Original article

Effect of castration on serum concentrations of gonadal hormones, insulin-like growth factor-I and its binding proteins in male pigs

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Summary – Ten male pigs (Large White × Landrace), 7 months old, were randomly allocated to two experimental groups. Five of them were castrated and the other five served as controls. Sera were collected on the day of castration and 1, 5, 6 and 7 weeks after castration for hormone assay. There was a significant rise in the splenic and pancreatic weights in the castrates ($P < 0.01$). The weights of prostate, seminal vesicles and bulbourethral glands were significantly decreased ($P < 0.01$) in the castrates, which is attributed to a fall in testosterone levels ($P < 0.001$). The fall in oestradiol concentrations ($P < 0.001$) in castrates confirms that the testis is the major source of oestrogens in males. Although there was no significant change in the body weight, serum IGF-I levels were elevated in the castrates as compared to the controls after 5, 6 and 7 weeks ($P < 0.001$). IGFBP bands of 43 and 39 kda predominate in both control and experimental groups indicating that castration had no effect on the IGFBP pattern. It is suggested that the increase in IGF-I levels may be due to uncoupling of GH/IGF-I axis induced by the decrease in steroid concentrations due to castration.

castration / IGF-I / IGF-I binding proteins / pig

Résumé – Effet, chez le porc, de la castration sur les stéroïdes gonadiques, l'*insulin-like growth factor 1* **et ses protéines de liaison.** Les effets de la castration ont été étudiés chez cinq mâles (Large White × Landrace) de 7 mois, par comparaison avec cinq témoins. Outre le poids de certains organes, les concentrations d'œstradiol, de testostérone, d'IGF$_1$ et de ses protéines de liaison ont été mesurées à partir de prélèvements obtenus le jour de la castration et 1, 5, 6 et 7 semaines après. La castration a produit une augmentation du poids de la rate et du pancréas et une réduction du poids des vésicules séminales, de la prostate et des glandes bulbouréthrales, par suite de la chute des concentrations de

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testostérone ($p < 0,001$). Malgré l'absence d'effet sur le poids corporel, la castration a induit une élévation des concentrations d'IGF$_1$ à 5, 6 et 7 semaines par rapport au contrôle. En revanche, les protéines de liaison des IGF de 43 et 39 kDa n'ont pas varié. La chute des niveaux de stéroïdes générée par la castration pourrait altérer les relations entre GH et IGF$_1$, produisant ainsi l'élévation de l'IGF$_1$ observée.

castration / porc / stéroïdes / IGF$_1$ / IGFBP

INTRODUCTION

Insulin-like growth factor-I (IGF-I) is a growth hormone (GH)-dependent peptide that plays an important role in growth and differentiation of many tissues (Florini et al, 1991). It circulates in blood bound to specific binding proteins (IGFBP) of approximately 18 and 43 kDa (Smith, 1984). IGF-I and IGFBPs in serum have been studied in gilts and sows under various experimental conditions including ovariectomy (Barb et al, 1992; Howard and Ford, 1992; Killen et al, 1992; Cox et al, 1994; Klindt et al, 1994). In boars, castration led to a fall in serum GH and IGF-I levels (Arbona et al, 1989; Dubreuil et al, 1989; Louveau et al, 1991). In rats, pre-pubertal castration led to a further increase rather than ablation of the pubertal IGF-I surge indicating an important functional relationship between hypothalamic–pituitary–gonadal function and hormonal regulation of peripubertal circulating IGF-I levels (Handelsman et al, 1987). Even when GH levels are high, serum IGF-I concentration is dependent on the nutritional state of the animal and is decreased with energy deficiency (Prewitt et al, 1982), protein deficiency (Maes et al, 1988) and in starvation (Maes et al, 1984). During nutritional restriction, the GH/IGF-I axis becomes uncoupled in swine, which may limit the expression of the anabolic effects of GH (Buonomo and Baile, 1991). It has been found that 43- and 39-kDa IGF-BPs decreased with weaning and fasting, while the 29-kDa band increased with weaning and fasting (White et al, 1991). Although changes in metabolic status can mediate short-term nutritional effects on reproductive function (Booth et al, 1994), the actual mechanisms mediating nutrition–reproduction interactions are not well-defined. In male calves, serum concentrations of testosterone, IGF-I and IGFBP-3 increase together, but independently, during the onset of puberty (Renaville et al, 1996). However, reports on castration-induced changes in the serum IGFBP levels in boars are scanty.

