Journal of Reproduction and Fertility (1993) **99**, 529–537

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

E. G. C. Regisford and L. S. Katz*

Department of Animal Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903-0231, USA

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 (Amann and Schanbacher, 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 Bethea, 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 *et al.*, 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,

*Correspondence.
Received 6 January 1993.

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 (Ssewannyana 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-I-2, NIADDK-oLH-I-3 and NIDDK-oFSH-I-1, respectively. The aforementioned antigens were iodinated with $Na^{125}I$ 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. NIDDK-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.

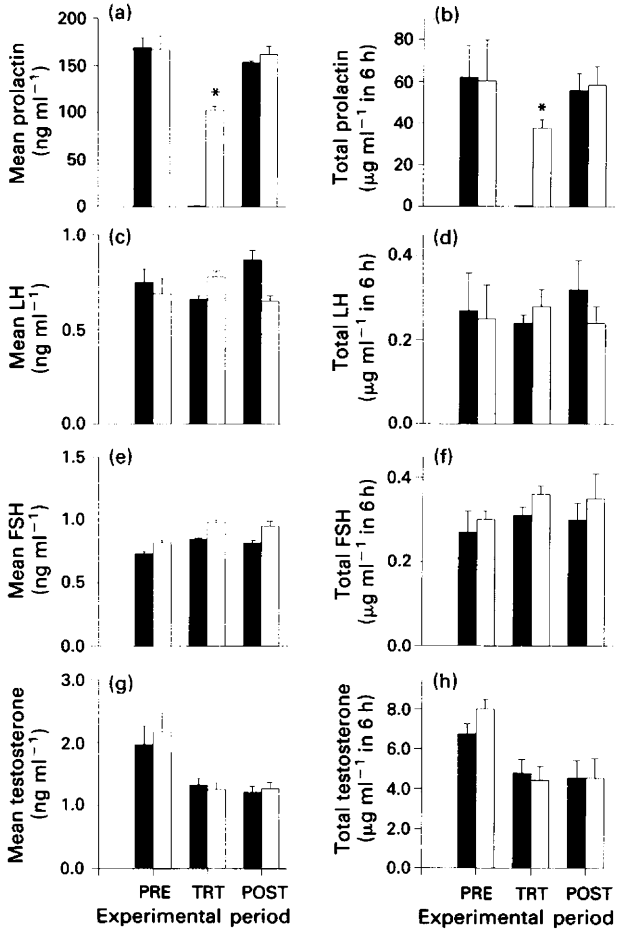

Fig. 1. Mean and total serum concentrations of (a) and (b) prolactin, (c) and (d) LH, (e) and (f) FSH and (g) and (h) testosterone, before (PRE), during (TRT) and after (POST) the treatment period, in rams (n = 11) treated with (■) bromocriptine (n = 6) or (□) ethanol–saline vehicle (n = 5) for 30 days in spring. Values are mean ± SEM. *Means are significantly different between treated and control rams within each experimental period ($P < 0.05$).

