Effect of hypoprolactinaemia and hyperprolactinaemia on LH secretion, endocrine function of testes and structure of seminiferous tubules in boars

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The influence of exogenous prolactin-induced hyperprolactinaemia and bromocriptine-induced hypoprolactinaemia on LH secretion, endocrine function of testes and the structure of seminiferous tubules was investigated in boars. Treatment with exogenous pig prolactin for 21 days ($0.07 \text{ mg kg}^{-1} \text{ day}^{-1}$; $n = 5$) caused a significant increase in mean prolactin concentrations during the experiment ($P < 0.05$) and during 4 h sampling ($P < 0.01$). Treatment with bromocriptine ($0.2 \text{ mg kg}^{-1} \text{ per os}$ and $0.05 \text{ mg kg}^{-1} \text{ i.m.}$; $n = 4$) decreased mean prolactin concentrations throughout the experiment as well as during two 4 h sampling periods ($P < 0.01$). Treatment with exogenous prolactin decreased mean LH concentrations during the treatment ($P < 0.01$) and during the first 4 h sampling period ($P < 0.05$), but did not affect the number of pulses, pulse frequency or amplitude. Bromocriptine did not affect LH concentrations. Prolactin increased testosterone concentrations during the treatment ($P < 0.01$), while boars treated with bromocriptine had lower testosterone concentrations ($P < 0.05$) than did controls. Both exogenous prolactin and bromocriptine treatments significantly decreased oestradiol concentrations during the course of the experiment. The injections of prolactin and administration of bromocriptine caused a decrease in LH/hCG receptor concentration ($P < 0.05$) in the cell membrane of testes, without causing a change in binding affinity. The bromocriptine treatment caused premature release of some spermatocytes and the presence of polynucleate cells in the seminiferous tubules. In the seminiferous epithelium of hyperprolactinaemic boars, more mature cells (mainly spermatids) were released into the lumen. In tubules in which many polynucleate cells were found, considerable regression of seminiferous epithelium occurred. It is concluded that both hyper- and hypoprolactinaemia disrupt testicular endocrine function and change the process of spermatogenesis in boars. Hyperprolactinaemia has a greater effect on the testes and may decrease the secretion of LH.

Introduction

Prolactin plays an important role in the regulation of testicular function in rodents (Bartke and Dalterio, 1976; Bartke *et al.*, 1977). The physiological role of prolactin in male laboratory mammals includes the regulation of growth and normal function of all tissues sensitive to androgens. Prolactin receptors have been found in Leydig cells of the testes, epididymides and accessory glands (Barkey *et al.*, 1977; Bouhdira *et al.*, 1989). Prolactin, together with LH and growth hormone (GH), controls the production and maintenance of the LH receptors in the testes (Zipf *et al.*, 1978). Prolactin also increases the capacity of LH receptors and the concentration of cholesterol esters in the testes, creating a pool of precursors used in steroidogenesis. Another important role of prolactin is the

activation of 3β - and 17β -hydroxysteroid dehydrogenase – the key enzymes in the synthesis of testosterone (Musto *et al.*, 1972; Amador and Bartke, 1991; Chandrashekar *et al.*, 1991).

Besides the effect on the endocrine function of the testes, prolactin significantly influences the process of spermatogenesis. There is a correlation between blood and seminal plasma concentrations of this hormone. Prolactin affects some parameters of semen quality such as the amount, motility and capacitation of spermatozoa and their ability to penetrate the ovum (Sheth *et al.*, 1975; Sueldo *et al.*, 1985; Eggert-Kruse *et al.*, 1991). The role of prolactin in male domestic ruminants has not yet been described, except in sheep. In rams, prolactin may affect LH secretion and influence testicular function (Ravault *et al.*, 1977, 1982a; Regisford and Katz, 1993).

