J. Reprod. Fert., Suppl. **30** (1981), 67–73 *Printed in Great Britain*

Cryptorchidism and the pituitary–testicular axis in bulls

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Summary. In calves made cryptorchid at birth, serum LH concentrations were elevated ($P < 0.01$) over those of intact controls by 7 weeks of age; a difference which persisted until after puberty. When intact and cryptorchid bulls were given LH-RH, i.v., at approximately 18 months of age, cryptorchid bulls were more responsive. Serum LH concentrations (mean ± s.e.m.) after 250, 1000 and 4000 ng LH-RH were 0·2 ± 0·03, 1·2 ± 0·3 and 12·1 ± 2·0 ng/ml for intact bulls and 1·0 ± 0·6, 5·1 ± 0·8 and 19·6 ± 2·1 ng/ml for cryptorchid bulls.

Testes from cryptorchid bulls weighed less ($P < 0.01$) than testes from intact bulls (68 ± 6 and 655 ± 36 g) at 24 months of age. Ability to secrete testosterone *in vitro* was determined by incubating minced pieces of testes at 36°C for 3 h in Krebs–Ringer–bicarbonate buffer (pH 7·2) containing 1 mg glucose/ml and 100 mi.u. hCG/ml. Cryptorchid testes secreted slightly more testosterone than scrotal testes on a unit weight basis, but when expressed on a paired testis weight basis, testosterone secretion by cryptorchid testes was less (8 ± 2 and 60 ± 10 mg, $P < 0.01$). Autoradiographic localization techniques were used to show the existence of LH/hCG binding sites in cryptorchid testes. These studies suggest that cryptorchid bull testes have a reduced capacity to secrete testosterone and the near-normal serum testosterone concentrations are maintained in the presence of increased serum gonadotrophins.

Introduction

Surgical translocation of the bovine testis to an intra-abdominal position results in disruption of the seminiferous epithelium (Skinner & Rowson, 1968) and an absence of spermatozoa in the ejaculate (Kellaway, Seamark & Farrant, 1971; Schanbacher, 1978). A 3°C higher internal temperature of the cryptorchid testis may be largely responsible for spermatogenic arrest (Kellaway *et al.*, 1971) but evidence for reduced testosterone production (Kellaway *et al.*, 1971; Schanbacher, 1978) suggests that the results obtained with cryptorchid bulls cannot be attributed solely to uncomplicated degeneration of the seminiferous epithelium. Impairment of the testosterone secretory response by cryptorchid bulls has also been demonstrated (Schanbacher, 1979a): neither elevated concentrations of endogenous gonadotrophins nor treatment with exogenous LH was able to increase serum concentrations of testosterone. Because serum concentrations of LH are elevated in cryptorchid bulls (Schanbacher, 1978, 1979a) and down-regulation of LH receptors by the high LH levels associated with cryptorchidism (de Kretser, Sharpe & Swanston, 1979) may render the cryptorchid testis unresponsive to additional gonadotrophic stimulation, the following study was undertaken to gain a better understanding of the pituitary–testicular endocrine axis in cryptorchid bulls. Specifically, pituitary sensitivity to testicular feedback was assessed in intact and cryptorchid bulls with an LH-RH challenge and the capacity to produce testosterone *in vitro* was determined from minces of scrotal and abdominal testes.

0449-3087/81/020067-08$02.00/0

Materials and Methods

Twenty newborn Hereford × Angus crossbred calves were assigned to this study. Between 3 and 5 days of age, each calf was anaesthetized with thiopentone sodium and prepared for inguinal surgery as described previously for young bulls (Schanbacher, 1978). A high lateral incision was made on the scrotum and the right and left testes were pushed through the inguinal canal and into the abdomen. In 10 of the calves cryptorchidism was ensured by closing the inguinal canal with surgical sutures. The testes in sham-treated calves were allowed to redescend into the scrotum. The scrotal incisions were sutured and allowed to heal.

