ELSEVIER

Animal Reproduction Science 66 (2001) 47–58

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Sustained testicular atrophy in bulls actively immunized against GnRH: potential to control carcase characteristics

Michael J. D'Occhio [a,*], William J. Aspden [a], Timothy E. Trigg [b]

[a] *Animal Sciences and Production Group, Central Queensland University, Bruce Highway, North Rockhampton, Queensland 4702, Australia*

[b] *Peptech Animal Health Pty Limited, North Ryde, Sydney, N.S.W. 2113, Australia*

Received 16 November 1999; received in revised form 2 January 2001; accepted 24 January 2001

Abstract

The objectives were to determine whether active immunization against gonadotrophin releasing hormone (GnRH) induced a long-term suppression of testicular function in bulls, and to ascertain the effects of immunization against GnRH on carcase and meat quality characteristics. In experiment 1, 6-month-old Zebu bulls were assigned to: control ($n = 25$), no treatment; immunized ($n = 31$), immunized against GnRH at 0 and 4 months (anti-$GnRH_2$), with a sub-set of bulls ($n = 17$) immunized again at 10 months (anti-$GnRH_3$). After the second immunization, testicular growth ceased for 2 months in 14/31 (45%) bulls and for at least 6 months in 17/31 (55%) bulls. Among the latter bulls (anti-$GnRH_3$) the testes did not grow for >1 year after the third immunization in 5/17 (30%) bulls. In experiment 2, 22-month-old Zebu bulls were assigned to: control ($n = 14$), no treatment; immunized ($n = 17$), immunized against GnRH at 0, 2 and 4 weeks. The testes decreased ($P < 0.05$) in size for 2 months after immunization in 11/17 (65%) bulls and then re-initiated growth, whilst in 6/17 (35%) bulls the testes continued to decrease in size for 4 months and did not re-initiate growth for 1 year. At slaughter, the latter immunocastrated bulls had carcase and meat quality characteristics the same as contemporary bulls that had been castrated before puberty. The findings demonstrated that active immunization against GnRH can induce a long-term suppression of testicular function in a proportion of bulls. Also, when bulls are immunocastrated after puberty, carcase and meat quality traits change from those typical of entire bulls to traits that are characteristic of long-term castrated bulls.

Keywords: GnRH immunization; Bull; Testes; Carcase; Meat quality

* Corresponding author. Tel.: +61-7-49306900; fax: +61-7-49309209.
E-mail address: m.docchio@cqu.edu.au (M.J. D'Occhio).

0378-4320/01/$ – see front matter
PII: S0378-4320(01)00091-4

1. Introduction

Gonadotrophin releasing hormone (GnRH) is released at the basal hypothalamus-median eminence and acts at the anterior hypophysis to stimulate the secretion of LH and FSH (D'Occhio, 1993, 1994). Accordingly, immunization against GnRH induces a hypogonadotrophic condition that is associated with gonadal atrophy (D'Occhio 1993, 1994). The potential to use immunization against GnRH as an alternative to gonadectomy for the control of sexual and aggressive behaviors, and fertility, has received particular attention in the major livestock species including cattle (Robertson et al., 1979, 1981, 1982, 1984; Adams and Adams, 1990; Hoskinson et al., 1990; Adams et al., 1993; Finnerty et al., 1994; Bell et al., 1997; Jago et al., 1997; Jeffery et al., 1997; Finnerty et al., 1998; Huxsoll et al., 1998), goats (Godfrey et al., 1996), pigs (Caraty and Bonneau, 1986; Molenaar et al., 1993; Meloen et al., 1994) and sheep (Clarke et al., 1978; Schanbacher, 1982; Brown et al., 1994, 1995; Clarke et al., 1998). In most studies, active immunization against GnRH induced only a temporary suppression of reproductive functions, after which animals returned to normal fertility (D'Occhio 1993, 1994). A return to normal fertility subsequent to immunization against GnRH is due to a decline in circulating anti-GnRH antibodies below a threshold required to neutralize GnRH in hypophysial portal blood.

In two studies in sheep, transient active immunization against GnRH was associated with a long-term suppression of reproduction. For example, 20–25% of rams immunized against GnRH before either 3 months or 7 months of age continued to have regressed testes at 2 years of age (Brown et al., 1994). When ewes were immunized at similar ages as above, only 20% displayed oestrous behavior at 2 years of age (Brown et al., 1995). In these studies, long-term suppression of reproductive function was not due to the maintenance of anti-GnRH antibodies in circulation in rams (Brown et al., 1994) or ewes (Brown et al., 1995). It would appear, therefore, that the transient induction of anti-GnRH antibodies, perhaps exclusively early in life, can induce a permanent suppression of reproduction. The basis for a sustained suppression of reproductive function subsequent to transient immunization against GnRH is poorly understood. However, ewes immunized against GnRH before 2 months of age showed reduced GnRH secretion at 3–4 years, at which time anti-GnRH antibodies could not be detected (Clarke et al., 1998). Also, boars immunized against GnRH at around 4 months of age had abnormalities of the median eminence at 6 months that included hypertrophy of the magnocellular neurons and infiltration of fibroblasts (Molenaar et al., 1993). It could be suggested from the latter studies that the basal hypothalamus-median eminence is a primary target site for anti-GnRH antibodies.