Therefore, the aim of the present study was to investigate the effect of castration on serum gonadal hormones (testosterone and estrogen), serum concentrations of IGF-I and serum IGFBP patterns in nutritionally deprived boars.

MATERIALS AND METHODS

Ten intact male pigs (Large White × Landrace), 7 months old, weighing 70–80 kg were randomly allocated to two experimental groups. These pigs were fed a raw soyabean-based diet from birth. Five of them were castrated by the following procedure. After anaesthetising the pigs with ketamine, both testes were gently displaced from the scrotal sacs and exteriorized through a median supra-pubic incision, 3 cm long. The blood supply to the testis was properly obliterated by ligating the spermatic cord. By using an orchidometer, the testes were incised and the spermatic cord returned to the scrotum. The skin and the tunica albuginea were sutured and antibiotic was sprayed at the site of the wound. They were then fed ad libitum a cereal–soya diet containing 17.3% crude protein and 0.8% lysine.

Body weights were recorded and live weight gain calculated weekly.

Blood samples were taken from the inferior vena cava on the day of castration and 1, 5, 6

and 7 weeks postcastration, for hormone assay. Serum samples were frozen at –80 °C until analysis. Sera were transported frozen to the School of Biological Sciences, University of Manchester, UK, where radioimmunoassay of IGF-I and western blot analysis of IGFBPs were carried out. Even though none of the extraction methods completely removed or inactivated binding proteins, all samples yielded IGF-I displacement curves that were parallel to that obtained for IGF-I standard (Frey et al, 1994). The acid–ethanol extraction efficiency was estimated to be 100 ± 12% and was successfully employed in gilts (Charlton et al, 1993).

IGF-1 assay

The IGF-I assay and the western ligand blotting of IGFBPs were as described elsewhere (Huang et al, 1994). IGF-I was determined by radioimmunoassay (RIA) after acid–ethanol extraction, applying a guinea-pig antiserum raised against human recombinant antigen (gift from Professor Bouilon and Professor Verhoven, University of Leuven, Belgium). The human recombinant IGF-1 (gift from Dr A White and Dr S Crosby, University of Manchester) was used as standard and tracer and iodinated using the chloramine-T method. The antiserum displayed < 0.01% cross-reaction with insulin and < 1% with IGF-2. The specific activity of iodinated IGF-1 was about 300–400 Bequerel/μg, the intra-assay coefficient of variation being 7.76%.

Western ligand blotting

Sera from five castrated animals collected before and after castration (5 weeks and 7 weeks postcastration) were subjected to ligand blotting. A 2.5-μL aliquot of each serum was mixed with 22.5-μL sample buffer [62.5 mM tris (pH 6.8), 5% SDS, 10% sucrose and 0.02% bromophenol blue], and heated to 60 °C for 10 min before being loaded onto a 12.5% discontinuous SDS-polyacrylamide gel. The samples were electrophoresed at 25 mamp (1.5–2.0 h), under nonreducing conditions. Protein M_r standards (Bio-Rad, Richmond, CA) were electrophoresed simultaneously in adjacent lanes. After electrophoresis, proteins were transferred to nitrocellulose membranes (0.2 μm; Schleicher and Schuell, Inc, Keene, NH) overnight at 200 mamp at 4 °C in Towbin buffer [25 mM Tris (pH 8.3), 192 mM glycine and 20% methanol] using a Hoefer (San Francisco, USA) electrophoresis transfer unit.