Sexual development

Starting at 1 week of age, all calves were weighed and a blood sample taken every other week until 41 weeks of age. Calves were maintained with their dams on grass pasture until weaned at 25 weeks of age. Testicular diameters of the sham-treated (intact) calves were measured to provide a record of testicular development (spermatogenesis). Pre- and post-weaning body weight gains were computed and serum LH changes recorded.

Episodic and LH-RH-induced LH release

At approximately 18 months of age, 10 cryptorchid and 9 intact bulls were placed in individual holding stalls and adapted to handling for 1 week. During the second week, each animal was fitted with an indwelling jugular canula. Blood samples were collected at 15-min intervals for 4 h (09:00–13:00 h, Day 1) from each animal. On the three subsequent days starting at 09:00 h, samples were collected at 15-min intervals for 3 h; synthetic LH-RH was administered i.v. at 09:15 h (250 ng, Day 2; 1000 ng, Day 3; 4000 ng, Day 4). Serum from each sample was frozen at −20°C until assayed for concentrations of LH.

Testicular and seminal vesicle weights

At approximately 24 months of age, 10 cryptorchid and 5 intact bulls were slaughtered and testes and seminal vesicles were collected, trimmed and weighed. Pieces of testicular tissue were fixed in Zenker–formol, embedded in paraffin wax, sectioned at 5 μm, stained with periodic acid–Schiff (PAS) and counterstained with haematoxylin. The histology of the seminiferous epithelium and the interstitial tissue (Leydig cells) in the cryptorchid and intact testes was compared.

Testicular binding sites for ^{125}I-labelled hCG

Autoradiographic localization studies were performed on testes of intact and cryptorchid bulls following in-vitro binding of ^{125}I-labelled hCG. Procedures were similar to those described earlier (Schanbacher, 1979b). Purified hCG (CR 119: 11 600 i.u./mg) was iodinated with chloramine T and 0·5 mCi ^{125}I. Sections (6 μm) were cut on a cryostat, mounted on glass slides and incubated with ^{125}I-labelled hCG alone or together with a 250-fold excess mass of unlabelled hCG. After 3 weeks exposure to Kodak NTB-2 emulsion, the slides were developed and the distribution of silver grains over the tissue sections was noted.

In-vitro testosterone production

Pieces of testicular tissue (approximately 2 g each) were minced into 1 mm^2 fragments and incubated in 5 ml Krebs–Ringer–bicarbonate buffer containing 1 mg glucose/ml as described for

rat testes (Bartke, Williams & Dalterio, 1977; Schanbacher, 1980a). Duplicate samples of each testis were incubated in the absence of gonadotrophin to estimate basal testosterone production and in the presence of 100 mi.u. hCG/ml to estimate stimulated testosterone production. Incubations were done in a shaking water bath at 36°C for 3 h. After centrifugation at 1500 *g* for 5 min, the supernatants were collected and assayed for testosterone.

