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GonaCon™ trial in bull calves

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Abstract

The project investigated the efficacy of the immunocontraceptive vaccine GonaCon™ to (1) induce antibodies against the key reproductive hormone gonadotrophin releasing hormone (GnRH) in bull calves and (2) suppress testicular growth longer-term. Brahman (*Bos indicus*) bull calves (n = 20) received a primary vaccination with GonaCon™ on Day 0 (4 to 6 months of age) and secondary vaccination on Day 55. Live weight and testicular size were monitored at regular intervals from Day 0 to Day 411 (356 days after secondary vaccination). A contemporary group of Brahman bull calves (n = 5) were not vaccinated and served as control animals. Vaccination with GonaCon™ did not have a significant effect ($P > 0.05$) on live weight gain from Day 0 to Day 411. Vaccinated bulls showed significantly reduced testicular growth and on Day 411 had a smaller ($P < 0.0001$) testicular diameter (39.7 ± 1.6 mm) compared with control bulls (65.0 ± 3.2 mm). On Day 411, all vaccinated bulls had a testicular diameter smaller than the lower 95% confidence interval for control bulls (95% confidence interval 58.6 to 71.3 mm). The duration of the immunocastration response in this project was significantly longer than previously reported for bulls vaccinated against GnRH. The project has shown that GonaCon™ has potential for longer-term immunocastration in bulls.

Executive summary

The castration of bulls is used to suppress aggressive and sexual behaviour and to influence growth and carcase characteristics. A goal of industry is to find a practical and acceptable alternative to surgical removal of the testes and the use of vascular constriction devices that cause testicular atrophy. The vaccination of bulls against gonadotrophin releasing hormone (GnRH, immunocastration) has been researched as an alternative to castration for over 35 years. GnRH has been the target of choice for an anti-reproductive vaccine as under normal circumstances GnRH initiates the reproductive-endocrine cascade responsible for the gametogenic and steroidogenic functions of the gonads, in both males and females. Vaccination against GnRH, and the neutralisation of GnRH action, achieves the dual objectives of controlling behaviour and carcase characteristics in bulls. A limited number of commercial vaccines that induce an immunocastration response in bulls are available but none is effective for sufficient duration to be considered suitable for broad-scale practical application in extensive beef production systems.

GonaCon™ is a GnRH vaccine that has induced longer-term suppression of gonadal function in wildlife including bison. The latter suggested that GonaCon™ could potentially also induce a longer-term immunocastration response in cattle. A previous MLA funded project (referenced below) investigated the utility of GonaCon™ to suppress ovarian function and prevent ovulation in heifers. It was found that approximately 50% of heifers continued to have suppressed ovaries at 331 days after secondary vaccination, at which time the project ended. The doses of GonaCon™ used in the above project could be considered relatively low for cattle which may have explained, in part, continued ovarian suppression in only about 50% of vaccinated heifers. Also, the heifer project utilised a relatively small number of animals. The question remained, therefore, whether higher doses of GonaCon™ would induce a more uniform and longer-term suppression of ovarian and testicular function in cattle. The project in heifers recommended that higher doses of GonaCon™ should be evaluated in cattle and this was addressed in the current project using bull calves.

Brahman (*Bos indicus*) bull calves (4 to 6 months of age) vaccinated with GonaCon™ showed suppressed testicular growth, and the suppression was relatively consistent amongst bulls. At Day 356 after secondary vaccination, treated bulls had a smaller ($P < 0.0001$) testicular diameter (39.7 ± 1.6 mm) compared with control bulls (65.0 ± 3.2

mm). Indeed, all vaccinated bulls had a testicular diameter smaller than the lower 95% confidence interval for control bulls (95% confidence interval 58.6 to 71.3 mm). The duration of the immunocastration response in this project was longer than previously reported for bulls vaccinated against GnRH. The project has therefore clearly shown that GonaCon™ has potential for longer-term immunocastration in bulls.

The GonaCon™ vaccine incorporates killed *Mycobacterium avium* (*M. avium*) which contributes to the induction of an immune response to GonaCon™. *Mycobacterium avium* subsp. *paratuberculosis* is the causative agent for Johne's Disease and cattle vaccinated with GonaCon™ can potentially test positive in the caudal-fold tuberculin test for tuberculosis. This issue also applies to the Silirum® vaccine for Bovine Johne's Disease which is presumed to have formulation components similar GonaCon™. A second issue is that the AdjuVac™ adjuvant component of GonaCon™ has the potential to cause site reactions. Site reactions have been observed with the Ovine Johne's Disease vaccine in sheep (Gudair™) that is also presumed to have formulation components similar to GonaCon™. Intellectual property, licensing, registration and manufacture are other areas that would need to be addressed in making GonaCon™ commercially available.

Notwithstanding, GonaCon™ warrants further investigation as the suppression of testicular growth in bulls in the present study persisted for significantly longer than previously reported. There are precedents with vaccines (Silirum®, Gudair™) that share similar formulations to GonaCon™ and the issues above would need to be balanced against the important animal welfare and production benefits of a potential practical alternative to castration in bulls.

Table of contents

1. Background

Vaccination against gonadotrophin releasing hormone (GnRH) has been explored for over 35 years as an alternative to castration and spaying in cattle (Appendix 1). GnRH has been a primary target for immunocontraception because under normal circumstances GnRH initiates the reproductive-endocrine cascade that is responsible for maintaining the gametogenic and steroidogenic functions of the gonads. Hence, in bulls, the effective neutralisation of GnRH with a vaccine would suppress the reproductive axis and render bulls essentially the same as castrates (steers). Vaccinated bulls would have similar behavioural and production characteristics as steers.