There is considerable interest in the potential use of GnRH immunization as an alternative to gonadectomy to control aggressive and sexual behaviors, fertility and meat quality in cattle. Accordingly, many studies have been conducted in bulls (Robertson et al., 1979, 1981, 1982, 1984; Adams et al., 1993; Finnerty et al., 1994; Jago et al., 1997; Finnerty et al., 1998; Huxsoll et al., 1998) and heifers (Adams and Adams, 1990; Hoskinson et al., 1990; Bell et al., 1997; Jeffery et al., 1997). Most studies in cattle have been of relatively short duration and long-term suppression of reproductive function after active immunization against GnRH has not been reported. Long-term suppression of gonadal activity is particularly relevant in cattle, however, as the majority of the world's cattle are managed for relatively long periods before marketing. The experiments in the present study examined whether transient

active immunization against GnRH, either before or after puberty, could induce a long-term suppression of testis growth and testosterone secretion in bulls. A second area addressed in the present study was whether immunocastration of bulls after puberty has the potential to allow the control of carcase type and meat quality characteristics.

2. Materials and methods

2.1. Animals and management

The animals used in the present study were Zebu (Brahman, *Bos indicus*) or Zebu crossbred. They were maintained on natural pastures and standard management except when required for experimental procedures.

2.2. GnRH vaccine

Bulls were immunized against GnRH using a previously described vaccine formulation (Hoskinson et al., 1990). In brief, a GnRH-ovalbumin conjugate was dissolved in saline and emulsified in an immunoadjuvant mixture that comprised DEAE-dextran, Arlocel 80 and Ondena mineral oil. Immunizations were subcutaneous on the dorsal surface of the neck.

2.3. Experimental design: experiment 1

About 6-month-old Zebu (Brahman, *Bos indicus*) and Zebu crossbred bulls were block randomized on liveweight (184 ± 3 kg) and genotype into two groups: control ($n = 25$), no treatment; anti-$GnRH_2$ ($n = 31$), received a primary and secondary immunization against GnRH at 0 (6-month-old) and 4 months, respectively. Bulls that had the highest anti-GnRH antibody titres two weeks after secondary immunization were given a tertiary immunization at 10 months of the experiment (anti-$GnRH_3$, $n = 17$). Diameters (mm) of the left and right testes were recorded using calipers at approximately monthly intervals during the experiment. Blood samples for testosterone assay were collected at 0, 6, 12, 17 and 21 months, and live weights were recorded at the same times. A single blood sample for anti-GnRH antibody titer measurement was collected 2 weeks after secondary immunization. All bulls were slaughtered at 25 months of the experiment (31 months of age) and the left and right testis weights recorded.

2.4. Experimental design: experiment 2

Zebu crossbred bulls (22-month-old) were block randomized on liveweight (363 ± 5 kg) into two groups: control ($n = 14$), no treatment; anti-$GnRH_3$ ($n = 17$), received a primary, secondary and tertiary immunization against GnRH at 0, 2 and 4 weeks, respectively. Six contemporary steers (castrated bulls) were included for comparisons of carcase characteristics at the end of the experiment. Diameters (mm) of the left and right testes of all bulls were recorded at 0, 4, 7, 14, 28, 32, 45, 50 and 54 weeks of the experiment. Blood samples for the assay of plasma concentrations of testosterone were collected at 0, 14, 32 and 54

weeks, at which times live weights were also recorded. Bulls and steers were slaughtered at 56 weeks of the experiment (approximately 36 months of age) and the left and right testes weights were recorded for bulls. Carcase characteristics and features of the *longissimus dorsi* muscle (Jeffery et al., 1997) were determined for six randomly selected control bulls, six contemporary steers and six immunized bulls that showed long term suppression of testicular size.

2.5. Testosterone assay

Plasma concentrations of testosterone were measured using an extraction, single-antibody radioimmunoassay (D'Occhio and Brooks, 1983). Intra- and inter-assay coefficients of variation were <10% based on duplicate samples. Sensitivity of the assay was 0.1 ng testosterone/ml.

2.6. Anti-GnRH antibody titres

In experiment 1, anti-GnRH antibody titres were determined as previously described, with titre defined as the dilution of sample plasma that bound 50% of a constant amount of ^{125}I-GnRH (Hoskinson et al., 1990).

2.7. Statistical analyses

Data at individual time points were analyzed by ANOVA using the general linear models (GLM) procedure of SAS/STAT (1990). Data analyses over time were done by repeated measures analysis using the MIXED procedure of SAS/STAT (1992) with REML estimation (autoregressive-1) and the model being y = treatment, time, treatment × time, with animal as the repeated subject. The CONTRAST statements of SAS/STAT GLM and MIXED were used for comparison between treatment group means. Where relevant, data were $\log_{10}$ transformed to achieve homogeneity of variances. For the purpose of analyzing the testicular data for long term effects of GnRH immunization on testis size, bulls in group anti-GnRH$_3$ in experiments 1 and 2 were further categorized into bulls with an average of left and right testis weight >100 g (anti-GnRH$_{3a}$) and bulls with an average of left and right testis weight <100 g (anti-GnRH$_{3b}$). All results are reported as untransformed arithmetic means±S.E.M.