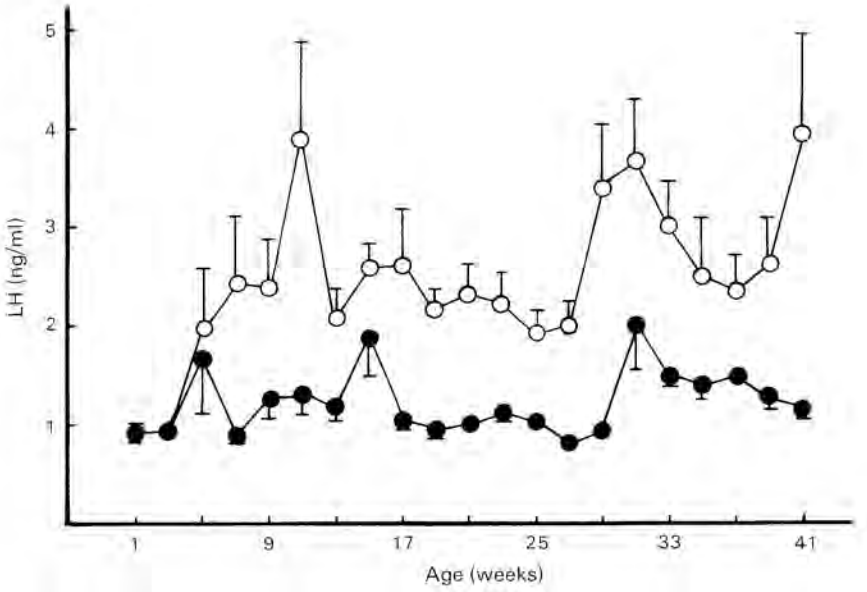
Hormone assays

Serum LH was assayed in duplicate by the double-antibody radioimmunoassay procedure described by Schanbacher & Echternkamp (1978). NIH-LH-B10 was used as the reference standard; the assay sensitivity was 0·5 ng/ml. Intra-assay coefficient of variation was <10% and inter-assay coefficient of variation was <13%. Testosterone values in serum and incubation media were measured in ether extracts without chromatography (Schanbacher, 1980b). Because the antiserum used cross-reacts with 5α-dihydrotestosterone approximately 70%, the assay values reflect concentrations of both androgens. The intra-assay coefficient of variation was <12% and assay sensitivity was 0·2 ng/ml serum.

Treatment means were examined either by split-plot analyses of variance or by Student's *t* test.

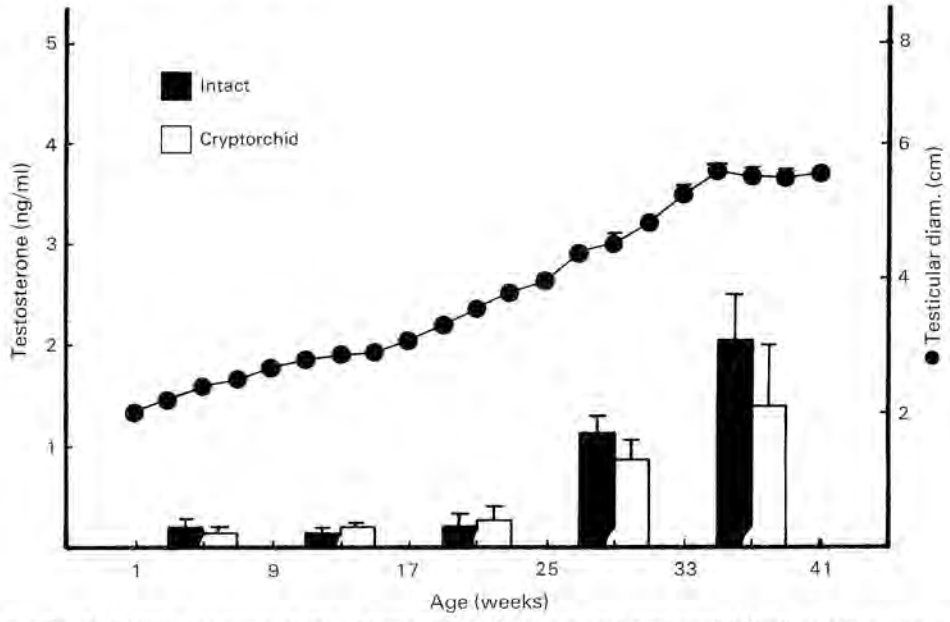
Results

Serum LH concentrations during sexual development in cryptorchid and intact bulls are shown in Text-fig. 1. Concentrations were similar during the first 5 weeks of age but were higher ($P < 0{\cdot}01$) in the cryptorchid than in the intact calves by 7 weeks of age. This treatment difference persisted for the duration of the study. In general, serum LH concentrations were near 1 ng/ml for intact calves and between 2 and 3 ng/ml for cryptorchid calves.



Text-fig. 1. Serum concentrations of LH in intact (●) and cryptorchid (○) bull calves at various ages. Each point represents the mean ± s.e.m. for 10 animals.

Serum testosterone concentrations were low up to 21 weeks of age (Text-fig. 2) and no differences were observed between cryptorchid and intact calves. A pubertal rise in serum testosterone concentrations was observed for the cryptorchid and intact calves at 29 weeks of age. Testosterone concentrations tended to be higher in intact calves at 37 weeks of age than in cryptorchid calves, but the difference was not statistically significant ($P > 0{\cdot}10$). Testicular diameter of intact calves increased with age but the increase appeared most rapid between 25 and 35 weeks of age (Text-fig. 2).



