td>
<td>Bromocriptine</td>
<td>PRE</td>
<td>159.94 ± 9.35</td>
<td>0.83 ± 0.21</td>
<td>178.09 ± 98.40</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>159.74 ± 13.04</td>
<td>0.40 ± 0.25</td>
<td>293.70 ± 26.29</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>TRT</td>
<td>1.29 ± 0.03*</td>
<td>0.95 ± 0.18</td>
<td>1.05 ± 0.20*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>POST</td>
<td>94.99 ± 3.1</td>
<td>0.90 ± 0.20</td>
<td>209.53 ± 56.99</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>POST</td>
<td>150.62 ± 12.1</td>
<td>0.50 ± 0.40</td>
<td>212.90 ± 182.1*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>150.99 ± 7.7</td>
<td>0.60 ± 0.20</td>
<td>803.57 ± 424.3</td>
</tr>
<tr>
<td>LH</td>
<td>Bromocriptine</td>
<td>PRE</td>
<td>0.71 ± 0.06</td>
<td>0.67 ± 0.20</td>
<td>1.57 ± 1.05</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.61 ± 0.70</td>
<td>1.00 ± 0.00</td>
<td>2.06 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>TRT</td>
<td>0.63 ± 0.02</td>
<td>0.71 ± 0.10</td>
<td>1.00 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>POST</td>
<td>0.76 ± 0.04</td>
<td>1.00 ± 0.15</td>
<td>1.33 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>POST</td>
<td>0.84 ± 0.02</td>
<td>0.83 ± 0.31</td>
<td>0.82 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.84 ± 0.08</td>
<td>0.40 ± 0.24</td>
<td>1.19 ± 0.64</td>
</tr>
<tr>
<td>FSH</td>
<td>Bromocriptine</td>
<td>PRE</td>
<td>0.72 ± 0.02</td>
<td>0.60 ± 0.25</td>
<td>0.58 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.81 ± 0.02</td>
<td>0.80 ± 0.20</td>
<td>0.52 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>TRT</td>
<td>0.83 ± 0.01</td>
<td>0.79 ± 0.14</td>
<td>0.75 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>POST</td>
<td>1.63 ± 0.43</td>
<td>0.70 ± 0.15</td>
<td>0.72 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>POST</td>
<td>0.81 ± 0.02</td>
<td>0.33 ± 0.22*</td>
<td>0.18 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>1.17 ± 0.27</td>
<td>1.20 ± 0.20</td>
<td>0.59 ± 0.21</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Bromocriptine</td>
<td>PRE</td>
<td>0.171 ± 0.03</td>
<td>0.50 ± 0.22</td>
<td>5.99 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.195 ± 0.03</td>
<td>0.60 ± 0.25</td>
<td>7.17 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>TRT</td>
<td>0.127 ± 0.01</td>
<td>0.39 ± 0.10</td>
<td>2.08 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>POST</td>
<td>0.122 ± 0.01</td>
<td>0.50 ± 0.11</td>
<td>4.00 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>POST</td>
<td>0.116 ± 0.01</td>
<td>0.17 ± 0.17</td>
<td>6.43 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.126 ± 0.01</td>
<td>0.20 ± 0.20</td>
<td>1.10 ± 0.00</td>
</tr>
</tbody>
</table>

Values presented are means ± SEM.
*Means are significantly different between treatment groups within an experimental period (P < 0.05).
PRE: period before the initiation of bromocriptine treatment (one week); TRT: period during bromocriptine administration (four weeks); and POST: period after the termination of bromocriptine treatment (one week).

Concentrations between treatment groups during the pretreatment and post-treatment experimental periods. Peak frequency and peak intervals were not different between treatment groups at any time during the experimental period (Table 1).

Luteinizing hormone. There was no significant difference in serum LH concentration between treated and control rams (Fig. 1c, d). Peak amplitude and peak frequency did not differ between treatment groups (Table 1). As photoperiod increased there was no difference in serum concentrations of LH in either treated or control rams.