The regulation of testicular activity by gonadotrophins has been well documented in boars (Allrich *et al.*, 1983; Berardinelli *et al.*, 1984; Ziecik *et al.*, 1989). The production of prolactin in

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pigs begins on about day 70 of fetal life (Dacheux, 1984; Meijer *et al.*, 1988). The prematurational surge of prolactin in boars, which occurs about 12 weeks after birth, is connected with the significant increase in gonadotrophin concentration (Meijer *et al.*, 1988). However, the importance of prolactin in the maintenance of testicular function in boars has not been elucidated. This study was therefore performed to determine the effects of exogenous prolactin-induced hyperprolactinaemia and bromocriptine-induced hypoprolactinaemia on LH secretion, and the endocrine function of the testes as well as on the structure of the seminiferous tubules in boars. Some of the results were communicated in a preliminary form at the 12th International Congress on Animal Reproduction, The Hague, The Netherlands, 23–27 August, 1992.

Materials and Methods

Animals and experimental procedures

Thirteen cross-bred boars, 7 months of age, were maintained under conditions of natural light and temperature (September) in individual stalls and fitted with indwelling vena caval cannulae (Kotwica *et al.*, 1978) 3 days before the experiment. The boars were assigned to three groups: (i) hyperprolactinaemic ($n = 4$) given exogenous porcine prolactin, (ii) hypoprolactinaemic ($n = 5$) given bromocriptine and (iii) control ($n = 4$) given saline.

Blood samples were collected twice a day (at 08:00 and 20:00 h) and every 20 min for 4 h (11:00–15:00 h) on days 4 and 17 of the experiment. The plasma obtained after centrifugation (1500 g, 4°C, 10 min) was stored at –20°C until prolactin, LH, testosterone and oestradiol concentrations were determined. Prolactin, LH and testosterone were determined in all the samples; oestradiol was measured in the samples taken at 08:00 and 20:00 h every day.

After the last blood collection, all boars were castrated and samples of testes were frozen immediately in liquid nitrogen and either kept until LH receptor assay or fixed in Bouin's solution.

Treatment

Boars were treated for 21 days. Prolactin purified from pig pituitaries (Biolactin; 19 iu mg⁻¹; a gift of F. Ryszka, Silesian School of Medicine, Zabrze, Poland) was injected i.m. (0.07 mg kg⁻¹ body mass) in 3 ml of saline. The same dose was used to stimulate lactation in sows (Dusza *et al.*, 1991). Bromocriptine (2-bromo- α -ergocriptine) was given twice a day: at 08:00 h per os in the form of Parlodel (Sandoz, 0.2 mg kg⁻¹) and at 20:00 h, 0.05 mg kg⁻¹ i.m. as bromocriptine CB-154 in 3 ml solution (a gift of D. Romer, Sandoz, Pharma). Bromocriptine CB-154 was dissolved in 96% ethanol and mixed 1:1 with saline before injection. At similar times control boars were given vehicle only. Doses of bromocriptine CB-154 were selected on the basis of earlier experiments in cows (Bevers and Dieleman, 1987) and sheep (Regisford and Katz, 1993).

Radioimmunoassays

The plasma concentrations of prolactin were determined by the double-antibody radioimmunoassay described by Dusza

and Krzymowska (1979) using the pig prolactin preparation KK-2 as a standard and for iodination. First, antibodies from a goat immunized against pig prolactin (Research Products Int. Corp., Mt Prospect, IL) were used at a titre of 1:40 000. Second, antibodies, anti-goat γ -globulin from immunized rabbits, were used at a 1:20 dilution. The sensitivity of the assay was 0.19 ng ml⁻¹. The intra- and interassay coefficients of variation were 4.2% and 9.8%, respectively.

Plasma concentrations of LH were determined by the double-antibody radioimmunoassay method described by Ziecik *et al.* (1978) with the following modifications. The primary antibodies (Sz/Z/89/396), from the immunized rabbit against a conjugate of pig LH with ovalbumin, were used at a final dilution of 1:1.800 000. The crossreactions of antiserum used with different antigens were 2.0% for pig GH, 1.5% for bovine TSH and 0.2% for porcine FSH, but 0.06% for prolactin and hCG. Purified pig LH (USDA-pLH-1) was used for the preparation of the radioiodinated antigen and USDA-pLH-B-1 as a standard. The sensitivity of the assay was 0.08 ng ml⁻¹. Intra- and interassay coefficients of variation were 6.7% and 11.3%, respectively.