3. Results

3.1. Experiment 1

About 2 weeks after secondary immunization against GnRH, anti-GnRH$_2$ bulls had higher ($P < 0.05$) anti-GnRH antibody titres compared with control bulls (2455±515 and 109±4, respectively), and both of these groups had lower ($P < 0.05$) antibody titres compared with anti-GnRH$_{3a}$ (9212 ± 3649) and anti-GnRH$_{3b}$ (11,839 ± 3366) bulls, which did not differ.

Results for testis diameter are shown in Fig. 1 and summarized in Table 1. At commencement of the experiment, there were no differences ($P > 0.05$) in testis diameter

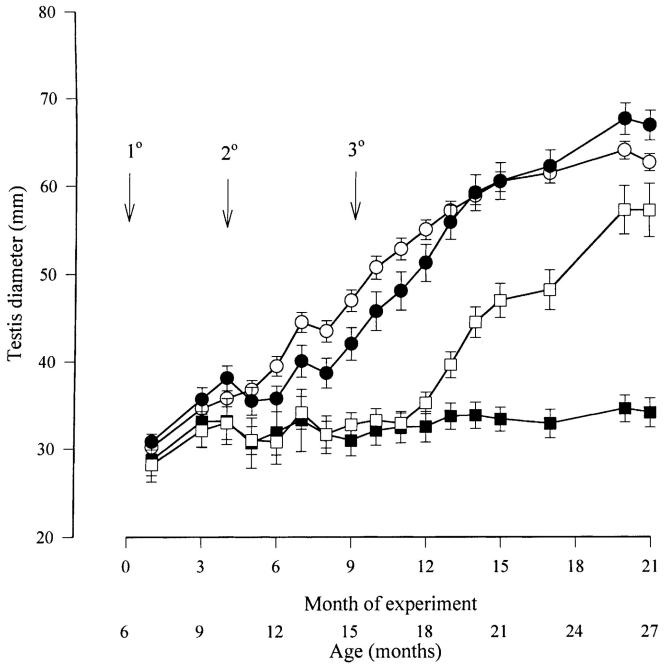

Fig. 1. Longitudinal changes in testis diameter (means ± S.E.M.) for control bulls (○), bulls that received a primary (1°) and secondary (2°) immunization against GnRH (●, anti-$GnRH_2$), bulls that received a 1, 2 and tertiary (3°) immunization against GnRH and had a testis weight >100 g at slaughter (□, anti-$GnRH_{3a}$), and bulls that received a 1, 2 and 3 immunization against GnRH and had a testis weight <100 g at slaughter (■, anti-$GnRH_{3b}$) in experiment 1. Arrows indicate the time of 1, 2 and 3° immunization.

between control bulls and bulls immunized against GnRH. Bulls that received a primary and secondary immunization against GnRH (group anti-$GnRH_2$) had a smaller ($P < 0.05$) testis diameter compared with control bulls from 6 to 12 months of the experiment, but these groups did not differ at 17 and 21 months (Fig. 1). Bulls in group anti-$GnRH_3$ that

Table 1
Testis diameter (means ± S.E.M., mm) for control bulls, bulls that received a primary (1°) and secondary (2°) immunization against GnRH (anti-$GnRH_2$), bulls that received a 1, 2 and tertiary (3°) immunization against GnRH and had a testis weight >100 g at slaughter (anti-$GnRH_{3a}$) and bulls that received a 1, 2 and 3° immunization against GnRH and had a testis weight <100 g at slaughter (anti-$GnRH_{3b}$) in experiment 1

Group	Month of experiment[a]					
	n	0	6	12	17	21
Control	25	30 ± 1 a; v	39 ± 1 a; w	55 ± 1 a; x	61 ± 1 a; y	63 ± 1 a,b; z
Anti-$GnRH_2$	14	31 ± 1 a; v	36 ± 1 a,b; w	51 ± 1 b; x	62 ± 1 a; y	67 ± 1 a; z
Anti-$GnRH_{3a}$	12	28 ± 1 a; v	31 ± 1 c; v,w	35 ± 1 c; w	48 ± 2 b; x	57 ± 3 b; y
Anti-$GnRH_{3b}$	5	29 ± 2 a; v	32 ± 4 b,c; v	33 ± 2 c; v	33 ± 2 c; v	34 ± 2 c; v
P value		0.314	<0.001	<0.001	<0.001	<0.001

[a] Means within columns without a common letter (a, b, c) differ ($P < 0.05$), means within rows without a common letter (v, w, x, y, z) differ ($P < 0.05$).