Follicle-stimulating hormone. Serum concentrations of FSH were not different between treatment groups throughout the experimental period. No change was observed in FSH concentration as photoperiod increased (Fig. 1e, f). Patterns of FSH secretion were also similar between treatment groups during the pretreatment and treatment experimental periods. However, peak frequency was lower in treated rams than in control rams during the post-treatment experimental period (P < 0.05; Table 1).

Testosterone. There was a decrease in serum testosterone concentration in both treated and control rams as the photoperiod increased (P < 0.05; Fig. 1g). However, there was no significant difference in mean and total serum concentrations (Fig. 1g, h) or patterns of hormone secretion between bromocriptine and control rams (Table 1).

Semen characteristics. No treatment differences in semen volume, sperm concentration and total number of spermatozoa collected were observed in rams throughout the experiment (Fig. 2). There was an increase in semen volume and total spermatozoa harvested in both groups of rams during treatment, but values returned to pretreatment values in all rams, during the post-treatment experimental period.

Scrotal circumference. The change in scrotal circumference was significantly different from zero in bromocriptine-treated rams (Fig. 3). Mean scrotal circumference decreased from 31.1 to 29.3 cm in treated rams, while there was a slight increase in testes size (from 30.8 to 31.0 cm) in control rams.

Autumn

Prolactin. As photoperiod decreased, mean serum concentrations of prolactin also decreased from 157.5 ng ml⁻¹ to 50.0 ng ml⁻¹ in all animals (P < 0.01). During the treatment period
Fig. 2. Semen characteristics: (a) semen volume; (b) sperm concentration and (c) total spermatozoa harvested of rams treated with (■) bromocriptine (n = 6) or (□) ethanol–saline vehicle (n = 5) for 30 days during spring. Values are means ± SEM.

period, prolactin concentrations were significantly lower in treated than in control rams. Concentrations of prolactin remained significantly lower in treated rams than in control rams during the post-treatment period (Fig. 4a, b). There was no difference in peak frequency between treatment groups, but basal and peak amplitude values were significantly higher in control rams during the treatment and post-treatment experimental periods (Table 2).

Luteinizing hormone. There was no effect of bromocriptine treatment on LH secretion. However, mean serum concentrations of LH were significantly lower in control rams than in treated rams in the treatment period (P < 0.05; Fig. 4c, d). Total LH release was slightly lower in control animals during the same treatment period (P < 0.06; Fig. 4d). There was no treatment difference in peak frequency or peak amplitude (P > 0.05; Table 2).

Follicle-stimulating hormone. Serum concentrations and the secretory patterns of FSH were not different between treatment groups at any time during the experimental period (Fig. 4e, f; Table 2).

Testosterone. Mean and total serum concentrations of testosterone were lower (P < 0.05) in bromocriptine-treated rams than in control rams during treatment (Fig. 4g, h). However, there was no treatment effect on the patterns of testosterone secretion (Table 2).

Semen characteristics. Semen volume was significantly lower in treated rams after the termination of bromocriptine administration (P < 0.05; Fig. 5). Sperm concentration and total semen production were highly variable among the rams and hence no treatment differences were detected.

Scrotal circumference. There was an increase in testis size in control rams, while there was a slight decrease in size in the bromocriptine-treated rams during treatment. However, neither of these changes was significantly different from zero (P > 0.05; Fig. 3).