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

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

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

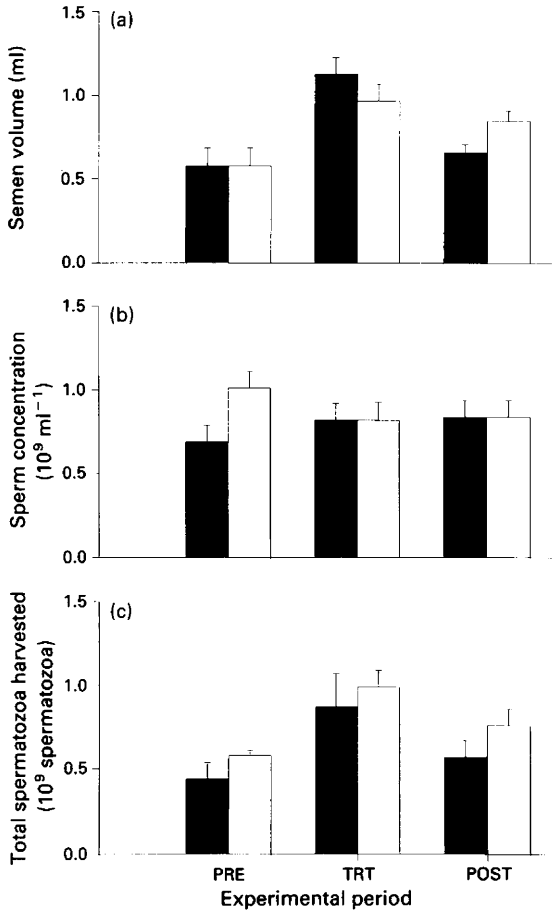

Fig. 2. Semen characteristics: (a) semen volume; (b) sperm concentration and (c) total spermatozoa harvested of rams treated with (■) bromocriptine ($n = 6$) or (□) ethanol–saline vehicle ($n = 5$) for 30 days during spring. Values are means ± SEM.

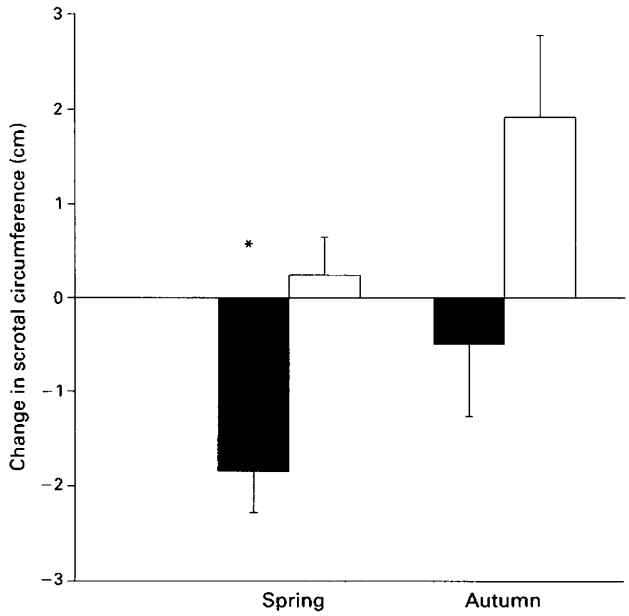

Fig. 3. Changes in scrotal circumference in rams treated with (■) 4 mg bromocriptine ($n = 6$ each season) or (□) ethanol–saline vehicle ($n = 5$ in spring; $n = 6$ in autumn) for 30 days during autumn and spring. Measurements were taken at the beginning and end of treatment. Values are mean differences ± SEM. *Mean is significantly different from zero ($P < 0.05$).