Table 2
Plasma concentrations of testosterone (means ± S.E.M., ng/ml) for control bulls, bulls that received a primary (1°) and secondary (2°) immunization against GnRH (anti-$GnRH_2$), bulls that received a 1, 2 and tertiary (3°) immunization against GnRH and had a testis weight >100 g at slaughter (anti-$GnRH_{3a}$) and bulls that received a 1, 2 and 3° immunization against GnRH and had a testis weight <100 g at slaughter (anti-$GnRH_{3b}$) in experiment 1

	Month of experiment[a]					
Group	*n*	0	6	12	17	21
Control	25	0.4 ± 0.1 a; x	1.4 ± 0.4 a; y	1.0 ± 0.2 a; y	2.5 ± 0.4 a,b; z	2.4 ± 0.4 a; z
Anti-$GnRH_2$	14	0.5 ± 0.1 a; x	0.7 ± 0.3 a; x	1.5 ± 0.4 a; y	3.5 ± 0.9 a; z	2.5 ± 0.6 a; z
Anti-$GnRH_{3a}$	12	0.4 ± 0.2 a; x	0.2 ± 0.1 b; x	0.4 ± 0.2 b; x	2.0 ± 0.6 b; y	2.7 ± 0.8 a; y
Anti-$GnRH_{3b}$	5	0.3 ± 0.1 a; x	0.2 ± 0.1 b; x,y	0.1 ± 0.0 c; y	0.1 ± 0.0 c; y	0.1 ± 0.0 b; y
P value		0.646	<0.001	<0.001	<0.001	<0.001

[a] Means within columns without a common letter (a, b, c) differ ($P < 0.05$), means within rows without a common letter (x, y, z) differ ($P < 0.05$).

were given a primary, secondary and tertiary immunization against GnRH had a smaller ($P < 0.05$) testis diameter than both control bulls and anti-$GnRH_2$ bulls at 6, 12 and 17 months. From 12 months onwards, anti-$GnRH_3$ bulls diverged into two groups based on testis growth. The majority of the latter bulls had an increase in testis diameter from 12 to 21 months (anti-$GnRH_{3a}$, $n = 12$), whilst testis diameter remained suppressed in the remaining bulls (anti-$GnRH_{3b}$, $n = 5$) (Fig. 1, Table 1). At the end of the experiment, anti-$GnRH_{3b}$ bulls had a smaller ($P < 0.001$) testis diameter compared with bulls in the other three groups, which did not differ from each other (Table 1).

At slaughter, testis weight (average of left and right testes) for control bulls (206 ± 6 g) did not differ ($P > 0.05$) from anti-$GnRH_2$ bulls (242 ± 16 g) and anti-$GnRH_{3a}$ bulls (180±6 g), whilst the latter two groups did differ ($P < 0.05$). Testis weight for anti-$GnRH_{3b}$ bulls (54 ± 15 g) was lighter ($P < 0.05$) than the other three groups. All bulls in the control, anti-$GnRH_2$, and anti-$GnRH_{3a}$ groups had a testis weight >100 g.

Plasma concentrations of testosterone did not differ ($P > 0.05$) throughout the experiment between control bulls and anti-$GnRH_2$ bulls (Table 2). Anti-$GnRH_3$ bulls had lower ($P < 0.05$) plasma concentrations of testosterone compared with control bulls at 6, 12 and 17 months, whilst anti-$GnRH_{3b}$ bulls had lower ($P < 0.05$) plasma testosterone than control, anti-$GnRH_2$ and anti-$GnRH_{3a}$ bulls at 12, 17 and 21 months (Table 2).

There were no treatment effects ($P > 0.05$) on changes in live weight during the experiment (Table 3).

3.2. Experiment 2

Longitudinal changes in testis diameter for control bulls and bulls immunized against GnRH are shown in Fig. 2 and the results are summarized in Table 4. At the start of the experiment there were no differences ($P > 0.05$) in testis diameter between control bulls and bulls immunized against GnRH. Testis diameter declined after immunization against GnRH, and at week 14, anti-$GnRH_3$ bulls had a smaller ($P < 0.05$) testis diameter than control bulls. After 14 weeks, the anti-$GnRH_3$ bulls diverged into two groups. In one group of immunized bulls (anti-$GnRH_{3a}$, $n = 11$), testis diameter started to increase, and by week

Table 3
Liveweight (means ± S.E.M., kg) for control bulls, bulls that received a primary (1°) and secondary (2°) immunization against GnRH (anti-$GnRH_2$), bulls that received a 1, 2 and tertiary (3°) immunization against GnRH and had a testis weight >100 g at slaughter (anti-$GnRH_{3a}$) and bulls that received a 1, 2 and 3° immunization against GnRH and had a testis weight <100 g at slaughter (anti-$GnRH_{3b}$) in experiment 1

Group	Month of experiment[a]					
	n	0	6	12	17	21
Control	25	187 ± 5 a	228 ± 6 b	341 ± 7 c	403 ± 7 d	490 ± 8 e
Anti-$GnRH_2$	14	191 ± 6 a	229 ± 8 b	340 ± 8 c	407 ± 10 d	491 ± 12 e
Anti-$GnRH_{3a}$	12	171 ± 6 a	212 ± 8 b	316 ± 10 c	400 ± 13 d	478 ± 14 e
Anti-$GnRH_{3b}$	5	178 ± 13 a	216 ± 18 b	333 ± 18 c	423 ± 14 d	492 ± 14 e
P value		0.268	0.434	0.211	0.686	0.850
Pooled	56	184 ± 3 a	224 ± 4 b	335 ± 5 c	405 ± 5 d	487 ± 6 e

[a] Means within columns with a common letter (a, b, c, d, e) do not differ ($P > 0.05$), means within rows without a common letter (a, b, c, d, e) differ ($P < 0.05$).