After the electrotransfer, the nitrocellulose membranes were air-dried, washed with TBS [10 mM Tris, (pH 7.4), 150 mM NaCl, and 0.05% sodium azide] and incubated with 1% nonfat dry milk in TBS on a rocking platform for 1 h at 24 °C. The membranes were washed with TBS containing 0.1% Tween-20 for 10 min and then incubated with [^{125}I] IGF-1 (0.6 × 10^6 cpm/mL) in TBS containing 0.1% Tween-20 and 1% BSA (Sigma) in plastic bags for 20–24 h at 4 °C on a rocking platform. After rinsing the membranes with Tween-20 and twice with TBS on a rocking platform for 15 min/wash, membranes were dried in air and exposed to X-Omat AR film (Eastman Kodak, Rochester, NY) at –80 °C for 7–14 days. IGFBPs were visualized by autoradiography and M_r estimated by comparison to prestained protein standards.

Testosterone and oestradiol-17ß assay

Serum testosterone and oestradiol levels were estimated by radioimmunoassay using specific kits (Code TRK 600, Amersham, UK and Kodak Amalide 8167356). The counting was carried out in a liquid scintillation counter programmed to calculate concentrations. The intra-assay coefficients of variation were 4.16 and 5.6%, and inter-assay coefficients of variation were 6.8 and 4.3%, respectively, as described earlier (Umapathy et al, 1993).

Collection of organs

The pigs were killed on the 7th week postcastration. The testes, the epididymis, the prostate, the seminal vesicles and the bulbourethral glands were dissected out, freed from adhering tissues and weighed. The liver, spleen, heart, kidneys and the pancreas were also dissected out, cleaned, blotted and weighed.

Statistical analysis

Data were analysed using Minitab statistical software, release 7, 8 and 9 and Students'*t*-test was

applied to compare means of hormonal concentrations between controls and castrates at each week of observation.

RESULTS

The influence of castration on live weight, growth rate and accessory sex gland weights are shown (table I). There was no significant change in the live weight gain between controls and castrates. However, the weights of prostate, seminal vesicles and bulbourethral glands were significantly decreased in the castrated group ($P < 0.01$). The weights of spleen and pancreas were increased in castrated pigs ($P < 0.01$).

The testosterone levels fell to zero 5 weeks postcastration ($P < 0.001$) (fig 1). The oestradiol concentrations were significantly lower in the 5th, 6th and 7th week postcastration samples ($P < 0.001$) compared to controls (fig 2).

IGF-I levels fluctuated between 45 and 62 ng/mL in both control and experimental pigs before castration. In the castrates, serum IGF-I concentrations showed a slight increase at 1 week postcastration ($P < 0.003$). Significant differences between control and castrate groups were seen in the 5th, 6th and 7th week samples ($P < 0.001$) (fig 3).

Ligand blot analysis revealed the presence of five IGFBP bands with apparent M_r of 43, 39, 34, 29 and 18 kDa in sera collected before and after castration (fig 4). The 43- and 39-kDa bands predominated in both pre- and postcastration samples.

DISCUSSION

The weight gain achieved by the entire and castrated pigs was low and live weight was well below the normal weight usually achieved by Large White × Landrace pigs at

Table I. Influence of castration on liveweight, growth rate and development of various organs at slaughter (means ± SEM).