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

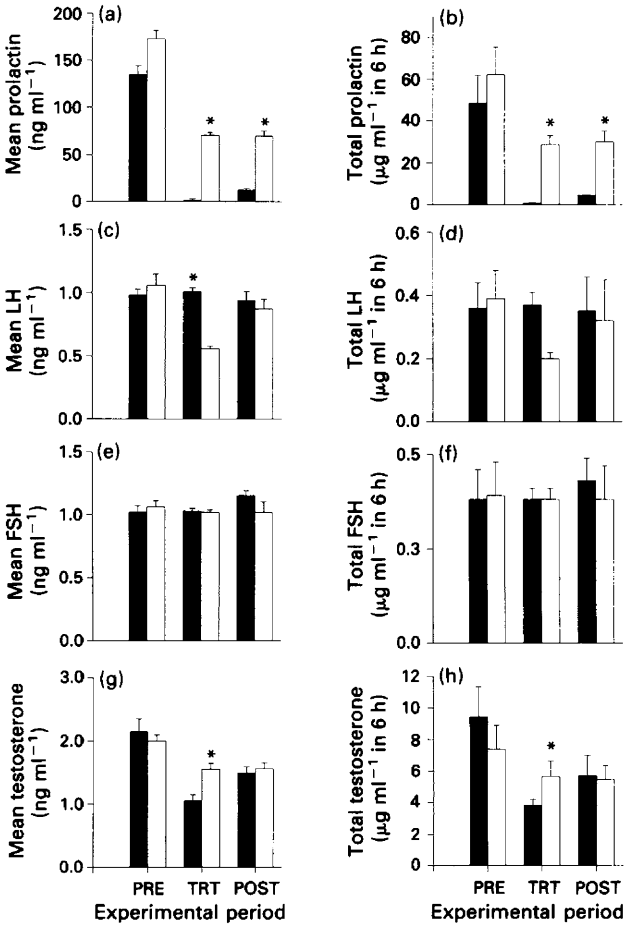

Fig. 4. Mean and total serum concentrations of (a) and (b) prolactin, (c) and (d) LH, (e) and (f) FSH and (g) and (h) testosterone, before (PRE), during (TRT) and after (POST) the treatment period, in rams ($n = 12$) treated with (■) bromocriptine ($n = 6$) or (□) ethanol–saline vehicle for 30 days in autumn. Values are means ± SEM. *Means are significantly different between treated and control rams within each experimental period ($P < 0.05$).

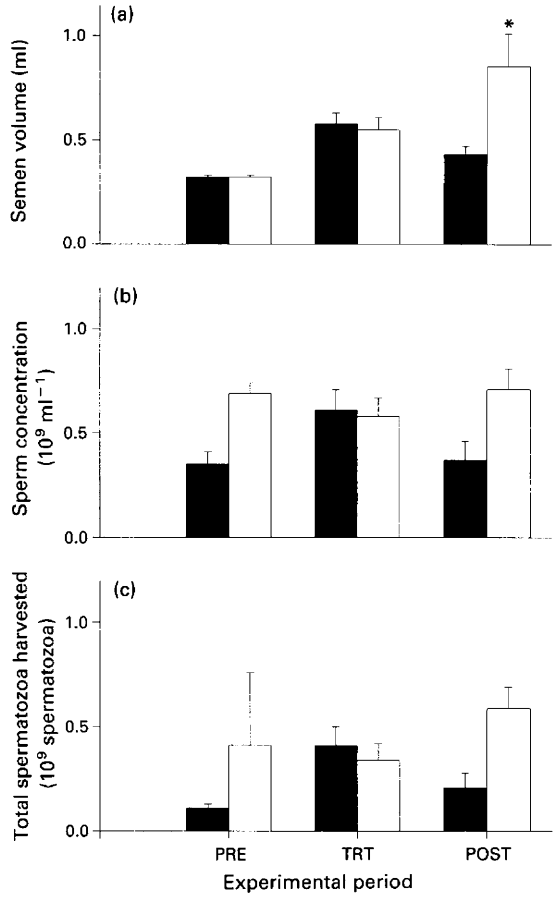

Fig. 5. Semen characteristics: (a) semen volume; (b) sperm concentration and (c) total spermatozoa harvested from rams treated with (■) bromocriptine ($n = 6$) or (□) ethanol–saline vehicle ($n = 6$) for 30 days during autumn. Values are means ± SEM. *Means are significantly different between treated and control rams within an experimental period ($P < 0.05$).

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 (Rjosk 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