Testosterone concentration was determined by direct assay in plasma diluted 10 times with antibodies from immunized rabbits against a conjugate of 4-androstene-17 β -ol-3-one-3-(carboxymethyl) oxime:BSA (B. Szafranska and A. J. Ziecik, unpublished data) with [1,2,6,7-³H]testosterone (Amersham, Buckinghamshire) as tracer. The crossreactions of antiserum with different antigens were: 5 α -androstane-17 β -ol-3-one: 33.3%; 4-androstane-11 β -ol-3,17-dione: 4.0%; 5 α -androstane-3,17-dione and 5 β -androstane-3 α -17 β -diol: 0.3%; epiandrosterone: 0.1% and progesterone, androsterone, 5 β -androstane-3 α -17 β -dione: <0.1%. No crossreactions were detected with dehydroepiandrosterone, 5 α -pregnan-3 α -ol-20-one, 5 α -pregnan-3 β -ol-20-one, 4-pregnan-20 β -ol-3-one, oestrone, oestradiol and β -oestradiol-17-propionate. This antibody was determined to have an affinity constant by Scatchard plot of 2.1×10^{10} l mol⁻¹. The antiserum was used at a titre of 1:20 000. The sensitivity of the assay was 0.12 ng ml⁻¹, and intra- and interassay coefficients of variation were 5.6% and 8.8%, respectively. Oestradiol concentration was determined using an extraction method (Szafranska and Tilton, 1993) with [2,4,6,7-³H]oestradiol (Amersham) as tracer. The recovery of oestradiol in the extracted samples was 88% and the sensitivity of the assay was 5 pg per tube. Intra- and interassay coefficients of variation were 9.0% and 10.2%, respectively.

Preparation of the membrane fraction and LH/hCG receptor analyses

Each testicular tissue was dissected, minced with scissors and weighed. The cell membrane fractions were obtained using the procedure described by Ziecik *et al.* (1986). Each tissue was homogenized twice in bursts of 10 s at 4°C with an Ultra-Turax homogenizer in four volumes (w/v) of 25 mmol Tris-HCl buffer l⁻¹, pH 7.4; containing 0.25 mol sucrose l⁻¹. The homogenate was then filtered through four layers of cheesecloth and the filtrate was centrifuged for 20 min at 800 g, at 4°C. The resulting supernatant was centrifuged for a further

1 h at 25 000 *g* at 4°C and the sediment suspended in 3 ml ice-cold 25 mmol Tris-HCl buffer l^{-1} , pH 7.2 containing 0.1% (w/v) BSA and 5 mmol $MgCl_2$ l^{-1} . Aliquots of 1 ml of the resulting suspension were stored in liquid nitrogen. A fraction of the receptor preparation was assayed for protein by the method of Lowry *et al.* (1951).

The cell membrane fraction was incubated with the tracer in polystyrene tubes. Human CG (CR-125) was labelled using the chloramine-T method described by Greenwood and Hunter (1963). The specific activity of [^{125}I]-labelled iodo-hCG was determined by a self-displacement in a radioligand receptor assay and was 55 μCi μg^{-1} . The maximum binding was 40%. Nonspecific binding was measured by the addition of 1 μg unlabelled hCG and was < 2% of the total [^{125}I]-labelled hCG added. The incubation mixture consisted of 0.1 ml 5 mmol Tris-HCl l^{-1} (pH 7.2) containing 5 mmol $MgCl_2$ l^{-1} and 0.1% (w/v) BSA (incubation buffer), 0.1 ml incubation buffer containing 20 000 c.p.m. [^{125}I]-labelled hCG min^{-1} , and 0.2 ml receptor preparation containing 0.6–1.0 mg protein. After incubation overnight at room temperature, 1.5 ml of ice-cold Tris-HCl buffer was added and the tubes were centrifuged at 1000 *g* for 30 min. The concentrations of unoccupied binding sites and equilibrium association constants (K_a) were determined by Scatchard analysis using the EBDA computer program (Elsevier, BIO-SOFT, Cambridge). Seven subsaturating quantities of unlabelled hCG (0.01–5 ng) were used for each receptor preparation. The sensitivity of the receptor assay was 0.15 fmol mg^{-1} protein.