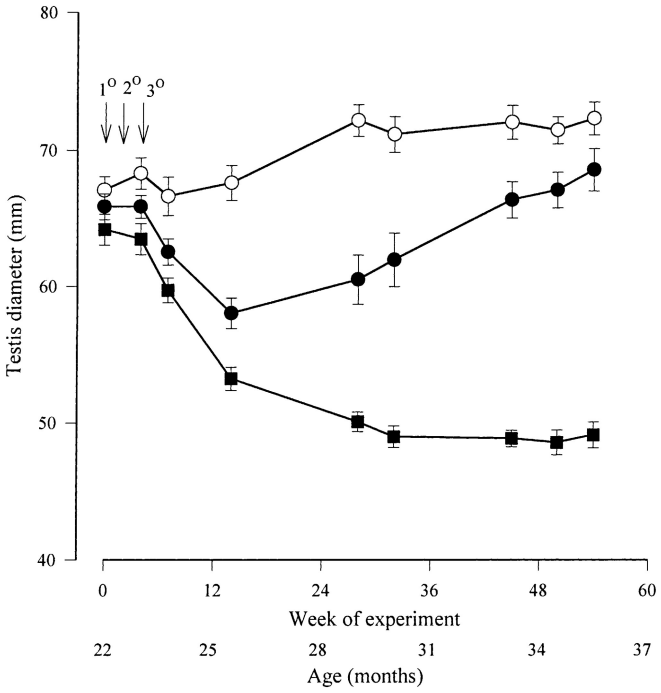

Fig. 2. Longitudinal changes in testis diameter (means±S.E.M.) for control bulls (○,), bulls that received a primary (1°), secondary (2°) and tertiary (3°) immunization against GnRH and had a testis weight >100 g at slaughter (●, anti-$GnRH_{3a}$), and bulls that received a 1, 2 and 3° immunization against GnRH and had a testis weight <100 g at slaughter (■, anti-$GnRH_{3b}$) in experiment 2. Arrows indicate the time of 1, 2 and 3° immunization.

Table 4
Testis diameter (means ± S.E.M., mm) for control bulls, bulls that received a primary (1°), secondary (2°) and tertiary (3°) immunization against GnRH and had a testis weight >100 g at slaughter (anti-$GnRH_{3a}$) and bulls that received a 1, 2 and 3° immunization against GnRH and had a testis weight <100 g at slaughter (anti-$GnRH_{3b}$) in experiment 2

Group	Week of experiment[a]				
	n	0	14	32	54
Control	14	67 ± 1 a; x	68 ± 1 a; x	71 ± 1 a; y	72 ± 1 a; y
Anti-$GnRH_{3a}$	11	66 ± 1 a; x,z	58 ± 1 b; y	62 ± 2 b; x	69 ± 2 a; z
Anti-$GnRH_{3b}$	6	64 ± 1 a; x	53 ± 1c; y	49 ± 1 c; z	49 ± 1 b; z
P value		0.224	<0.001	<0.001	<0.001

[a] Means within columns without a common letter (a, b, c) differ ($P < 0.05$), means within rows without a common letter (x, y, z) differ ($P < 0.05$).

54, testis diameter for anti-$GnRH_{3a}$ bulls did not differ ($P > 0.05$) from that of control bulls (Table 4). For the second group of bulls (anti-$GnRH_{3b}$, $n = 6$), testis diameter continued to decline to around week 30 after which time it remained relatively constant to week 54. At week 54, testis diameter for anti-$GnRH_{3b}$ bulls (49 ± 1 mm) was smaller ($P < 0.001$) than that of control bulls (72 ± 1 mm) and anti-$GnRH_{3a}$ bulls (69 ± 2 mm).

Testis weight (average of left and right testes) at slaughter (week 56) did not differ ($P > 0.05$) for control bulls (284 ± 11 g) and anti-$GnRH_{3a}$ bulls (266 ± 31 g), whilst testis weight of anti-$GnRH_{3b}$ bulls (78 ± 4 g) was lighter ($P < 0.001$) than both control and anti-$GnRH_{3a}$ bulls. All control and anti-$GnRH_{3a}$ bulls had a testis weight >100 g.

Plasma concentrations of testosterone at the start of the experiment did not differ ($P > 0.05$) between control bulls and bulls immunized against GnRH (Table 5). Plasma testosterone in anti-$GnRH_{3a}$ bulls declined to concentrations typical of castrated bulls at week 14 (control, 5.6 ± 2.4 ng/ml; anti-$GnRH_{3a}$, 0.3 ± 0.1 ng/ml; $P < 0.05$). Anti-$GnRH_{3a}$ bulls showed an increase in plasma testosterone after week 14, commensurate with an increase in testis size. Plasma testosterone in anti-$GnRH_{3b}$ bulls was below the minimum detectable concentration (0.1 ng/ml) from week 14 to week 54 (Table 5).