Hormone	Treatment	Experimental period	Basal concentration (ng ml^{-1})	Peak frequency (peaks in 6 h)	Peak amplitude (ng ml^{-1})
Prolactin	Bromocriptine		130.91 ± 9.5	0.50 ± 0.25	225.07 ± 69.75
	Control	PRE	171.06 ± 8.5	0.67 ± 0.21	103.73 ± 20.07
	Bromocriptine		1.8 ± 0.05*	0.79 ± 0.10	2.18 ± 0.84*
	Control	TRT	75.25 ± 2.70	0.58 ± 0.10	137.54 ± 38.6
	Bromocriptine		11.77 ± 1.6*	0.67 ± 0.21	17.98 ± 15.59*
	Control	POST	80.70 ± 5.65	0.50 ± 0.34	124.45 ± 29.53
LH	Bromocriptine		0.93 ± 0.05	1.33 ± 0.21	1.51 ± 0.31
	Control	PRE	0.95 ± 0.08	1.17 ± 0.17	2.80 ± 0.87
	Bromocriptine		0.96 ± 0.03*	1.21 ± 0.17	1.55 ± 0.23
	Control	TRT	0.50 ± 0.02	1.04 ± 0.13	1.39 ± 0.19
	Bromocriptine		0.89 ± 0.06	0.83 ± 0.31	1.91 ± 1.53
	Control	POST	1.04 ± 0.25	0.83 ± 0.31	1.73 ± 1.01
FSH	Bromocriptine		0.96 ± 0.04	1.17 ± 0.31	1.06 ± 0.86
	Control	PRE	1.01 ± 0.05	1.67 ± 0.17	1.14 ± 0.49
	Bromocriptine		1.02 ± 0.02	0.63 ± 0.10	2.06 ± 1.56
	Control	TRT	1.01 ± 0.02	0.71 ± 0.14	0.98 ± 0.28
	Bromocriptine		1.12 ± 0.04	0.83 ± 0.31	0.84 ± 0.39
	Control	POST	1.09 ± 0.16	0.50 ± 0.22	3.77 ± 3.15
Testosterone	Bromocriptine		0.207 ± 0.01	1.00 ± 0.36	5.72 ± 2.91
	Control	PRE	0.187 ± 0.01	0.83 ± 0.31	2.82 ± 4.65
	Bromocriptine		0.100 ± 0.01	0.63 ± 0.11	2.02 ± 0.34
	Control	TRT	0.151 ± 0.01	0.38 ± 0.11	3.02 ± 0.83
	Bromocriptine		0.150 ± 0.01	0.67 ± 0.21	2.62 ± 0.62
	Control	POST	0.150 ± 0.01	0.33 ± 0.21	2.90 ± 0.82

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; Moult *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 (Amann 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; Hafiez *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 (Eggert-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 D_1-dopamine receptors on GT_1 GnRH cell lines. However, bromocriptine, the dopamine D_2 receptor agonist, had no effect on GnRH secretion. This suggests that in our study bromocriptine selectively inhibited prolactin secretion via dopamine D_2 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.

This research was supported by the New Jersey Agricultural Experiment Station (publication D-06117-01-93). The authors are grateful to the National Hormone and Pituitary Program and the NIDDK of the NIH for purified antigens and antisera used in the radioimmunoassays. The bromocriptine was generously provided by the Sandoz Research Institute (East Hanover, NJ). The authors are also grateful to M. Schwartz for typing the manuscript, B. Bilney, K. Bodden, C. Croney, C. Horbacewicz and J. Wendolowski for help with blood sample collection and radioimmunoassays.

References

Aiman J, McAsey M and Harms L (1988) Serum and seminal plasma prolactin concentrations in men with normospermia, oligospermia or azoospermia *Fertility and Sterility* **49** 133–137

Amann RP and Schanbacher BD (1983) Physiology of male reproduction *Journal of Animal Science* **57** 380–403

Barenton B and Pelletier J (1980) Prolactin, testicular growth and LH receptors in the ram following light and 2-Br-α-ergocryptine (CB-154) treatments *Biology of Reproduction* **22** 781–790

Barenton B and Hochereau-de Reviers MT, Perreau C and Poirier JC (1982) Effects of induced hypoprolactinemia in the ram: plasma gonadotrophin levels, LH and FSH receptors and histology of the testis *Reproduction, Nutrition and Development* **22** 621–630

Bartke A (1971) Effects of prolactin on spermatogenesis in hypophysectomized mice *Journal of Endocrinology* **49** 311–316

Ben-David M, Danon A and Sulman FG (1971) Evidence of antagonism between prolactin and gonadotrophin secretion: effect of methallibure or perphenazine-induced prolactin secretion in ovariectomized rats *Endocrinology* **51** 719–725