Histology

Tissue specimens were fixed with Bouin's solution, embedded, serially sectioned at 7 μm and stained routinely with haematoxylin-eosin and periodic acid-Schiff's reagent. Examination of the morphology of the testes was based on the cycle of the seminiferous epithelium described in boars by Swierstra (1968). The stage of epithelium cycle was assessed by analysis of the transverse sections of seminiferous tubules.

Statistical analyses

The mean hormone concentrations were obtained by averaging the values, for each animal, for all 42 samples taken every day at 08:00 and 20:00 h. The concentrations of plasma prolactin, LH and testosterone were also averaged for the two 4 h sampling periods (13 samples) for each animal. The number of peaks, hormone peak frequency and peak amplitude during the 4 h were determined using the EPISTAT programme (Statistical Package, Version 3.0, Round Rock, TX).

Consecutively, the collected data were compared by analysis of variance (ANOVA) to establish the overall effects of treatment (prolactin-treated, bromocriptine-treated and control). In addition, comparisons of means were performed by Duncan's multiple-range test.

Fig. 1. Mean concentrations of prolactin in boars treated with (○) pig prolactin ($n = 4$; SEM ± 0.2), (■) bromocriptine ($n = 5$; SEM ± 0.04) or (▲) saline vehicle ($n = 4$; SEM ± 0.1).

Results

Effect of exogenous prolactin and bromocriptine on prolactin, LH, testosterone and oestradiol concentrations

Prolactin. The analysis of plasma samples taken twice a day demonstrated that both exogenous prolactin and bromocriptine affected prolactin concentrations (Fig. 1). The injections of exogenous prolactin caused a significant increase ($P < 0.05$) in mean (2.2 ± 0.2 ng ml^{-1}) plasma concentrations of prolactin compared with control values of 1.6 ± 0.2 ng ml^{-1} . Higher concentrations of prolactin ($P < 0.01$) were found during the 4 h sampling period on day 4 and day 17 of the experiment (Fig. 2). The bromocriptine treatment significantly decreased ($P < 0.01$) mean prolactin concentrations to 0.50 ± 0.06 ng ml^{-1} during the whole period of the experiment. A similar depletion of prolactin concentrations was found during both 4 h sampling periods ($P < 0.01$; Fig. 2).

Luteinizing hormone. Exogenous prolactin clearly decreased ($P < 0.01$) the mean LH concentrations from 0.53 ± 0.03 ng ml^{-1} to 0.34 ± 0.01 ng ml^{-1} during the whole period of the experiment (Fig. 3). The injections of prolactin caused a decrease in mean LH concentrations on day 4 ($P < 0.05$) but did not affect them on day 17 (0.64 ± 0.03 compared with 0.72 ± 0.04 ng ml^{-1} in control) (Table 1). The number of pulses, pulse frequency and amplitude remained unchanged throughout both sampling periods. Bromocriptine did not affect LH concentrations during the whole period of the experiment (Fig. 3) or during the 4 h sampling periods.

Testosterone and oestradiol. Exogenous prolactin increased testosterone concentrations during the treatment ($P < 0.01$) from 1.9 ± 0.1 to 2.8 ± 0.02 ng ml^{-1} . Boars treated with bromocriptine preparations had lower testosterone concentrations (1.6 ± 0.09 ng ml^{-1} ; $P < 0.05$) than did controls. The twice daily recorded data revealed variable testosterone secretion in boars tested. The daily range of blood testosterone concentrations varied from 0.25 ± 0.01 to 3.76 ± 0.16 , 0.52 ± 0.02 to 3.10 ± 0.20 and 0.75 ± 0.03 to 7.20 ± 0.52 ng ml^{-1} in control, bromocriptine- and prolactin-treated animals, respectively, over the 21 days of the experiment. Analysis of