Discussion

The study reported here determined the effect of bromocriptine-induced hypoprolactinaemia on gonadotrophin secretion and testicular function in rams during two different seasons. In general, serum prolactin concentrations were higher during spring than in autumn (85.79 ng ml⁻¹ versus 60.19 ng ml⁻¹). Ravault and Ortavant (1977) observed that as the photoperiod decreased, there was a corresponding decrease in circulating prolactin concentrations. This observation was confirmed by our studies. Furthermore, we observed that bromocriptine

Fig. 3. Changes in scrotal circumference in rams treated with (■) 4 mg bromocriptine (n = 6 each season) or (□) ethanol–saline vehicle (n = 5 in spring; n = 6 in autumn) for 30 days during autumn and spring. Measurements were taken at the beginning and end of treatment. Values are mean differences ± SEM. *Mean is significantly different from zero (P < 0.05).
inhibited prolactin secretion during spring and autumn. This finding is consistent with results obtained by Ravault et al. (1977), Barenton and Pelletier (1980), Barenton et al. (1982) and Ravault et al. (1982). The recovery period for increased prolactin secretion was found in this study to be shorter in rams treated during spring than during autumn. It is possible that upon termination of bromocriptine treatment, dopamine, an inhibitor of prolactin secretion (Weiner and Bethea, 1981), was released, resulting in the continued depression of prolactin secretion. Furthermore, other factors, such as high ambient temperature and relative humidity (Neill, 1970; Raud et al., 1971) may augment the photoperiodic effect (Pelletier, 1973) on prolactin secretion during spring, resulting in an immediate increase in prolactin secretion after withdrawal of bromocriptine. However, Jackson and Jansen (1991) reported that in ewes the circannual rhythm of serum prolactin concentrations persisted under constant temperature conditions. Furthermore, rams kept at natural ambient temperatures with alternating periods of different photoperiod had a serum prolactin cycle coincident with that of photoperiod and not of temperature (Lincoln, 1979).

The secretion of LH and FSH was slightly higher during the autumn than in the spring (0.84 versus 0.72 ng ml\(^{-1}\) for LH and 1.04 versus 0.88 ng ml\(^{-1}\) for FSH), confirming previous reports that photoperiod affects the secretion of gonadotrophins (Schanbacher and Ford, 1976; Lincoln and Davidson, 1977; Lincoln and Short, 1980). Exposure of rams to short days induced increases in circulating concentrations of gonadotrophins and a decrease in prolactin secretion. Subsequent exposure of these animals to long days resulted in a decrease in gonadotrophins and an increase in prolactin (Lincoln et al., 1978; Langford et al., 1987). This inverse relationship between gonadotrophins and prolactin secretion was also observed in female (Ben-David et al., 1971; Huang et al., 1978) and male (Hodson et al., 1980) rats. Conversely, other investigators have found no correlation between prolactin and gonadotrophin secretion in humans (Rjost and Schill, 1979) and rams (Lincoln, 1990).

In our study, there were no prolactin-related changes in FSH secretion in spring or autumn. This finding is consistent with
Table 2. Basal hormone concentrations, peak frequency and peak amplitude in bromocriptine-treated and control rams during pretreatment, treatment and post-treatment experimental periods in autumn