Control bulls and bulls immunized against GnRH showed a progressive increase in live weight during the experiment and there was no effect ($P > 0.05$) of treatment on live

Table 5
Plasma concentrations of testosterone (means ± S.E.M., ng/ml) for control bulls, bulls that received a primary (1°), secondary (2°) and tertiary (3°) immunization against GnRH and had a testis weight >100 g at slaughter (anti-$GnRH_{3a}$) and bulls that received a 1, 2 and 3° immunization against GnRH and had a testis weight <100 g at slaughter (anti-$GnRH_{3b}$) in experiment 2

Group	Week of experiment[a]				
	n	0	14	32	54
Control	14	6.3 ± 2.3 a; x	5.6 ± 2.4 a; x	3.1 ± 0.6 a; x	4.6 ± 1.4 a; x
Anti-$GnRH_{3a}$	11	5.2 ± 2.3 a; x	0.3 ± 0.1 b; y	2.4 ± 0.7 a; x	1.3 ± 0.3 b; x
Anti-$GnRH_{3b}$	6	3.6 ± 1.6 a; x	0.1 ± 0.0 b; y	0.1 ± 0.0 b; y	0.1 ± 0.0 c; y
P value		0.743	<0.001	<0.001	<0.001

[a] Means within columns without a common letter (a, b, c) differ ($P < 0.05$), means within rows without a common letter (x, y, z) differ ($P < 0.05$).

Table 6
Liveweight (means ± S.E.M., kg) for control bulls, bulls that received a primary (1°), secondary (2°) and tertiary (3°) immunization against GnRH and had a testis weight >100 g at slaughter (anti-$GnRH_{3a}$) and bulls that received a 1, 2 and 3° immunization against GnRH and had a testis weight <100 g at slaughter (anti-$GnRH_{3b}$) in experiment 2

Group	Week of experiment[a]				
	n	0	14	32	54
Control	14	360 ± 8 a	424 ± 8 b	525 ± 8 c	528 ± 9 c
Anti-$GnRH_{3a}$	11	360 ± 7 a	413 ± 8 b	516 ± 9 c	527 ± 9 c
Anti-$GnRH_{3b}$	6	374 ± 12 a	426 ± 12 b	505 ± 14 c	516 ± 15 c
P value		0.543	0.552	0.398	0.767
Pooled	31	363 ± 5 a	420 ± 5 b	518 ± 5 c	525 ± 6 c

[a] Means within columns with a common letter (a, b, c) do not differ ($P > 0.05$), means within rows without a common letter (a, b, c) differ ($P < 0.05$).

Table 7
Carcase and *logissimus dorsi* muscle characteristics (means ± S.E.M.) for representative steers, control bulls and bulls in group anti-$GnRH_{3b}$ in experiment 2

Group	*n*	Carcase weight (kg)	Dressing (%)	Carcase fat depth (mm)	*longissimus dorsi*[a]	
					Fat (%)	Marbling
Bulls	6	261 ± 6 a	48.6 ± 0.9 a	1.5 ± 0.3 a	0.3 ± 0.03 a	1.25 ± 0.11 a
Anti-$GnRH_{3b}$	6	246 ± 6 a	48.7 ± 0.8 a	5.0 ± 1.7 b	1.4 ± 0.03 b	1.91 ± 0.15 b
Steers	6	259 ± 4 a	49.7 ± 0.8 a	6.5 ± 1.2 b	1.1 ± 0.10 c	1.50 ± 0.12 a
P value		0.140	0.622	<0.001	<0.001	0.009

[a] Means within columns without a common letter (a, b, c) differ ($P < 0.05$).

weight (Table 6). Carcase weight and dressing percentage also did not differ ($P > 0.05$) between control bulls, anti-$GnRH_{3b}$ bulls and steers (Table 7). Anti-$GnRH_{3b}$ bulls had a similar carcase fat depth as steers which was greater ($P < 0.001$) than the carcase fat depth in control bulls. Marbling and percentage fat of the *Longisimus dorsi* muscle were similar for anti-$GnRH_{3b}$ bulls and steers (Table 7).

4. Discussion

Immunization of bulls against GnRH before puberty caused a retardation of testicular growth. In bulls that had relatively low anti-GnRH antibody titres (14/31 (45%) bulls) testicular growth ceased for approximately 2 months after secondary immunization and then showed a pattern of growth similar to that of non-immunized bulls. For bulls that had relatively high antibody titres (17/31 (55%) bulls) growth of the testes was prevented for 6 months after secondary immunization, at which time these bulls received a tertiary immunization against GnRH. Following tertiary immunization, testicular growth was re-initiated after 3 months in 12/17 (70%) bulls, whilst 5/17 (30%) bulls (16% of original immunized group) did not show any testicular growth for >1 year after tertiary immunization. In sexually mature bulls, immunization against GnRH caused a regression in testis size and

suppression of plasma testosterone to concentrations typical of steers. In 11/17 (65%) of these bulls, the testes decreased in size for around 2 months and then gradually returned to the pre-immunization size. For 6/17 (35%) bulls, the testes continued to decrease in size for 4 months and did not show any re-initiation of growth for 1 year after immunization.

The proportion (16–35%) of bulls that showed a long-term suppression of testicular function after immunization against GnRH in the present study was similar to the proportion (20–25%) of rams immunized against GnRH early in life that had regressed testes at 2 years (Brown et al., 1994). In the latter study, anti-GnRH antibody titres could not be detected at 2 years of age in rams previously immunized against GnRH. Anti-GnRH antibody titres were not measured at the conclusion of the experiments in the present study. However, from observations in related studies in cattle using the same GnRH-ovalbumin immunogen formulation, it was considered unlikely that bulls would have measurable antibody titres 1 year after immunization against GnRH (Hoskinson et al., 1990). It was concluded, therefore, that transient active immunization against GnRH can induce a long-term, and possibly permanent, suppression of reproductive function in bulls. Continuing suppression of reproduction occurred in a proportion of bulls immunized against GnRH either before or after puberty which indicated that the phenomenon is not age-related in cattle.