Fig. 2. Mean concentrations of prolactin in boars treated with (○) pig prolactin ($n = 4$), (■) bromocriptine ($n = 5$) or (▲) saline vehicle ($n = 4$) during frequent sampling periods on (a) day 4 and (b) day 17 of the experiment. Values are means $\pm$ SEM.

the 4 h periods of collection did not show changes in pulse characteristics. Exogenous prolactin reduced ($P < 0.01$) oestradiol concentrations in the blood by about 20% compared with saline-treated boars (76.4 ± 2.4 versus 61.9 ± 1.7 pg ml $^{-1}$; Fig. 3). In addition, the bromocriptine treatment significantly decreased ($P < 0.05$) oestradiol concentrations during the course of the experiment.

LH/hCG receptors in cell membrane preparations of boar testes

The injections of exogenous prolactin as well as the administration of bromocriptine caused a decrease in LH/hCG receptor content ($P < 0.05$) in the cell membranes of the testes of the treated boars compared with the control group. In hyperprolactinaemic boars, LH/hCG receptor content decreased from 13.5 ± 4.2 to 7.6 ± 1.3 fmol mg $^{-1}$ protein. The content of LH/hCG receptors in animals given bromocriptine was 50% of control values (6.8 ± 1.2 fmol mg $^{-1}$ protein). In both groups, the decrease in LH binding by receptors occurred without a change in binding affinity. Association contents (K_d) for prolactin, bromocriptine and the control group were 5.0 ± 1.1 , 5.3 ± 0.9 and $4.6 \pm 1.0 \times 10^{10}$ l mol $^{-1}$, respectively.

Histology

The examination of seminiferous epithelium in control boars (Fig. 4a) revealed the presence of all generations and layers of germinal cells in stages from I to VIII. The bromocriptine treatment did not cause many changes on the seminiferous tubule transections. The premature release of spermatocytes into the lumen of the tubule and the presence of polynucleate cells was sporadically observed. One boar from the bromocriptine-treated group showed more intensive changes and there were more polynucleate cells than in the other animals (Fig. 4b).

The injections of exogenous prolactin caused the most intensive changes in the testes. Seminiferous epithelium showed empty sites left by released cells (Fig. 4c). The more mature cells, mainly spermatids at various stages of differentiation but also occasional pachytene spermatocytes, were flaked off. Many polynucleate cells were observed in the seminiferous epithelium as well as in the lumen of the tubule. In some tubules, especially those in which many polynucleate cells were found, considerable regression of seminiferous epithelium occurred (Fig. 4d).

Discussion

The study reported here determined the effect of prolactin-induced hyperprolactinaemia and bromocriptine-induced hypoprolactinaemia on LH secretion and testicular function in boars. The experiment was performed on mature animals when the prolactin concentration in the blood had stabilized after a prepubertal period (10–16 weeks) and pubertal peaks (20–22 weeks; Meijer *et al.*, 1988). Significant changes in the concentration of circulating prolactin in treated animals were then induced by exogenous prolactin or administration of bromocriptine. The concentrations of LH in control animals were similar to those reported by Liptrap *et al.* (1986) in adult boars. The results from twice daily sampling show that injections of exogenous prolactin decreased mean plasma concentration of LH by 34%; however, this suppression was short lived, presenting at 4 but not at 17 days.