Bulls vaccinated against GnRH have anti-GnRH antibodies in circulation. These antibodies bind GnRH that is released into the hypothalamo-hypophyseal portal blood system which links the base of the brain (basal hypothalamus-median eminence) with the pituitary gland. The binding of GnRH by anti-GnRH antibodies prevents GnRH from acting at gonadotrope cells which are located in the anterior pituitary and this, in turn, prevents the release of LH and FSH. The latter two hormones together normally stimulate the production of sperm and steroid hormones (mainly testosterone) by the testes. Steroid hormones are responsible for aggressive and sexual behaviour in bulls and also have anabolic actions that largely cause the distinction in carcases between bulls and steers.

Whilst vaccination against GnRH has been researched for over 35 years in cattle, an issue that remains unresolved is the relatively short duration of immunocontraception (26 to 30 weeks) after vaccination against GnRH (Appendix 1). This is largely due to the inability of GnRH vaccines to maintain adequate levels of anti-GnRH antibodies in circulation (1-6, 8-12, 14-17, 34-44) (Appendix 1). Anti-GnRH antibody titres can be maintained to a degree with repeated vaccinations (6, 29) (Appendix 1) but this is impractical, particularly in extensive beef production systems.

The GonaCon™ vaccine has been shown to induce sustained anti-GnRH antibodies and an immunocontraceptive condition for a relatively long period (up to 2 to 3 years) in both males and females of avian and mammalian species (7, 22-27, 31, 33, 45). The features of the GonaCon™ vaccine, and the immunocontraceptive response in a range of species, were reviewed in the Final Report for MLA Project GonaCon™ trial

in heifers, B.AWW.0194: http://www.mla.com.au/Research-and-development/Final-report-details?projectid=15388. See also: http://www.aphis.usda.gov/wildlife_damage/nwrc/publications/13pubs/miller134.pdf

A notable and distinctive feature of GonaCon™ is that it has been shown to induce a long-term immunocontraceptive response after a single vaccination (24-27). This occurs, in part, because the GonaCon™ formulation includes killed *Mycobacterium avium* (*M. avium*). *M. avium* and related mycobacteria are considered to be endemic in many areas and cause exposure in wildlife, domestic animals and livestock. In Australia, for example, *M.avium* subsp. *paratuberculosis* is the causative agent for Johne's Disease (paratuberculosis) and can be found in livestock and wildlife, predominantly in southeast and southern Australia. In animals previously exposed to *M. avium* and/or related organisms, vaccination with GonaCon™ evokes a humoral immune memory response and the generation of antibodies that include anti-GnRH antibodies. Evidence for the latter was provided by the long-term immunocastration response of kangaroos in Canberra to a single vaccination with GonaCon™ (see www.aphis.usda.gov/wildlife site above).

Single vaccination with GonaCon™ did not induce significant anti-GnRH antibodies in heifers in southern Queensland and this was consistent with the apparent absence of *M.avium* in northern Australia (28) (GonaCon™ trial in heifers, B.AWW.0194 Final Report). An immunocontraceptive response did, however, occur in heifers subjected to a primary and secondary vaccination with GonaCon™ (28) (GonaCon™ trial in heifers, B.AWW.0194 Final Report). With two vaccinations, immune memory processes would have occurred after primary vaccination with GonaCon™ and a humoral antibody response would have been invoked after secondary vaccination, leading to anti-GnRH antibodies.

In GonaCon™ trial in heifers, B.AWW.0194, an immunocontraceptive response occurred in around 50% of vaccinated heifers. It was proposed that the 50% response could be explained, in part, by the relatively low doses of GonaCon™ utilised. It was further proposed that higher doses of GonaCon™ would result in a more uniform response that would be maintained longer-term in cattle. GonaCon™ trial in heifers, B.AWW.0194, involved a relatively small number of animals.

The present project was designed to test the hypothesis that the use of GonaCon™ at doses greater than in Project B.AWW.0194 would induce (1) a relatively high and

uniform immunocontraceptive response in cattle and (2) the response would be maintained longer-term. Bull calves were used to test this hypothesis and they received a primary vaccination at 4 to 6 months of age and secondary vaccination 55 days later. In industry, a two vaccination schedule with GonaCon™ could potentially be incorporated into existing management of young cattle at branding (primary vaccination) and weaning (secondary vaccination). After treatment with GonaCon™, bull calves were monitored for live weight and testicular growth and were compared to contemporary bulls that were not vaccinated.

2. Project objectives

The objectives of this project were:

1. Measure and report on the *Mycobacterium avium paratuberculosis* (MAP) antibody titres and anti-GnRH antibody titres in bull calves vaccinated with GonaCon™; and
2. Measure and report on the suppression of testicular function in beef bull calves through the use of a primary and booster vaccination with GonaCon™.

3. Methodology

Approvals

Animal ethics

The project was approved by the Production and Companion Animal, Animal Ethics Committee (PCA AEC) of the Animal Welfare Unit, The University of Queensland (Permit SAFS/019/13/MLA).

Australian Pesticides and Veterinary Medicines Authority (APVMA)

The project was approved by APVMA (Permit 13971).

Australian Quarantine and Inspection Service (AQIS)

The project was approved by AQIS (Permit IP12022209).