| Parameters	Control	Castrated
Body weight	71.5 ± 2.4	73.4 ± 3.1
ADG (kg) (week 1)	0.07 ± 0.01	0.07 ± 0.01
(week 2)	0.2 ± 0.08	0.2 ± 0.07
(week 3)	0.3 ± 0.07	0.4 ± 0.09
(week 4)	0.4 ± 0.09	0.5 ± 0.09
(week 5)	0.4 ± 0.09	0.6 ± 0.08
(week 6)	0.6 ± 0.08	0.7 ± 0.09
(week 7)	0.5 ± 0.07	0.75 ± 0.09
Body weight (kg)	93.5 ± 3.4	100.4 ± 4.3
Liver (g)	1964.3 ± 212.3	1598.1 ± 198.6
Heart (g)	455.7 ± 10.8	434.2 ± 14.4
Kidneys (g)	271.3 ± 8.7	232.3 ± 6.8
Spleen (g)	110.4 ± 6.7	151.1 ± 7.5*
Pancreas (g)	115.3 ± 7.2	173.7 ± 10.3*
Seminal vesicles (g)	121.3 ± 12.3	62.6 ± 7.9*
Prostate (g)	10.7 ± 0.9	5.6 ± 0.2*
Bulbourethralglands (g)	124.9 ± 12.2	75.6 ± 8.9*

* $P < 0.01$.
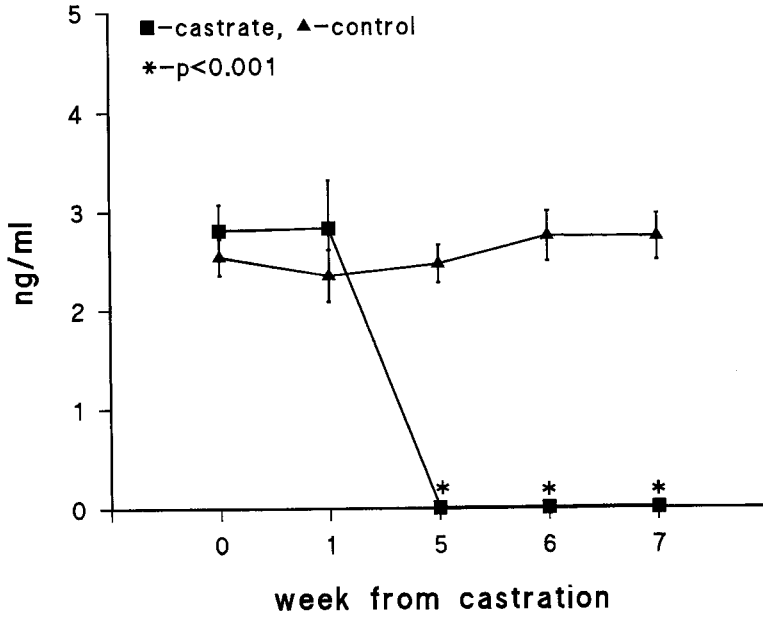
Fig 1. Effect of castration on serum testosterone.
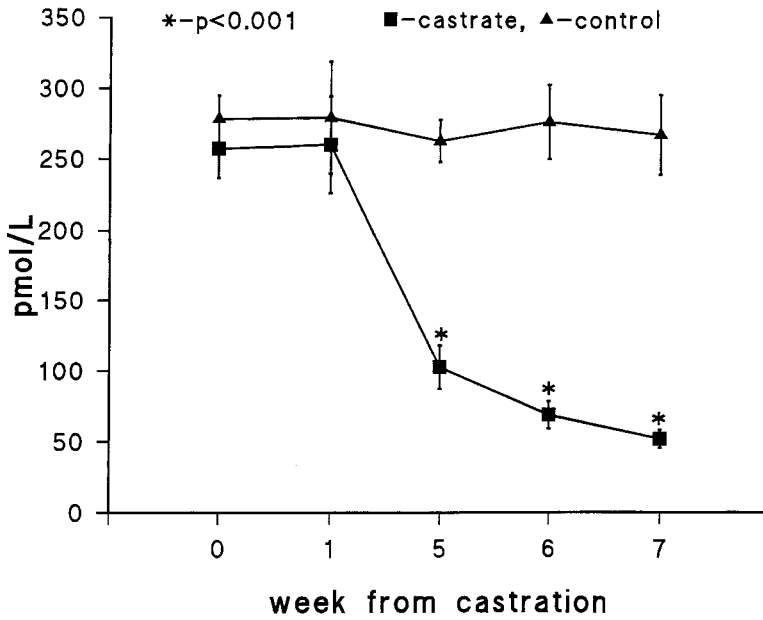