Bex FJ, Bartke A, Goldman BD and Dalterio S (1978) Prolactin, growth hormone, luteinizing hormone receptors and seasonal changes in testicular activity in the golden hamster *Endocrinology* **103** 2069–2080

Chandrashekar V and Bartke A (1988) Influence of endogenous prolactin on the luteinizing hormone stimulation of testicular steroidogenesis and the role of prolactin in adult male rats *Steroids* **51** 559–576

D'Occhio MJ, Schanbacher BD and Kinder JE (1984) Profiles of luteinizing hormone, follicle stimulating hormone, testosterone and prolactin in rams of diverse breeds: effects of contrasting short (8L:16D) and long (16L:8D) photoperiods *Biology of Reproduction* **30** 1039–1054

Eggert-Kruse W, Schwalbach B, Gerhard I, Tilgen W and Runnebaum B (1991) Influence of serum prolactin on semen characteristics and sperm function *International Journal of Fertility* **36** 243–251

Farrell WE, Clark AJL, Stewart MF, Crosby SR and White A (1992) Bromocriptine inhibits pro-opiomelanocortin mRNA and ACTH precursor secretion in small cell lung cancer cell lines *Journal of Chemical Investigation* **90** 705–710

Gudelsky GA, Simpkins J, Mueller GP, Meites J and Moore KE (1976) Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH *Endocrinology* **22** 206–215

Greenwood FC and Hunter WM (1963) The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity *Biochemical Journal* **89** 114–123

Hafiez AA, Lloyd CW and Bartke A (1972) The role of prolactin in the regulation of testis function: the effects of PRL and LH on the plasma levels of testosterone and androstenedione in hypophysectomized rats *Journal of Endocrinology* **52** 327–332

Hodson CA, Simpkins JW, Pass KA, Aylsworth CF, Steger RW and Meites J (1980) Effects of a prolactin-secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in male rats *Neuroendocrinology* **30** 7–10

Howles CM, Webster GM and Haynes NB (1980) The effect of rearing under a long or short photoperiod on testis growth, plasma testosterone and prolactin concentrations and the development of sexual behavior in rams *Journal of Reproduction and Fertility* **60** 437–447

Howles CM, Craigon J and Haynes NB (1982) Long-term rhythms of testicular volume and plasma prolactin concentrations in rams reared for 3 years in constant photoperiod *Journal of Reproduction and Fertility* **65** 439–446

Huang HH, Steger RW, Bruni JF and Meites J (1978) Patterns of sex steroid and gonadotropin secretion in aging female rats *Endocrinology* **103** 1855–1859

Jackson GL and Jansen HT (1991) Persistence of a circannual rhythm of plasma prolactin concentration in ewes exposed to a constant equatorial photoperiod *Biology of Reproduction* **44** 469–475

Karsch RJ, Bittman EL, Foster DL, Goodman RL, Legan SJ and Robinson JE (1984) Neuroendocrine basis of seasonal reproduction *Recent Progress in Hormone Research* **40** 185–232

Klemcke HG, Bartke A and Borer KT (1984) Regulation of testicular prolactin and luteinizing hormone receptors in golden hamsters *Endocrinology* **114** 594–603

Koike K, Miyake A, Aono T, Sakumoti T, Ohmichi M, Yomaguchio M and Tanizawa O (1991) Effect of prolactin on the secretion of hypothalamic GnRH and pituitary gonadotropins *Hormone Research* **35** 5–12

Lackritz RM and Bartke A (1980) The effect of prolactin on the androgen response to human chorionic gonadotropin in normal men *Fertility and Sterility* **34** 140–143

Land RB, Carr WR, McNeilly AS and Preece RD (1980) Plasma FSH, LH and positive feedback of oestrogen, ovulation and luteal function in the ewe given bromocriptine to suppress prolactin during seasonal anoestrus *Journal of Reproduction and Fertility* **59** 73–78