Animals

Brahman (*Bos indicus*) bull calves (n = 25; 4 to 6 months old; live weight 139 ± 3 kg) were obtained from a commercial breeder. The bulls were maintained on standard pasture that was predominantly Rhodes grass and a relatively small proportion of native bluegrass. They received approximately 1.0 kg/head/day milled grain (barley and/or sorghum).

Treatment

Bulls were randomly allocated on live weight to (1) control (n = 5), vaccinated with Adjuvac™ adjuvant (without GnRH antigen) or (2) GonaCon™ (n = 20), vaccinated with GonaCon™ (complete vaccine containing Adjuvac™ and GnRH antigen). Both groups of bulls were treated on Day 0 (primary vaccination, 5 mg GonaCon™) and Day 55 (secondary vaccination, 3 mg GonaCon™). They were monitored on Days 0, 30, 55, 90, 127, 155, 183, 211, 232, 285, 316, 361, 383 and 411 for live weight and testicular size (Figure 1). Blood samples were obtained on Days 0, 30, 55, 90, 127, 155, 183 and 211 for the analysis of antibody titres to *M. avium* and GnRH. Blood samples were placed on ice at collection, centrifuged at 800 x g within 2 h of collection, and the plasma was stored at -20^{0}C until required for analysis.

GonaCon™ vaccine

Details on the formulation of the GonaCon™ vaccine are available in GonaCon™ trial in heifers, B.AWW.0194 Final Report and:
http://www.aphis.usda.gov/wildlife_damage/nwrc/publications/13pubs/miller134.pdf

Vaccination was by intramuscular injection at the rump site.

Figure 1. Technique for measuring testis size (diameter) in bulls using vernier callipers

Johne's titres

Details on the determination of Johne's (*M. avium*) titres are available in GonaCon™ trial in heifers, B.AWW.0194 Final Report (see also 22-27).

Anti-GnRH antibody titres

Details on the determination of anti-GnRH antibody titres are available in GonaCon™ trial in heifers, B.AWW.0194 Final Report (see also 22-27).

Antibody titres to *M. avium* (Johne's titres) and GnRH were determined by ELISA and further details are available at:
http://www.aphis.usda.gov/wildlife_damage/nwrc/publications/13pubs/miller134.pdf

Statistical analyses

Data were analysed using a repeated measures analysis of variance (ANOVA) carried out with MIXED procedures in SAS-STAT version 9.3. The model estimated effects of treatment group, day of observation, and the interaction between the two. Within subject variation was modelled using an ante-dependence covariance

structure. Least squares means, standard errors and 95% confidence intervals were estimated and comparisons carried out both between treatment groups each time, and between days within treatment group. Probability levels less than 0.05 were considered statistically significant.

4. Results

Johne's titres

The immune response to the Adjuvac™ component of GonaCon™ is assessed using the IDEXX MAP assay for anti-*M. avian* spp *paratuberculosis* (Johne's titres) (22-27). Both control bulls and bulls vaccinated with GonaCon™ received Adjuvac™.

Results for Johne's titres are shown in Table 1. All bulls were negative for Johne's titres at the time of primary vaccination (Day 0, 0.07 ± 0.01). Johne's titres showed a marginal increase at Day 30 (0.62 ± 0.10) and Day 55 (0.63 ± 0.10) but remained in the equivocal titre category (> 0.60 and < 0.70). Notwithstanding the latter, 32% and 48% of bulls vaccinated with GonaCon™ were positive for Johne's titres on Day 30 and Day 55, respectively (Table 1). There was a significant increase in Johne's titres after secondary vaccination on Day 55 and at Day 90 titres were 1.46 ± 0.14. Johne's titres remained elevated to Day 211 (Table 1).

Table 1. Anti-Johne's titres in young Brahman bulls that received a primary vaccination on Day 0 and secondary vaccination on Day 55. Titres ≤ 0.60 are negative, titres > 0.60 and < 0.70 are equivocal, and titres ≥ 0.70 are positive. Control bulls are grey rows.

Animal	Anti-Johne's titre							
	Day							
	0	30	55	90	127	155	183	211
1	0.03	0.25	0.14	0.87	0.69	0.84	0.98	0.79
2	0.15	1.35	1.00	2.42	2.47	2.29	2.07	1.98
3	0.02	0.69	1.01	1.61	1.78	1.59	1.39	1.56
4	0.08	0.12	0.15	1.16	1.07	0.92	0.86	-
5	0.15	0.97	1.64	2.30	2.37	2.13	2.08	1.02
6	0.02	0.45	0.43	1.31	1.47	1.54	1.50	2.03
7	0.10	0.10	0.03	0.09	0.19	0.42	0.52	0.56
8	0.03	1.18	1.55	2.23	2.04	2.01	1.97	1.87
9	0.03	0.06	0.08	1.23	1.26	1.09	1.32	1.47
10	0.15	0.97	0.83	1.28	1.85	2.05	1.89	1.71
11	0.03	0.24	0.23	0.43	1.05	1.05	1.10	0.97
12	0.08	1.88	1.88	1.85	1.37	1.07	1.22	1.13
14	0.25	1.31	0.91	1.06	1.36	1.42	1.65	1.96
16	0.02	0.12	0.05	1.18	0.90	0.66	1.06	1.57
17	0.03	0.44	0.55	0.93	0.80	1.19	2.11	1.85
18	0.01	0.03	0.02	0.17	0.32	0.29	0.35	0.32
19	0.08	0.14	0.17	1.78	2.36	2.34	2.21	2.06
20	0.16	1.11	0.71	2.20	1.92	1.51	1.84	1.86
21	0.04	0.20	0.22	1.34	1.43	-	1.32	1.23
22	0.04	0.34	0.42	0.76	1.18	1.11	1.01	0.90
23	0.02	1.05	1.20	2.41	2.43	2.18	2.22	2.09
24	0.02	0.43	0.89	1.22	1.05	0.70	0.70	0.59
25	0.11	0.18	0.24	2.41	2.57	2.45	2.40	2.14
26	0.16	0.47	1.13	2.29	2.30	2.41	2.36	2.26
27	0.04	1.62	1.40	2.06	2.27	2.03	1.91	1.89
mean ± sem	0.07 ± 0.01	0.62 ± 0.10	0.63 ± 0.10	1.46 ± 0.14	1.54 ± 0.13	1.47 ± 0.13	1.52 ± 0.11	1.49 ± 0.11