The mechanism for sustained suppression of reproductive function after active immunization against GnRH is poorly understood. Ewes actively immunized against GnRH before 2 months of age had normal levels of GnRH in the median eminence, but showed reduced secretion of GnRH into hypophysial portal blood, at 3–4 years of age (Clarke et al., 1998). Boars immunized against GnRH between 3 and 4 months of age showed pathological changes in the median eminence at around 6 months that included oedemas, hypertrophy of the magnocellular neurons, and infiltration of fibroblasts (Molenaar et al., 1993). These preliminary findings in sheep and swine would suggest that the basal hypothalamus-median eminence is a primary target site for anti-GnRH antibodies. This might be anticipated as GnRH secreting neurones terminate in this region of the brain, which is outside the blood-brain barrier. An understanding of the mechanisms that lead to an apparent disruption of the integrity of the basal hypothalamus-median eminence is of fundamental importance. This information would direct future research on immunogen formulation and immunization strategies that induced a long-term suppression of reproduction in the majority of immunized animals. In experiment 1, continued suppression of testicular growth after secondary immunization predictably occurred in bulls with the highest anti-GnRH antibody titres. It is possible, therefore, that the effects of anti-GnRH antibodies at tissues in the basal hypothalamus-median eminence are dependent on the attainment of a threshold level of antibodies in hypophysial portal blood. An ideal response to immunization against GnRH in cattle would appear to involve two phases; a short-term (months) direct immunoneutralization of GnRH in circulation by anti-GnRH antibodies and a longer-term (years) disruption of tissues in the median eminence associated with GnRH secretion into hypophysial portal blood.

A component of the present study examined the effects of active immunization of sexually mature bulls against GnRH on carcase and meat characteristics. In experiment 2, bulls that showed a long-term suppression of testis function after immunization at 22 months of age had carcase and meat quality characteristics the same as steers when slaughtered at 36 months of age. Suppression of testicular steroid secretion in mature bulls by immunization

against GnRH, therefore, caused body composition to change from that typical of entire bulls to that of castrated bulls. In experiment 2, this change occurred over a period of around 1 year. It could be predicted that graded changes in body composition between that of entire and castrated bulls would be achieved if mature bulls were slaughtered at different times after immunization against GnRH. This approach may provide a technology for improved control of carcase and meat quality traits in both male and female cattle. In bulls, it should be possible to utilize the increased growth potential of entire bulls early in life, and to then apply immunocastration at an appropriate time to influence behavior and to also control carcase and meat quality traits before slaughter.

5. Conclusions

The present study has shown that active immunization of bulls against GnRH, before or after puberty, can result in a long-term suppression of reproductive function in a proportion of animals. Other preliminary studies in sheep and swine have provided evidence that sustained suppression of gonadal function after immunization against GnRH is associated with a disruption of the integrity of the basal hypothalamus-median eminence. It will be necessary to establish the mechanisms that induce an apparent permanent disruption of GnRH secretion subsequent to immunization against GnRH, as there are important potential applications in production, recreational and domestic animals. An application demonstrated in the present study is the ability to closely control carcase and meat quality characteristics in cattle by active immunization against GnRH after puberty.

Acknowledgements

The authors thank Mr. G. Halford and Mr. J. Quilty ('Belmont' National Cattle Breeding Station) for expert management of the animals and assistance in experimental procedures. The cooperation of the Queensland Department of Primary Industries (Queensland Beef Industry Institute) and assistance of staff at 'Brigalow' Research Station are gratefully acknowledged.

References

Adams, T.E., Adams, B.M., 1990. Reproductive function and feedlot performance of beef heifers actively immunized against GnRH. J. Anim. Sci. 68, 2793–2802.

Adams, T.E., Daley, C.A., Adams, B.M., Sakurai, H., 1993. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of implants containing progesterone and estradiol benzoate. J. Anim. Sci. 71, 811–817.

Bell, M., Daley, C.A., Berry, S.L., Adams, T.E., 1997. Pregnancy status and feedlot performance of beef heifers actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 75, 1185–1189.

Brown, B.W., Mattner, P.E., Carroll, P.A., Holland, E.J., Paull, D.R., Hoskinson, R.M., Rigby, R.D.G., 1994. Immunization of sheep against GnRH early in life: effects on reproductive function and hormones in rams. J. Reprod. Fert. 101, 15–21.

Brown, B.W., Mattner, P.E., Carroll, P.A., Hoskinson, R.M., Rigby, R.D.G., 1995. Immunization of sheep against GnRH early in life: effects on reproductive function and hormones in ewes. J. Reprod. Fert. 103, 131–135.

Caraty, A., Bonneau, M., 1986. Active immunization of male pigs against gonadoliberin: effect on the secretion of gonadotropic hormones and on 5 alpha-androst-16-ene-one levels in adipose tissue. C.R. Acad. Sci. III 303, 673–676.