Langford GA, Ainsworth L, Marcus GJ and Shiestha JNB (1987) Photoperiod entrainment of testosterone, luteinizing hormone, follicle-stimulating hormone, and prolactin cycles in rams in relation to testis size and semen quality *Biology of Reproduction* **37** 489–499

Lincoln GA (1979) Light-induced rhythms of prolactin secretion in the ram and the effect of cranial sympathectomy *Acta Endocrinologica* **91** 421–427

Lincoln GA (1990) Correlation with changes in horns and pelage, but not reproduction, of seasonal cycles in the secretion of prolactin in rams of wild, feral and domesticated breeds of sheep *Journal of Reproduction and Fertility* **90**, 285–296

Lincoln GA and Davidson W (1977) The relationship between sexual and aggressive behaviour, and pituitary and testicular activity during the seasonal sexual cycle of rams, and the influence of photoperiod *Journal of Reproduction and Fertility* **49** 267–276

Lincoln GA and Short RV (1980) Seasonal breeding: nature's contraceptive *Recent Progress in Hormone Research* **36** 1–52

Lincoln GA, McNeilly AS and Cameron CL (1978) The effects of a sudden decrease or increase in daylength on prolactin secretion in the ram *Journal of Reproduction and Fertility* **52** 305–311

Martinez de la Escalera G, Gallo F, Choi ALH and Weiner RI (1992) Dopaminergic regulation of the GT_1 gonadotropin-releasing hormone (GnRH) neuronal cell lines: stimulation of GnRH release via D_1-receptors positively coupled to adenylate cyclase *Endocrinology* **131** 2965–2971

Meites J, Lu KH, Wuttke W, Welsch CW, Nagawasa A and Quadri SK (1972) Recent studies on functions and control of prolactin secretion in rats *Recent Progress in Hormone Research* **38** 471–526

Mickelson WD, Paisley LG and Dahmen JJ (1982) Seasonal variations in scrotal circumferences, sperm quality and sexual ability in rams *Journal of the American Veterinary Medical Association* **181** 376–380

Moult PJA, Rees LH and Besser GM (1982) Pulsatile gonadotropin secretion in hyperprolactinemic amenorrhea and the response to bromocriptine therapy *Clinical Endocrinology* **16** 153–162

Neill JF (1970) Effect of 'stress' on serum prolactin and luteinizing hormone levels during the estrous cycle of the rat *Endocrinology* **87** 1192–1197

Ohlson DL, Spicer LJ and Davis SL (1981) Use of active immunization against prolactin to study the influence of prolactin on growth and reproduction in the ram *Journal of Animal Science* **52** 1350–1359

Pan J-T, Tian Y, Lookingland KJ and Moore KE (1992) Neurotensin-induced activation of hypothalamic dopaminergic neurons is accompanied by a decrease in pituitary secretion of prolactin and α-melanocyte-stimulating hormone *Life Sciences* **50** 2011–2017

Park S-K and Selmanoff M (1991) Dose-dependent suppression of post-castration luteinizing hormone secretion exerted by exogenous prolactin administration in male rats: a model for studying hyperprolactinemic hypogonadism *Neuroendocrinology* **53** 404–410

Pelletier J (1973) Evidence for photoperiodic control of prolactin release in rams *Journal of Reproduction and Fertility* **35** 143–147

Poulton AL and Robinson TJ (1987) The response of rams and ewes of three breeds to artificial photoperiod *Journal of Reproduction and Fertility* **79** 609–626

Raud HR, Kiddy CA and Odell WD (1971) The effect of stress upon the determination of serum prolactin by radioimmunoasay *Proceedings of the Society of Experimental Biology and Medicine* **136** 689–693

Ravault JP and Ortavant R (1977) Light control of prolactin secretion in sheep. Evidence for a photoinducible phase during a diurnal rhythm *Annales de Biologie, Animale Biochimie et Biophysique* **17** 459–473