Text-fig. 2. Serum concentrations of testosterone in intact and cryptorchid bull calves at various ages. Mean testicular diameter for intact calves is also shown. Each point represents the mean ± s.e.m. for 10 animals.

Growth rate was essentially identical for cryptorchid and intact calves. Pre-weaning average daily gains were $1{\cdot}02 \pm 0{\cdot}03$ and $1{\cdot}00 \pm 0{\cdot}04$ kg/day; whereas post-weaning average daily gains were $1{\cdot}19 \pm 0{\cdot}05$ and $1{\cdot}19 \pm 0{\cdot}05$ kg/day.

Serum concentrations of LH and testosterone in samples collected at frequent intervals from cryptorchid and intact bulls at approximately 18 months of age were similar to those determined at younger ages. Concentrations of LH but not testosterone were significantly higher in cryptorchid bulls as compared to intact control bulls (Table 1). Episodic (pulsatile) secretion of LH was not observed in cryptorchid and intact bulls during the 4 h bleeding schedule of Day 1, but challenges with submaximal doses of LH-RH gave dose-dependent release of LH into the systemic circulation (Table 1). Cryptorchid bulls not only had higher preinjection concentrations of LH but released more LH in response to a given LH-RH injection. Two cryptorchid bulls exhibited an episodic release of LH on Day 3 in addition to that induced by 1000 ng LH-RH.

Testicular and seminal vesicle weights were considerably lower ($P < 0{\cdot}01$) for cryptorchid bulls than for intact bulls (Table 2). Failure of the germinal epithelium to develop was primarily responsible for the reduced testes weight (see Pl. 1, Figs 1 and 2). The scrotal testes contained large seminiferous tubules with active spermatogenesis and healthy interstitial cells, but the abdominal testes contained collapsed seminiferous tubules lined with a single layer of spermatogonia and supporting cells and abundant Leydig cells scattered in the interstitial spaces and surrounded by an abundance of capillaries and connective tissue.