Fig 2. Effect of castration on serum estradiol.
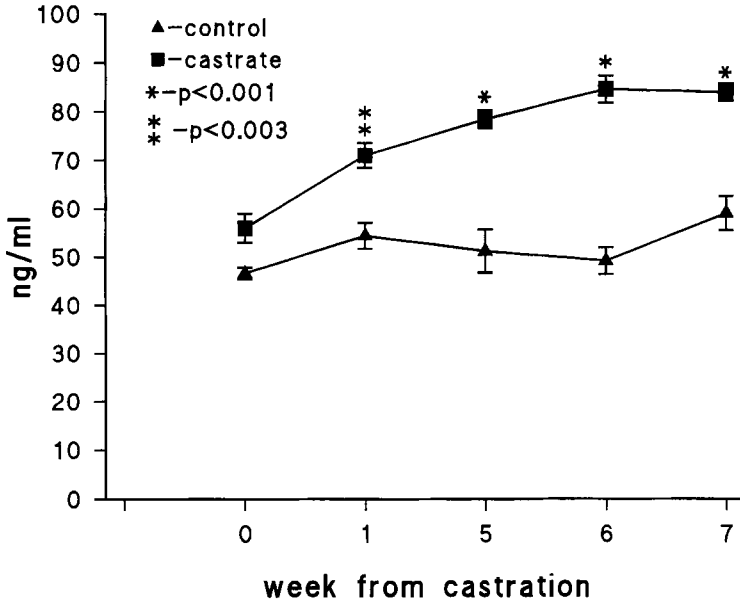
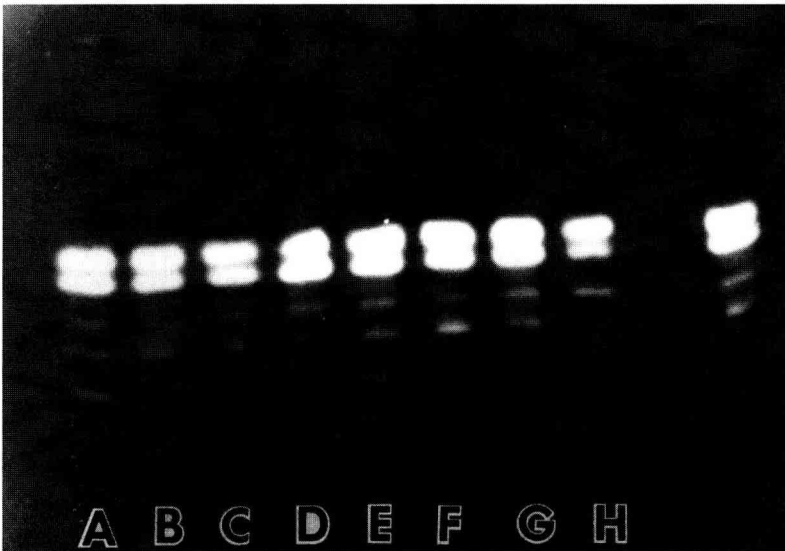**Fig 3.** Effect of castration on serum insulin-like growth factor-I.

Fig 4. Autoradiogram of a ^{125}I-labelled IGF-1 ligand blot of the porcine serum. Animal No 1, on the day of castration (lane A); 5 weeks after castration (lane B); 7 weeks after castration (lane C). Animal No 2, on the day of castration (lane D); 5 weeks after castration (lane E); 7 weeks after castration (lane F); Animal No 3, 7 weeks after castration (lane G). Animal No 4, 7 weeks after castration (lane H).