An inhibitory effect of exogenous prolactin on concentrations of LH is found in pregnant gilts (Szafranska and Ziecik, 1990). Hyperprolactinaemia, induced by haloperidol during the second half of pregnancy, also drastically suppresses LH secretion in pigs (Szafranska and Tilton, 1993). Exogenous prolactin exerts a dose-dependent suppression of postcastration LH secretion in male rats (Park and Selmanoff, 1991). Prolactin is thus involved in the control of the hypothalamo-pituitary axis. Its effect on LH secretion is probably transmitted via activation of a dopaminergic part of the hypothalamus. High concentrations of prolactin cause 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). It is also possible that LH concentrations in the prolactin-injected boars have been reduced owing to increased plasma concentrations of testosterone exerting negative feedback at the hypothalamus or pituitary. The interrelation between LH and prolactin seems to differ

Fig. 3. Mean plasma concentrations of (a) prolactin, (b) LH, (c) testosterone and (d) oestradiol during 21 days of the experiment in boars treated with (□) pig prolactin ($n = 4$), (▨) bromocriptine ($n = 5$), or (■) saline vehicle ($n = 4$). Values are means $\pm$ SEM. Means are significantly different between treated and control boars within the experimental period (* $P < 0.05$, ** $P < 0.01$).

Table 1. Mean hormone concentrations, peak frequency and peak amplitude in prolactin-treated, bromocriptine-treated and control boars during the 4 h sampling period on day 4 of the experiment

Hormone	Treatment	Day 4		
		Mean (ng ml ⁻¹)	Peak frequency (peaks in 4 h)	Peak amplitude (ng ml ⁻¹)
LH	Prolactin	0.51 $\pm$ 0.04*	2.75 $\pm$ 0.25	0.91 $\pm$ 0.22
	Bromocriptine	1.04 $\pm$ 0.06	3.20 $\pm$ 0.37	1.39 $\pm$ 0.24
	Control	0.93 $\pm$ 0.10	2.25 $\pm$ 0.62	1.74 $\pm$ 0.66
Testosterone	Prolactin	0.72 $\pm$ 0.04	2.50 $\pm$ 0.29	0.67 $\pm$ 0.17
	Bromocriptine	1.09 $\pm$ 0.04	2.00 $\pm$ 0.70	1.36 $\pm$ 0.17
	Control	1.12 $\pm$ 0.07	1.75 $\pm$ 0.25	1.46 $\pm$ 0.48

Values are means $\pm$ SEM.

*Means are significantly different between treatment groups within the sampling period.

depending on the age of the animal. In prepubertal rats, neither hyper- nor hypoprolactinaemia affects the concentration of LH in serum. In these animals, hyperprolactinaemia reduces only the secretion of FSH (Chandrasekar *et al.*, 1987). In 12-week-old boars, the pubertal surge of prolactin occurs together with increased concentrations of gonadotrophins but low concentrations of testosterone (Meijer *et al.*, 1988).

In the study reported here exogenous prolactin increased the concentrations of testosterone but decreased the concentra-

tions of oestradiol in plasma in blood samples taken twice a day but no treatment effect was found during intensive sampling periods. The discrepancies between the data obtained twice a day and the data based on intensive sampling can be explained by the variable pulsatile secretion of testosterone in intact boars (Liptrap *et al.*, 1986) and by the different daily hours of blood collection. It is suggested that prolactin may have a direct effect on the aromatization of androgens in the human testes (Magrini *et al.*, 1976; Martikainen and Vihko,

Fig. 4. Photomicrographs of testicular tissue from (a) a normal control boar. Histology of seminiferous epithelium (E) is normal and germ cells conform to normal cellular associations of the spermatogenic cycle; (b) bromocriptine-treated boar showing presence of polynucleate cells (PC) in the lumen of seminiferous tubules; (c, d) hyperprolactinaemic boars. Exogenous prolactin caused: (c) premature release of germ cells and (d) the appearance of many polynucleate cells (PC) and regression of the seminiferous epithelium (E). Scale bars represent 25 μm .