Clarke, I.J., Fraser, H.M., McNeilly, A.S., 1978. Active immunization of ewes against luteinizing hormone releasing hormone, and its effects on ovulation and gonadotrophin, prolactin and ovarian steroid secretion. J. Endocrinol. 78, 39–47.

Clarke, I.J., Brown, B.W., Tran, V.V., Scott, C.J., Fry, R., Millar, R.P., Rao, A., 1998. Neonatal immunization against gonadotropin-releasing hormone (GnRH) results in diminished GnRH secretion in adulthood. Endocrinology 139, 2007–2014.

D'Occhio, M.J., 1993. Immunological suppression of reproductive functions in male and female mammals. Anim. Reprod. Sci. 33, 345–372.

D'Occhio, M.J., 1994. Endocrine, gamete and conceptus antigens as targets for immunocontraceptive vaccines: applications in farm animals. In: Wood, P.R., Willadsen, P., Vercoe, J.E., Hoskinson, R.M., Demeyer, D. (Eds.), Vaccines in Agriculture: Immunological Implications to Animal Health and Production, CSIRO, Melbourne, Australia, pp. 71–80.

D'Occhio, M.J., Brooks, D.E., 1983. Seasonal changes in plasma testosterone concentration and mating activity in Border Leicester, Poll Dorset, Romney and Suffolk rams. Aust. J. Exp. Anim. Husb. 23, 248–253.

Finnerty, M., Enright, W.J., Morrison, C.A., Roche, J.F., 1994. Immunization of bull calves with a GnRH analogue-human serum albumin conjugate: effect of conjugate dose, type of adjuvant and booster interval on immune, endocrine, testicular and growth responses. J. Reprod. Fert. 101, 333–343.

Finnerty, M., Enright, W.J., Roche, J.F., 1998. Testosterone, LH and FSH episodic secretory patterns in GnRH-immunized bulls. J. Reprod. Fert. 114, 85–94.

Godfrey, S.I., Walkden-Brown, S.W., Martin, G.B., Speijers, E.J., 1996. Immunization of goat bucks against GnRH to prevent seasonal reproductive and agonistic behaviour. Anim. Reprod. Sci. 44, 41–54.

Hoskinson, R.M., Rigby, R.D., Mattner, P.E., Huynh, V.L., D'Occhio, M.J., Neish, A., Trigg, T.E., Moss, B.A., Lindsey, M.J., Coleman, G.D., Schwartzkoff, C.L., 1990. Vaxstrate: an anti-reproductive vaccine for cattle. Aust. J. Biotechnol. 4, 166–170.

Huxsoll, C.C., Price, E.O., Adams, T.E., 1998. Testis function, carcass traits, and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 76, 1760–1766.

Jago, J.G., Cox, N.R., Bass, J.J., Matthews, L.R., 1997. The effect of prepubertal immunization against gonadotropin-releasing hormone on the development of sexual and social behaviour of bulls. J. Anim. Sci. 75, 2609–2619.

Jeffery, M., Loxton, I., Van der Mark, S., James, T., Shorthose, R., Bell, K., D'Occhio, M., 1997. Liveweight gains, and carcass and meat characteristics of entire, surgically spayed or immunologically spayed beef heifers. Aust. J. Exp. Agric. 37, 719–726.

Meloen, R.H., Turkstra, J.A., Lankhof, H., Puijk, W.C., Schaaper, W.M., Dijkstra, G., Wensing, C.J., Oonk, R.B., 1994. Efficient immunocastration of male piglets by immunoneutralization of GnRH using a new GnRH-like peptide. Vaccine 12, 741–746.

Molenaar, G.J., Lugard-Kok, C., Meloen, R.H., Oonk, R.B., de Koning, J., Wensing, C.J., 1993. Lesions in the hypothalamus after active immunisation against GnRH in the pig. J. Neuroimmunol. 48, 1–11.

Robertson, I.S., Wilson, J.C., Fraser, H.M., 1979. Immunological castration in male cattle. Vet. Rec. 105, 556–557.

Robertson, I.S., Wilson, J.C., Rowland, A.C., 1981. Further studies on immunological castration in male cattle. Vet. Rec. 108, 381–382.

Robertson, I.S., Fraser, H.M., Innes, G.M., Jones, A.S., 1982. Effect of immunocastration on sexual and production characteristics in male cattle. Vet. Rec. 111, 529–531.

Robertson, I.S., Wilson, J.C., Fraser, H.M., Innes, G.M., Jones, A.S., 1984. Immunological castration of young bulls for beef production. In: Roche, J.F., O'Callaghan, D. (Eds.), Manipulation of Growth in Farm Animals. Martinus Nijhoff, Boston, MA, pp. 137–146.

Schanbacher, B.D., 1982. Responses of ram lambs to active immunization against testosterone and luteinizing hormone-releasing hormone, Am. J. Physiol. 242, E201–E205.

SAS/STAT, 1990. SAS/STAT User's Guide, Vol. 2, GLM-VARCOMP, Version 6, 4th Edition. Statistical Analysis System Institute, Inc., Cary, NC, pp. 891–996.

SAS/STAT, 1992. SAS/STAT Technical Report p-229, SAS/STAT Software: Changes and Enhancements, Release 6.07. Statistical Analysis System Institute, Inc., Cary, NC, pp. 287–368.