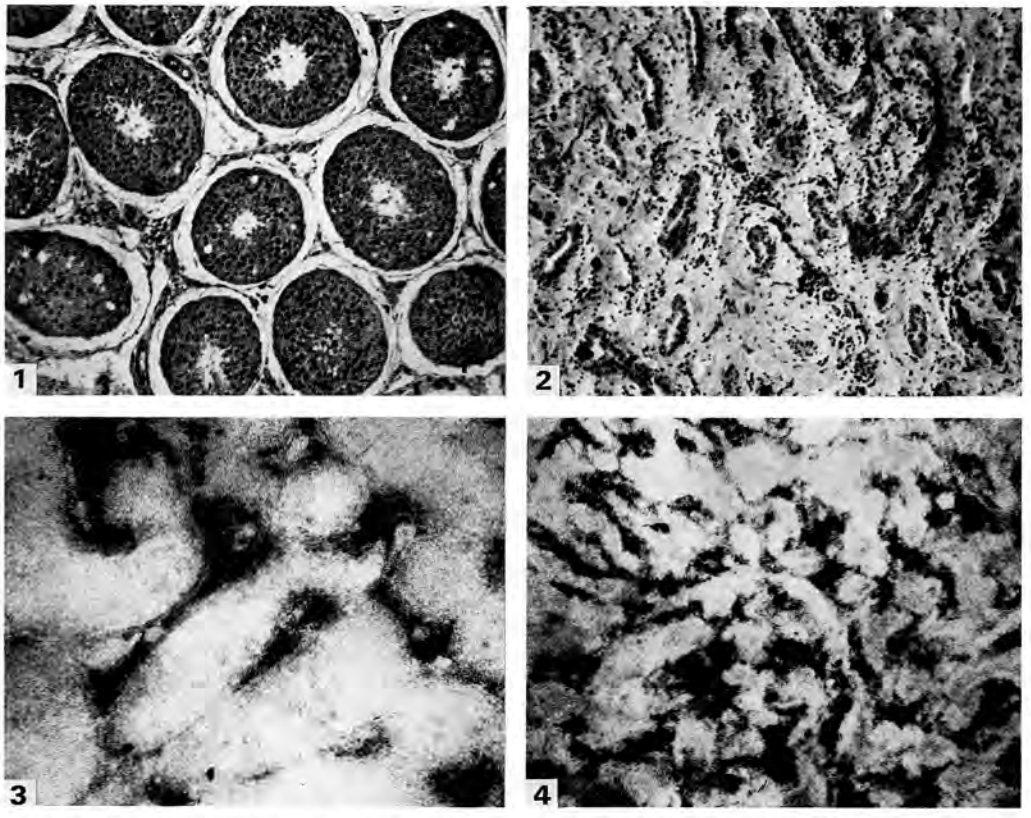


Fig. 1. Photomicrograph of a testis section from an intact bull, showing large and distinct seminiferous tubules and active spermatogenesis. PAS-haematoxylin, ×80.

Fig. 2. Photomicrograph of a testis section from a cryptorchid bull showing collapsed seminiferous tubules lacking mature germinal elements. PAS-haematoxylin, ×80.

Figs 3 and 4. Photomicrographs of frozen sections from the testis of an intact bull (Fig. 3) and a cryptorchid bull (Fig. 4) showing the concentration of silver grains over the interstitial compartment of the testis after processing for topical autoradiographic localization of ^{125}I-labelled hCG. ×80.

(*Facing p.* 70)

Table 1. Mean ± s.e.m. concentrations of serum LH and testosterone in cryptorchid and intact bulls and their response to intravenous challenge of different doses of LH-RH

	No. of bulls	LH (ng/ml)†	Testosterone (ng/ml)†	LH response (ng/ml)‡		
				250 ng	1000 ng	4000 ng
Cryptorchid	10	1·6 ± 0·1*	2·5 ± 0·3	1·0 ± 0·6*	5·1 ± 0·8*	19·6 ± 2·1*
Intact	9	0·9 ± 0·04	1·8 ± 0·5	0·2 ± 0·03	1·2 ± 0·3	12·1 ± 1·0

* Significantly different from value for intact bulls, $P < 0·01$.

† Calculated from the average of a 4-h intensive bleed (Day 1). One of the original 10 intact bulls died before these data were collected.

‡ LH responses to LH-RH challenge (Days 2, 3 and 4) were calculated as peak amplitude (peak minus preinjection concentration).

Table 2. Testicular and seminal vesicle weights and in-vitro testicular testosterone production of cryptorchid and intact bulls at 24 months of age

	No. of bulls	Seminal vesicle wt (g)	Paired testes wt (g)	Without hCG		With 100 mi.u. hCG/ml	
				µg/g testis	mg/paired testes	µg/g testis	mg/paired testes
Cryptorchid	10	22 ± 3*	69 ± 6*	116 ± 9*	7 ± 1*	138 ± 15	8 ± 2*
Intact	5	97 ± 9	655 ± 36	51 ± 5	30 ± 3	101 ± 15	60 ± 10

Values are mean ± s.e.m.

* Significantly different from value for intact bulls, $P < 0·01$.

Autoradiographs developed after topical application of ^{125}I-labelled hCG to cryosections of a scrotal testis (Pl. 1, Fig. 3) and an abdominal testis (Pl. 1, Fig. 4) showed that silver grains were concentrated over the interstitial tissue of both testes. The density of these grains was markedly reduced when cryosections from the same testes were co-incubated with a 250-fold excess mass of unlabelled hCG (not shown).

In-vitro testosterone production by abdominal and scrotal bull testes is shown in Table 2. In the absence of hCG, the abdominal testis secretes approximately twice as much testosterone as the scrotal testis when expressed on a unit weight basis but in the presence of 100 mi.u. hCG testosterone secretion was similar. This similarity is brought about by a two-fold stimulation of the scrotal testis by hCG. Total testosterone production by paired testes *in vitro* is small for abdominal testes when compared to scrotal testes, both in the absence and presence of hCG.