8–9 months. This is probably because these animals were fed a raw soybean-based diet in the initial stages of growth (from birth to about 7 months of age). Concomitant to these body weight changes, serum IGF-I levels were also much lower compared to other studies using males of similar ages or slightly younger males and the same method of extraction (Louveau et al, 1991). This is also probably due to the fact that our pigs were underfed from birth. Such a hypothesis agrees well with earlier reports showing that feed restriction is susceptible to a decrease in circulating IGF-I in pigs (Buonomo and Baile, 1991; Cosgrove et al, 1992; Charlton et al, 1993). Similarly, underfeeding significantly reduces plasma IGF-I concentrations in heifers (Spicer et al, 1992). Lower concentrations of IGF-I under energy deficit result probably from an uncoupling of IGF-I secretion from GH secretion (Thissen et al, 1994).

The decrease in the accessory sex gland weights and the fall in testosterone levels to zero in castrated pigs confirms earlier observations that the structural integrity and functional aspects of the accessory sex glands are dependent on the androgens (Negro-Vilar et al, 1977). Present data do not show a fall in serum testosterone levels 1 week after castration, which is contrary to results from Bonneau et al (1982), who reported a sharp decrease in testosterone levels as early as 1 day after castration. However, Bonneau et al (1982) have also observed that plasma testosterone declines more slowly in lean than in fatty boars. The leanness of the boars from the present study might explain why the fall in serum testosterone was very slow in our boars compared to previous results.

Higher weights of the spleen and of the pancreas in castrated pigs is, to our knowledge, a new finding. Although raw soybean feeding decreased pancreatic enzyme activities, it did not cause pancreatic hypertrophy (Yen et al, 1977). This is in contrast to the response noted in the chick (Yen et al, 1973) and in the rat (Yen et al, 1971). In the present study, pancreatic hypertrophy seen only in castrated pigs suggests that the mechanism of compensatory hypertrophy of the pancreas in pigs may interact with the presence of the testicular hormones.

In the present study, serum IGF-I levels increased in the operated pigs after castration and became higher than in controls. This effect of castration is contrary to that observed by Louveau et al (1991) and Arbona et al (1989) in male pigs. In rats, prepubertal castration has been shown to increase serum IGF-I levels and the involvement of hypothalamic and/or hepatic programming in this process has been proposed (Handelsman et al, 1987). The involvement of gonadotrophins in prepubertal rise of IGF-I has also been suggested in bulls (Renaville et al, 1996).

Discordance between our results and those of earlier reports in pigs could be attributed to the fact that Louveau et al (1991) as well as Arbona et al (1989) realized castration at a younger age than us, well before puberty (30 days of age for Louveau et al, 1991; just 1 week of age for Arbona et al, 1989). Therefore, a possible impuniting of the somatotrophin axis during pubertal development could have occurred in our study in contrast to previous ones. Factors such as nutritional status, insulin and thyroid hormones are important modulators of circulating IGF-I levels (Buonomo and Baile, 1991). Therefore, the effect of undernutrition should also be taken into account to explain the differences between studies. Since fasting or restricted feeding uncouple the GH/IGF-I axis (Thissen et al, 1994), the influence of steroid hormones on IGF-I secretion may be altered in our pigs suffering chronically from undernutrition.

The pattern of binding proteins in our boars was similar to that reported in the plasma of sows and younger pigs of unspec-

ified sex (McCusker et al, 1988; Lee et al, 1991) with the 43- and 39-kd proteins being predominant. The IGFBP pattern does not seem to be affected by castration, in spite of an increase in IGF-I levels. Independent variations in concentrations of testosterone, IGF-I and IGFBP-3 were also observed during the onset of puberty in bulls (Renaville et al, 1996). This suggests that IGFBP-3 is not affected by gonadal hormones.

CONCLUSION

Our experiment shows an unexpected increase in plasma IGF-I after castration in boars. Two hypotheses may explain this phenomenon: 1) the influence of testicular hormones is modified in slow-growing pigs; 2) testicular hormones have an impuniting effect and the influence of castration depends on the age at which it is performed.

ACKNOWLEDGEMENTS

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