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Treatment</th>
<th>Experimental period</th>
<th>Basal concentration (ng ml⁻¹)</th>
<th>Peak frequency (peaks in 6 h)</th>
<th>Peak amplitude (ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin</td>
<td>Bromocriptine</td>
<td>PRE</td>
<td>130.91 ± 9.5</td>
<td>0.50 ± 0.25</td>
<td>225.07 ± 69.75</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>171.06 ± 8.5</td>
<td>0.67 ± 0.21</td>
<td>103.73 ± 20.07</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>TRT</td>
<td>1.8 ± 0.05*</td>
<td>0.79 ± 0.10</td>
<td>2.18 ± 0.84*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>75.25 ± 2.70</td>
<td>0.58 ± 0.10</td>
<td>137.54 ± 38.6</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>POST</td>
<td>11.77 ± 1.6*</td>
<td>0.67 ± 0.21</td>
<td>17.98 ± 15.59*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>80.70 ± 5.65</td>
<td>0.50 ± 0.34</td>
<td>124.45 ± 29.53</td>
</tr>
<tr>
<td>LH</td>
<td>Bromocriptine</td>
<td>PRE</td>
<td>0.93 ± 0.05</td>
<td>1.33 ± 0.21</td>
<td>1.51 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.95 ± 0.08</td>
<td>1.17 ± 0.17</td>
<td>2.80 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>TRT</td>
<td>0.96 ± 0.03*</td>
<td>1.21 ± 0.17</td>
<td>1.55 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.50 ± 0.02</td>
<td>1.04 ± 0.13</td>
<td>1.39 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>POST</td>
<td>0.89 ± 0.06</td>
<td>0.83 ± 0.31</td>
<td>1.91 ± 1.53</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>1.04 ± 0.25</td>
<td>0.83 ± 0.31</td>
<td>1.73 ± 1.01</td>
</tr>
<tr>
<td>FSH</td>
<td>Bromocriptine</td>
<td>PRE</td>
<td>0.96 ± 0.04</td>
<td>1.17 ± 0.31</td>
<td>1.06 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>1.01 ± 0.05</td>
<td>1.67 ± 0.17</td>
<td>1.14 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>TRT</td>
<td>1.02 ± 0.02</td>
<td>0.63 ± 0.13</td>
<td>2.06 ± 1.63</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>1.01 ± 0.02</td>
<td>0.71 ± 0.14</td>
<td>0.98 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
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<td>1.12 ± 0.04</td>
<td>0.83 ± 0.31</td>
<td>0.84 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>1.09 ± 0.16</td>
<td>0.50 ± 0.22</td>
<td>3.77 ± 3.15</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Bromocriptine</td>
<td>PRE</td>
<td>0.207 ± 0.01</td>
<td>1.00 ± 0.36</td>
<td>5.72 ± 2.91</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.187 ± 0.01</td>
<td>0.83 ± 0.31</td>
<td>2.82 ± 4.65</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>TRT</td>
<td>0.100 ± 0.01</td>
<td>0.63 ± 0.11</td>
<td>2.02 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.151 ± 0.01</td>
<td>0.38 ± 0.11</td>
<td>3.02 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>Bromocriptine</td>
<td>POST</td>
<td>0.150 ± 0.01</td>
<td>0.67 ± 0.21</td>
<td>2.62 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>0.150 ± 0.01</td>
<td>0.33 ± 0.21</td>
<td>2.90 ± 0.82</td>
</tr>
</tbody>
</table>

Values presented are means ± SEM.
*Means are significantly different between treatment groups within an experimental period (P < 0.05).
PRE: period before the initiation of bromocriptine treatment (one week); TRT: period during bromocriptine administration (four weeks); and POST: period after the termination of bromocriptine treatment (one week).

reports that treatment of ewes (Land et al., 1980) and male lambs (Ravault et al., 1977) with bromocriptine does not affect FSH secretion. Inhibition of prolactin secretion by bromocriptine resulted in a decrease in FSH secretion in men (Lackritz and Bartke, 1980), an increase in women (Seki et al., 1974) and hyperprolactinaemic rams (Barenton et al., 1982; Ravault et al., 1982). Furthermore, Sanford and Dickson (1980) determined that the seasonal increase in FSH secretion was delayed in bromocriptine-treated rams while temporal changes in LH secretion occurred normally. The latter studies suggest that the effect of prolactin on FSH secretion in rams may depend on season, but this was not supported by our findings.

In the present study, a decrease in prolactin secretion was associated with an increase in LH secretion. In contrast, other investigators reported that induced hypoprolactinaemia in rams had no effect on LH secretion (Ravault et al., 1977; Ohlson et al., 1981; Barenton et al., 1982) in any season. However, there is evidence indicating that prolactin inhibits LH secretion in certain endocrine states in other species (Meites et al., 1972; Park and Selmanoff, 1991), for example, hyperprolactinaemia caused a decrease in LH secretion in male rats (Smith and Bartke, 1987). The inhibitory effect of prolactin on LH secretion may be via a central action; high concentrations of prolactin caused an increase in dopamine turnover in the medial basal hypothalamus, and a decrease in GnRH secretion from the hypothalamus and, consequently, LH release from the pituitary (Gudelsky et al., 1976; Moulton et al., 1982; Koike et al., 1991).