Discussion

Serum concentrations of LH are elevated in the cryptorchid bull whereas serum testosterone is in the near-normal range. During the prepubertal period serum LH values are elevated when testosterone concentrations are normal. The reason(s) for elevated LH is unknown but at least three explanations might be considered. First, LH of intact and cryptorchid bulls may differ such that a discrepancy between biologically active and immunologically reactive LH exists. Secondly, a change in the secretory pattern of testosterone may alter the effectiveness by which this steroid regulates LH secretion. This possibility would suggest that mean serum testosterone concentrations provide limited insight into feedback regulation of LH secretion. Thirdly, increased intratesticular temperature and the resultant disruption of the seminiferous epithelium may affect the secretion of steroids other than testosterone that may play an important role in the regulation of LH secretion. Although oestrogen production by the cryptorchid bull may be impaired (Schanbacher, 1978) and testosterone secretory response to gonadotrophin stimulation

suppressed (Schanbacher, 1979a), the explanation(s) for the change in the relationships of the pituitary–testicular endocrine axis remains to be determined.

Mean serum concentrations of LH are elevated in cryptorchid bulls and these animals respond to exogenous LH-RH by releasing more LH than do intact bulls. Feedback inhibition of gonadotrophin secretion at the level of the pituitary may involve oestrogen (Schanbacher, 1978) as serum testosterone is nearly normal. The equivalent growth performance until 41 weeks of age for intact and cryptorchid bulls suggests that testosterone and/or other anabolic steroids were secreted by scrotal and abdominal testes in amounts adequate to enhance the growth potential compared with that of castrated bulls. However, the low testosterone concentrations previously reported for cryptorchid bulls (Schanbacher, 1978) and reduced seminal vesicle weights indicate that low or abnormal testosterone secretion went undetected in this study and that this may be the primary reason for elevated serum concentrations of LH in cryptorchid bulls.

The cryptorchid bull was previously shown to be unable to secrete androgen in response to endogenous or exogenous gonadotrophin above a basal level (Schanbacher, 1979a). In this study, we also report that the testes of cryptorchid bulls secrete only a fraction of the testosterone produced *in vitro* by testes of intact bulls. Furthermore, basal production cannot be increased by levels of hCG stimulation that significantly enhance production by scrotal testes. A diminished testosterone response *in vivo* to hCG administration in cryptorchid rats has been reported by Kerr, Rich & de Kretser (1979). Contrary to our findings with the bull, testes from cryptorchid rats secrete significantly more testosterone in response to hCG stimulation *in vitro* than testes from intact rats (de Kretser *et al.*, 1979; Schanbacher, 1980a). In-vitro binding of ^{125}I-labelled hCG to testicular tissue of cryptorchid rats showed that LH/hCG binding sites were present in abdominal testes, but only at 43% (de Kretser *et al.*, 1979) and 34% (Schanbacher, 1980a) of that found in scrotal testes. While a similar reduction in hCG receptors may occur in the cryptorchid bull testis, these numbers have not been determined.

The abundance of interstitial tissue in the cryptorchid bull testis and the presence of LH/hCG binding sites suggest that steroid secretion by the abdominal testis might be enhanced. The reason for an apparent block in testosterone production is unclear but insensitivity of the Leydig cell within abdominal testes to circulating LH is most likely involved. The relationship between testosterone production by abdominal testes and LH secretion is likewise unclear. Involvement of testicular hormones, other than testosterone, or a change in the processing of testosterone feedback information by cryptorchid animals is perhaps responsible for elevated serum LH.

We thank Ms Donna Taubenheim and Ms Marilyn Bierman for technical assistance; the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, for co-operation; Dr D. J. Bolt, U.S.D.A., for LH antiserum (DJB 3-12/11); Dr R. H. Rippel, Abbott Laboratories, for LH-RH and NIAMDD, Bethesda, Maryland, for purified LH (NIH-LH-B10).

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