Ravault JP, Courot M, Garnier D, Pelletier J and Terqui M (1977) Effect of 2-bromo-α-ergocryptine (CB-154) on plasma prolactin, LH and testosterone levels, accessory reproductive glands and spermatogenesis in lambs during puberty *Biology of Reproduction* **17** 192–197

Ravault JP, Barenton B, Blanc M, Daveau A, Garnier DH, Ortavant R, Pelletier I, de Reviers M-M and Terqui M (1982) Influence of 2-Br-α-ergocryptine (CB154) on the secretion of prolactin, LH, FSH and testosterone and on testicular growth in rams subjected to different photoperiods *Reproduction, Nutrition and Development* **22** 989–998

Rjosk HK and Schill WB (1979) Serum prolactin in male infertility *Andrologia* **11** 297–304

Salisbury GW, Beck GH, Elliot I and Willett EL (1943) Rapid methods for estimating the number of spermatozoa in bull semen *Journal of Dairy Science* **26** 69–78

Sanford LM and Dickson KA (1980) Seasonality in reproductive processes in rams with suppressed prolactin secretion *Fertility and Sterility* **34** 192–193

Sanford LM, Palmer WM and Howland BE (1977) Changes in the profiles of serum LH, FSH and testosterone and in mating performance and ejaculate volume in the ram during the ovine breeding season *Journal of Animal Science* **45** 1382–1391

Sanford LM, Beaton BD, Howland BE and Palmer WM (1978) Photoperiod-induced changes in LH, FSH, prolactin and testosterone secretion in the ram *Canadian Journal of Animal Science* **58** 123–128

Sanford LM, Howland BE and Palmer WM (1984a) Seasonal changes in the endocrine responsiveness of the pituitary and testes of male sheep in relation to their patterns of gonadotropic hormone and testosterone secretion *Canadian Journal of Physiology and Pharmacology* **62** 827–833

Sanford LM, Howland BE and Palmer WM (1984b) Seasonal changes in the secretion of gonadotropic hormones and in the endocrine response of the pituitary of male sheep in the absence of gonadal influence *Canadian Journal of Physiology and Pharmacology* **62** 834–839

SAS (1988) *SAS Statistics User's Guide* (Release 6.03 Edition) SAS Institute, Inc., Cary, NC

Schanbacher BD and Ford JJ (1976) Seasonal profiles of plasma luteinizing hormone, testosterone and estradiol in the ram *Endocrinology* **99** 752–757

Seki K, Seki M and Omura T (1974) Serum FSH rise induced by CB-154 in post-partum women *Journal of Clinical Endocrinology and Metabolism* **39** 206–208

Smith SM and Bartke A (1987) Effects of hyperprolactinemia on the control of luteinizing hormone and follicle stimulating hormone secretion in the male rat *Biology of Reproduction* **36** 138–147

Ssewannyana E and Lincoln GA (1990) Regulation of the photoperiod-induced cycle in the peripheral blood concentrations of β-endorphin and prolactin in the ram: role of dopamine and endogenous opioids *Journal of Endocrinology* **127** 461–469

Takase M, Tsutsui K and Kawashima S (1990) Effects of prolactin and bromocriptine on the regulation of testicular luteinizing hormone receptors in mice *Journal of Experimental Zoology* **256** 200–209

Weiner RI and Bethea CI (1981) Hypothalamic control of prolactin secretion. In *Prolactin* pp 19–26 Ed. R-B Jaffe. Elsevier, New York

Yarney TA and Sanford LM (1989) Pubertal changes in the secretion of gonadotropic hormones, testicular gonadotropic receptors and testicular function in the ram *Domestic Animal Endocrinology* **6** 219–229

Xu ZZ, McDonald SN, McCutcheon and Blair HT (1992) Effect of season and testosterone treatment on gonadotrophin secretion and pituitary responsiveness to gonadotrophin-releasing hormone in castrated Romney and Dorset rams *Journal of Reproduction and Fertility* **95** 183–190