Anti-GnRH antibody titres

Results for anti-GnRH antibody titres are shown in Table 2. At Day 30 after primary vaccination, 15/20 bulls that received GonaCon™ had low anti-GnRH titres (12 ± 1 $x10^{-3}$) and 5/20 bulls had relatively high titres (76 ± 12 $x10^{-3}$). The mean anti-GnRH titre for vaccinated bulls on Day 30 was 28 ± 7 $x10^{-3}$ and had declined marginally at Day 55 (17 ± 4 $x10^{-3}$). Anti-GnRH titres increased significantly after secondary vaccination on Day 55 and at Day 90 the mean titre was 118 ± 5 $x10^{-3}$. The mean titre was maintained to Day 211 at which time 12/20 vaccinated bulls had an anti-GnRH titre at the upper limit of the assay (128 $x10^{-3}$) and 6/20 vaccinated bulls had a relatively high titre (32-64 $x10^{-3}$). Two bulls had a relatively low titre (8 $x10^{-3}$) (Table 2). It was concluded that vaccination with GonaCon™ induced a high and consistent anti-GnRH antibody response that was maintained longer-term in most bulls.

Live weight

Results for live weight are shown in Figure 2 and Table 3 the data are summarised in Table 4. There was a significant ($P < 0.001$) effect of day on live weight but there was no effect of treatment on this parameter.

There was a significant ($P = 0.0193$) day x group interaction for live weight that had no apparent explanation as the two groups appeared to have similar patterns of growth. The live weight gain (∆ LW) from Day 0 to Day 411 was: control bulls, 277 ± 15 Kg; GonaCon™ bulls, 266 ± 6 kg ($P > 0.05$). The daily live weight gain was: control bulls, 0.68 ± 0.06 kg/day; GonaCon™ bulls, 0.65 ± 0.01 kg/day ($P > 0.05$). Control bulls appeared to show a marginally greater increase in live weight from Day 285 although this was not significant and not as apparent on Day 411 (Figure 2, Figure 3, Table 3).

Testicular size

Results for testicular size are shown in Figure 4 and Table 5 and the data are summarised in Table 6. There were significant ($P < 0.0001$) effects of day, group, and day x group on testicular size. Control bulls showed a progressive increase in testicular size from Day 0 to Day 411. Vaccination with GonaCon™ was associated with a significant ($P < 0.0001$) suppression of testicular growth after Day 55. From Day 55 to Day 411, control bulls had an increase in testicular diameter of 33.0 ± 0.8 mm and vaccinated bulls 9.0 ± 1.7 mm ($P < 0.001$) (Figure 5).

Table 2. Anti-GnRH titres ($x10^{-3}$) in young Brahman bulls that received a primary vaccination on Day 0 and secondary vaccination on Day 55. Bulls 5, 10, 12, 14 and 17 were control and did not receive GnRH antigen. A titre of 128 was the upper limit of the titre assay. Control bulls (grey rows) are not included in the mean ± sem; nt, no titre.

Animal	Anti-GnRH titre							
	Day							
	0	**30**	**55**	**90**	**127**	**155**	**183**	**211**
1	nt	8	16	128	128	128	128	128
2	nt	64	32	128	128	128	128	32
3	nt	64	64	128	128	128	128	128
4	nt	4	0	128	128	128	128	128
5	nt	0	0	0	0	0	0	0
6	nt	64	64	128	128	128	128	128
7	nt	128	0	128	64	64	64	128
8	nt	16	16	128	64	128	128	128
9	nt	16	0	128	32	16	16	8
10	nt	0	0	0	0	0	0	0
11	nt	64	1	128	128	128	128	128
12	nt	0	0	0	0	0	0	0
14	nt	0	0	0	0	0	0	0
16	nt	16	0	64	32	64	64	64
17	nt	0	0	0	0	0	0	0
18	nt	8	8	64	64	128	128	128
19	nt	8	0	128	64	32	8	8
20	nt	8	0	128	128	128	128	128
21	nt	16	16	128	64	-	128	128
22	nt	8	32	128	128	128	128	64
23	nt	16	16	128	128	128	128	32
24	nt	16	32	128	64	128	128	128
25	nt	8	16	128	64	64	64	64
26	nt	16	8	64	-	64	64	32
27	nt	16	32	128	128	128	128	128
mean ± sem	nt	28 ± 7	17 ± 4	118 ± 5	94 ± 8	103 ± 8	103 ± 9	92 ± 10