1982). The increase of testosterone during hyperprolactinaemia is also found in men (Magrini *et al.*, 1976), rats (Hafiez *et al.*, 1972; Bartke *et al.*, 1977) and hamsters (Bartke *et al.*, 1985). A high concentration of prolactin can also disrupt the conversion of testosterone to its active metabolite 5- α -dihydrotestosterone (Katovich *et al.*, 1985). However, in prepubertal rats the increase in oestradiol concentrations induced by prolactin is preceded by an increase in the concentration of testosterone in the plasma, but only in the presence of FSH (Pomerantz, 1983). Prolactin alone does not induce a synthesis of steroids in hypophysectomized animals (Klemcke *et al.*, 1990). The administration of bromocriptine did not induce any significant changes in the secretion of LH in boars. A similar lack of effect of bromocriptine on LH is found in cyclic sows (Dusza *et al.*, 1983). However, bromocriptine treatment did decrease the plasma concentrations of testosterone and oestradiol. This may indicate the direct testicular effect of bromocriptine. There were no differences in the profiles of LH and testosterone secretion during the 4 h period of blood sampling on days 4 and 17 of the experiment between the treated and control boars. The main reason for this is, perhaps, the variability of the pulsatile secretion of LH (Liptrap *et al.*, 1986).

The concentrations of LH receptor sites in boar testes reached a maximum at the age of between 20 and 70 days, decreased at the onset of puberty (up to 110 days) and reached a stable concentration in adults (Peyart *et al.*, 1981). The binding capacity of testicular tissue, expressed as fmol mg^{-1} membrane protein, is greatest at the age of 100 days, decreases linearly until day 190 and then remains stable for the next 250 days (Berardinelli *et al.*, 1984). In the present study, both prolactin and bromocriptine caused a significant decrease in concentrations of LH/hCG receptors in the boar testes. The stimulatory effect of prolactin on LH/hCG receptors may have been overridden by the consequences of reduced LH concentrations. The above results can be explained not only by the direct effect of prolactin, but also by changes in gonadotrophin and steroid hormone secretion. The direct effect of bromocriptine on LH/hCG binding sites cannot be excluded (Amador and Bartke, 1991).

Hyperprolactinaemia has different effects on testicular LH/hCG receptor concentrations in various species, causing a decrease in mice numbers (Klemcke and Bartke, 1981; Takase *et al.*, 1990), no effect in rams (Barenton and Pelletier, 1980) and an increase in rats (Sharpe *et al.*, 1980). Bromocriptine affects LH/hCG receptors more uniformly, causing a decrease in their

concentration in the testes of adult mice (Takase *et al.*, 1990), rats (Aragona *et al.*, 1977), hamsters (Amador and Bartke, 1991) and pigs (this study) but not in rams (Barenton and Pelletier, 1980).

High concentrations of prolactin are often associated with regression of the seminiferous epithelium, oligospermia and a decrease in the lifespan of spermatozoa (Cameron *et al.*, 1984; Chandrashekar and Bartke, 1988). Hyperprolactinaemia causes a reduction in fertility by producing changes in the process of spermatogenesis, low quality semen and a decrease in libido in many species (Segal *et al.*, 1979; Katovich *et al.*, 1985; Eggert-Kruse *et al.*, 1991; Gonzales *et al.*, 1992). The study reported here showed for the first time that prolactin-induced hyperprolactinaemia causes a strong disturbance of spermatogenesis in boars. The bromocriptine treatment did not influence the seminiferous epithelium to the same extent. In wild boars, in contrast to males of domesticated breeds of pig, plasma concentrations of prolactin vary with season; and the highest concentrations occur in July and the lowest during the winter months (Ravault *et al.*, 1982b). During the summer months, wild boars as well as sows show sexual inactivity. It has not been clearly demonstrated that prolactin causes anoestrus in wild pigs. Normal physiological plasma concentrations of prolactin, as have been shown in this study, are important in the regulation of gonadotrophin and testicular steroid secretion and in the function of seminiferous epithelium in boars. It is, therefore, possible that hyperprolactinaemia observed during long daylength is a reason for seasonal infertility in wild boars and the sporadically reported decrease in semen quality in some domesticated pigs during the summer months.

In summary, this study has shown the importance of normal physiological secretion of prolactin on reproduction in boars. Both hyper- and hypoprolactinaemia disrupt testicular endocrine function and change the process of spermatogenesis. However, hyperprolactinaemia causes a more severe effect on the testes and also influences the secretion of LH. These results support the hypothesis that there is an inverse relationship between LH and prolactin secretion in pigs.

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