In this study, treatment with bromocriptine and the resulting decrease in prolactin secretion was correlated with a decrease in concentrations of testosterone in serum and semen volume in autumn. Scrotal circumference of bromocriptine-treated rams decreased during both seasons, but the reduction was more marked in the spring. Testicular function is affected by both LH and FSH (Aamann and Schanbacher, 1983); testosterone production, spermatogenesis and semen production, and testicular growth are therefore optimized during the breeding season, when photoperiod is short (Lincoln and Davidson, 1977; Sanford et al., 1978; Karsch et al., 1984; Lincoln, 1990). In some species, prolactin is an important component in the complex of physiological and environmental factors that influence testicular function (Bartke, 1971; Hafez et al., 1972; Bex et al., 1978). In rams, circulating prolactin concentration is inversely related to testosterone production and testes growth (Lincoln et al., 1978; Barenton and Pelletier, 1980; Poulton and Robinson, 1987). Other investigators have postulated that the rise in prolactin during long photoperiods is necessary for the timely onset of...
testicular growth and activity during the short photoperiod (Barenton and Pelletier, 1980; Barenton et al., 1982; Howles et al., 1982; Ravault et al., 1982).

The observations in this study support the hypothesis that there is a positive relationship between prolactin secretion and testicular growth (Sanford and Dickson, 1980; Ohlson et al., 1981), and testosterone production in rams (Yarney and Sanford, 1989). Furthermore, we observed a decrease in testosterone production with a concomitant increase in LH secretion when prolactin secretion was inhibited. Normal physiological serum concentrations of prolactin may be important in regulating testosterone secretion in rams during the breeding season. It has been documented that prolactin affects testosterone production via the maintenance and stimulation of testicular LH receptors in mice (Takase et al., 1990) and in golden hamsters (Klemcke et al., 1984), or by regulating specific enzymatic steps in androgen biosynthesis (Chandrashekar and Bartke, 1988). The exact mechanism whereby prolactin affects testosterone production in rams needs further study.

Bartke (1971) reported that prolactin and LH acted synergistically to restore spermatogenesis in rats. In lambs, prolactin may also be necessary for normal growth and secretory activity of the vesicular glands (Ravault et al., 1977). Semen volume was affected in our study following bromocriptine, in contrast to observations reported for men, in whom semen volume was not influenced by bromocriptine (Eggett-Kruse et al., 1991). Our observation suggests that the accessory glands may have been affected by induced hypoprolactinaemia in the autumn. However, our data do not suggest that prolactin directly affects sperm production as observed in men (Aiman et al., 1988).

However, this study does not rule out the possibility that the dopamine agonist had effects on the reproductive axis other than via inhibition of prolactin secretion. As previously mentioned, dopamine may affect GnRH secretion, which may in turn affect testicular function. Martinez et al. (1992) reported that dopamine directly stimulated GnRH secretion via pharmacologically characteristic D2 dopamine receptors on GT1 GnRH cell lines. However, bromocriptine, the dopamine D2 receptor agonist, had no effect on GnRH secretion. This suggests that in our study bromocriptine selectively inhibited prolactin secretion via dopamine D2 receptors.

In summary, the present study has shown that prolactin may be one part of the complex multifaceted system that regulates reproduction in rams. Evidence that the effects of reduced prolactin secretion on LH and testicular function are more marked during the autumn suggests that the pituitary testicular axis may be more sensitive to abnormal changes in the secretion of prolactin at this time in the annual reproductive cycle. These studies support the hypothesis that there is a synergistic relationship between prolactin and LH on Leydig cell function in rams. Future studies must involve prolactin replacement in bromocriptine-treated animals or prolactin inactivation by immunoneutralization or other methods, to determine the direct effect of prolactin on reproduction in rams.

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