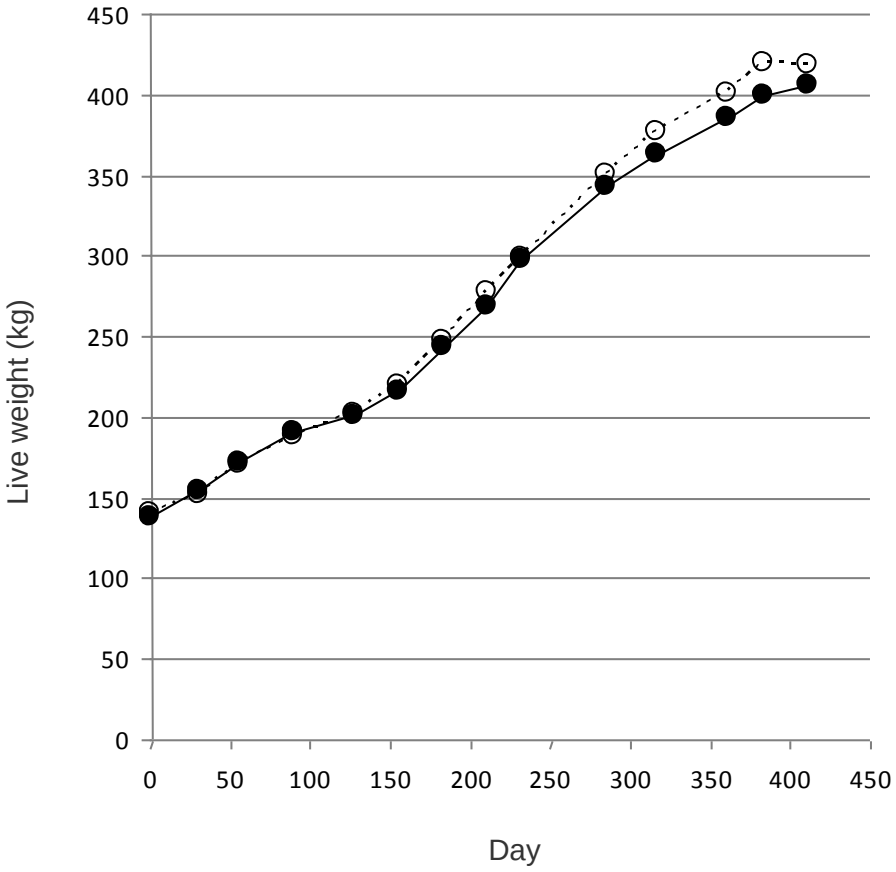

Figure 2. Longitudinal changes (mean) in live weight for control Brahman bulls (○) and bulls vaccinated with GonaCon™ (●). Vaccinated bulls received a primary vaccination on Day 0 and secondary vaccination on Day 55. Results are shown as means and summary data and statistical analyses are presented in Table 4.

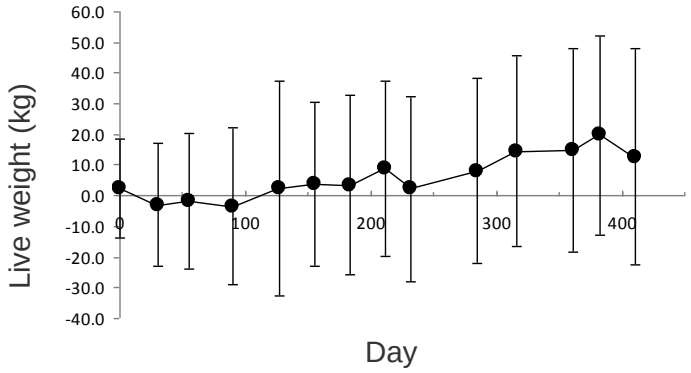

Figure 3. Longitudinal trend for the difference in live weight between control Brahman bulls and bulls vaccinated with GonaCon™. There was some evidence that control bulls showed a greater increase in live weight gain after Day 285 although this was not significant. Results are the difference in live weight and 95% confidence interval.

Table 3. Live weight for individual control bulls (grey rows) and bulls vaccinated with GonaCon™.

Animal	Live weight (kg)													
	Day													
	0	**30**	**55**	**90**	**127**	**155**	**183**	**211**	**232**	**285**	**316**	**361**	**383**	**411**
1	166	194	214	238	246	260	286	306	342	390	416	444	454	468
2	142	166	184	210	216	232	256	274	298	350	368	404	410	424
3	146	162	186	212	232	242	264	288	318	344	376	404	410	422
4	154	168	192	210	234	248	276	296	332	384	398	428	448	454
5	140	148	168	180	185	208	218	246	272	318	346	370	394	382
6	114	126	136	149	152	162	186	210	236	282	294	328	340	338
7	132	138	158	178	190	202	220	250	284	344	368	400	418	412
8	148	156	182	202	220	228	262	284	316	354	384	392	412	418
9	118	132	150	161	171	185	222	244	274	332	362	378	396	404
10	152	168	188	210	220	238	274	310	328	382	402	416	434	434
11	144	162	180	204	216	224	258	290	314	360	368	404	408	412
12	116	118	136	150	169	177	198	228	252	298	330	350	360	364
14	174	190	214	236	258	268	308	334	358	412	440	478	494	500
16	144	166	182	202	208	222	256	286	312	362	374	398	406	414
17	122	134	146	164	182	206	240	272	288	346	368	392	416	412
18	118	134	148	166	184	192	234	256	282	342	360	386	408	422
19	130	134	146	150	154	165	193	226	248	304	316	344	358	368
20	128	134	152	175	189	202	226	254	274	304	328	342	358	358
21	142	160	168	184	197	208	226	250	270	314	334	346	364	364
22	128	152	168	179	208	212	240	262	290	334	348	366	388	392
23	126	140	160	187	204	220	252	280	320	362	380	404	418	426
24	164	182	198	214	234	238	266	284	322	366	388	400	418	418
25	134	154	174	192	212	218	246	272	304	340	360	384	394	396
26	140	160	180	210	228	228	260	296	306	350	366	388	392	400
27	148	172	184	206	216	224	254	274	306	354	366	386	398	406

Table 4. Live weight for control Brahman bulls and bulls vaccinated with GonaCon™. Vaccinated bulls received a primary vaccination on Day 0 and secondary vaccination on Day 55. Results are presented as means ± SEM.

Day	Live weight (kg) †		
	Control (n = 5)	GonaCon™ (n = 20)	P value
0	140 ± 7 [a]	138 ± 4 [a]	0.76
30	151 ± 9 [b]	154 ± 4 [b]	0.76
55	170 ± 10 [c]	172 ± 5 [c]	0.87
90	188 ± 11 [d]	191 ± 6 [d]	0.78
127	202 ± 12 [e]	205 ± 6 [e]	0.89
155	219 ± 12 [f]	215 ± 6 [f]	0.78
183	247 ± 13 [g]	244 ± 7 [g]	0.81
211	278 ± 13 [h]	269 ± 6 [h]	0.54
232	299 ± 14 [i]	297 ± 7 [i]	0.88
285	351 ± 14 [j]	343 ± 7 [j]	0.59
316	377 ± 14 [k]	362 ± 7 [k]	0.35
361	401 ± 15 [l]	386 ± 7 [l]	0.37
383	419 ± 15 [m]	399 ± 7 [m]	0.23
411	418 ± 16 [m]	405 ± 8 [m]	0.48

† there were no significant differences in live weight between control and vaccinated bulls

[a-m] means within column with a different superscript differ (P < 0.001)

On Day 411, bulls vaccinated with GonaCon™ had a smaller (P < 0.0001) testicular size compared with control bulls (39.7 ± 1.6 mm and 65.0 ± 3.2 mm, respectively) (Table 6). All vaccinated bulls had a testicular diameter smaller than the lower 95% confidence interval for control bulls (95% confidence interval 58.6 to 71.3 mm) on Day 411 (356 days after secondary vaccination). Two bulls (10%) vaccinated with GonaCon™ had an apparent lesser response to vaccination and showed increased testis growth after Day 127 (Bull 16) and Day 155 (Bull 9) (Table 5). Representative control and vaccinated bulls are shown in Figure 6.

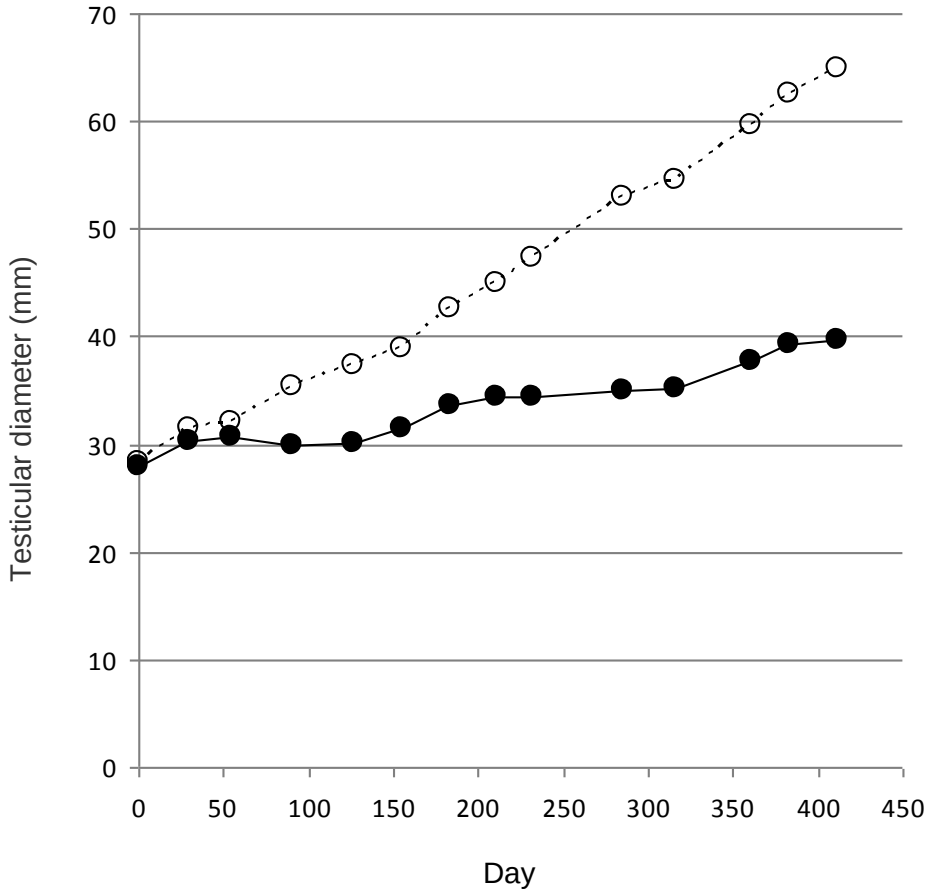

Figure 4. Longitudinal changes (mean) in testicular diameter for control bulls (○) and bulls vaccinated with GonaCon™ (●). Testicular diameter is the average diameter for the left and right testes. Vaccinated bulls received a primary vaccination on Day 0 and secondary vaccination on Day 55.

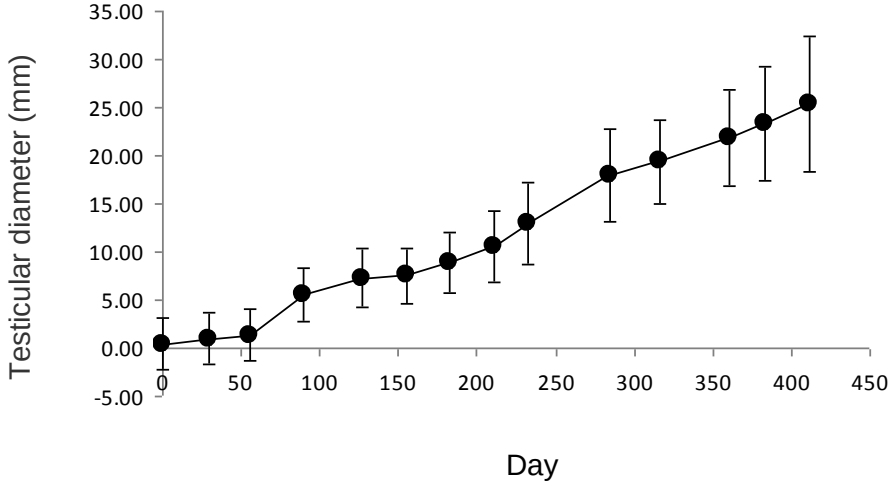

Figure 5. Longitudinal trend for the difference in testicular size between control bulls and bulls vaccinated against GonaCon™. Results are the difference between control and vaccinated bulls and 95% confidence intervals

Table 5. Testicular size for individual control bulls (grey rows) and bulls vaccinated with GonaConTM. vaccinated bulls received a primary vaccination on Day 0 and secondary vaccination on Day 55. Testicular size is the average diameter of the right and left testes.

Animal	Testicular size (mm)													
	Day													
	0	**30**	**55**	**90**	**127**	**155**	**183**	**211**	**232**	**285**	**316**	**361**	**383**	**411**
1	32	31	30	30	29	31	33	35	33	34	32	34	34	33
2	30	31	32	31	32	32	34	35	36	37	40	43	44	46
3	29	31	30	29	32	32	34	34	33	32	33	40	38	37
4	28	31	34	31	32	32	34	35	36	35	35	36	36	36
5	28	32	32	33	35	38	41	41	45	50	50	57	59	62
6	27	27	26	26	28	27	30	30	30	31	32	34	34	34
7	27	29	29	28	28	31	32	34	35	36	36	42	41	39
8	27	30	32	29	28	32	34	34	33	37	36	38	37	35
9	25	33	30	30	30	33	37	38	40	46	47	52	56	58
10	28	31	33	38	40	39	44	46	50	57	56	58	62	67
11	30	31	30	31	32	32	35	34	37	37	37	39	39	38
12	24	27	27	31	32	34	38	39	40	43	48	54	57	61
14	33	36	36	41	43	45	47	52	55	61	61	67	69	71
16	26	32	30	32	33	37	42	44	46	43	42	42	47	51
17	29	31	33	34	37	39	43	47	47	54	58	62	66	64
18	24	26	28	26	26	30	31	32	32	34	35	41	46	51
19	29	29	30	28	27	30	31	32	33	34	34	38	45	47
20	25	27	28	27	27	28	31	30	29	28	30	32	33	33
21	32	36	37	35	34	36	38	38	37	39	37	39	41	41
22	23	27	28	27	26	28	29	29	28	29	30	30	30	29
23	29	31	32	32	30	34	35	35	35	35	34	36	37	37
24	26	29	29	29	29	29	30	31	31	30	29	31	32	32
25	31	34	33	34	35	33	35	35	36	35	36	39	42	46
26	29	31	33	31	31	31	36	36	34	36	35	34	37	36
27	30	33	33	32	33	33	34	36	35	33	34	34	36	34

Table 6. Testicular diameter for control bulls and bulls vaccinated with GonaCon. Testicular diameter is the average diameter for the left and right testes. Vaccinated bulls received a primary vaccination on Day 0 and secondary vaccination on Day 55. Results are presented as means ± SEM.

Day	Testicular diameter (mm)		P value
	Control (n = 5)	GonaCon™ (n = 20)	
0	28.4 ± 1.2	27.9 ± 0.6	0.89
30	31.4 ± 1.2	30.4 ± 0.6	0.37
55	32.0 ± 1.2	30.6 ± 0.6	0.37
90	35.4 ± 1.3	29.9 ± 0.6	0.002
127	37.4 ± 1.4	30.1 ± 0.7	< 0.0001
155	39.0 ± 1.3	31.5 ± 0.6	< 0.0001
183	42.6 ± 1.4	33.7 ± 0.7	< 0.0001
211	45.0 ± 1.7	34.4 ± 0.8	< 0.0001
232	47.4 ± 1.9	34.5 ± 0.9	< 0.0001
285	53.0 ± 2.2	35.0 ± 1.1	< 0.0001
316	54.6 ± 2.0	35.2 ± 1.0	< 0.0001
361	59.6 ± 2.3	37.7 ± 1.1	< 0.0001
383	62.6 ± 2.7	39.3 ± 1.3	< 0.0001
411	65.0 ± 3.2	39.7 ± 1.6	< 0.0001

Figure 6. Representative control bull (left) and bull vaccinated with GonaCon™ (right) on Day 383 of the project (Day 328 after secondary vaccination).

5. Discussion

The aim of the present project was to determine whether active immunization with the GonaCon™ vaccine would induce a longer-term suppression of testicular growth in young bulls. The suppression of testicular growth would prevent the initiation of spermatogenesis and also markedly reduce the secretion of gonadal steroids which are required for the display of aggressive and sexual behaviour in bulls. Gonadal steroids (androgens and oestrogens) also influence growth performance and carcase characteristics in cattle.

The doses of GonaCon™ utilised in the present project were greater than in the previous project GonaCon™ trial in heifers, B.AWW.0194. In the latter project, longer-term suppression of ovarian function (330 days) was observed in 50% of heifers vaccinated with 3 mg (primary vaccination) and 1 mg (secondary vaccination) of GonaCon™. The suggestion was made in GonaCon™ trial in heifers, B.AWW.0194, that higher doses of GonaCon™ would likely induce a more uniform and longer-term suppression of gonadal function in cattle. The present project therefore utilised 5 mg (primary vaccination) and 3 mg (secondary vaccination) of GonaCon™.

Young bulls vaccinated with GonaCon™ developed significant antibody titres to the Adjuvac™ component of the vaccine (anti-Johne's titres) and also to the GnRH-conjugate component of the vaccine (anti-GnRH titres). A notable feature of the immune response to GonaCon™ in the current project was the maintenance of relatively high anti-Johne's and anti-GnRH titres longer-term. This was consistent with the underlying rationale of the project that doses of GonaCon™ greater than previously used in cattle would induce a more uniform and longer-term immune response. The duration of the immune response was significantly longer than previously reported for cattle with vaccines produced by conventional conjugation chemistry (1-6, 8-12, 14-17, 34-44) and bacterial expression systems (40-41) (Appendix 1).

The immune response to GonaCon™ was associated with a relatively uniform and sustained suppression of testicular growth in young bulls. At 356 days after secondary vaccination with GonaCon™ all vaccinated bulls had a testicular size smaller than the lower 95% confidence interval for testicular size in unvaccinated bulls. The duration of testicular suppression was substantially longer than previously

reported in bulls subjected to a similar vaccination protocol (1-6, 8-12, 14-17, 34-44) (Appendix 1).

It can be concluded from the immune and testicular findings in the current project that GonaCon™ is a potent immunocastration vaccine in bulls.

Immunisation against GnRH in pigs produced long-term suppression of reproductive function which was associated with lesions at the basal hypothalamus (30). The lesions could not be explained but it was suggested that the response to vaccination included the formation of immune complexes that caused a disruption of tissue surrounding GnRH neuron terminals, and interfered with the release of GnRH (30). This potential mechanism(s) for longer-term suppression of reproductive function after vaccination against GnRH warrants further investigation.

As GnRH is structurally the same in males and females, and has the same biological function, it can be inferred that GonaCon™, at the doses used in the present project, would have the same immunocontraceptive action in heifers and cows. Indeed, it could be proposed that GnRH should be the biological target of choice for an immunocontraceptive vaccine in cattle as it can be applied to males and females, and it can be used to manage both behaviour and fertility. Notwithstanding the relative merits of vaccination against GnRH, there are other potential strategies to replace castration and spaying that deserve attention (13, 29, 32) (Review of the alternatives to castration and spaying of ruminants, B.AWW.0225 Final Report).

Whilst the response of young bulls to GonaCon™ in the current project was better than previously reported (1-6, 8-12, 14-17, 34-44) (Appendix 1), 2/20 (10%) bulls had a lesser response and initiated testicular growth after Day 127 and Day 155. One of these bulls (Bull 9) showed low anti-GnRH titres (8-16 $x10^{-3}$) after Day 127 whilst the second bull (Bull 16) had relatively high anti-GnRH titres (64 $x10^{-3}$) to Day 211. It is possible that the occurrence of individual variation in the response to GonaCon™ could be further reduced with higher doses of vaccine. Higher doses of GonaCon™ could also potentially result in an even longer duration of testicular suppression in bulls (and ovarian suppression in heifers and cows) than observed in the present project.

The Adjuvac™ component of GonaCon™ contains killed *Mycobacterium avium* (*M. avium*) which contributes to the induction of an immune response to GonaCon™.

Mycobacterium avium subsp. *paratuberculosis* is the causative agent for Johne's Disease in cattle (BJD) and cattle vaccinated with GonaCon™ could potentially test positive in the caudal-fold tuberculin test for tuberculosis (TB). This issue also applies to the Silirum® vaccine for Bovine Johne's Disease which is presumed to have formulation components similar to GonaCon™:

http://www.apvma.gov.au/consultation/public/2014/tan_silirum.php

http://www.apvma.gov.au/registration/assessment/docs/tan_silirum_february_2014.pdf

Silirum® is not recommended for use in cattle that are destined for live export given the tuberculosis test requirements of some countries. The potential cross-reactivity with the TB test could be addressed by the recent development of a Bovine Johne's Disease test with greater discrimination and specificity:

http://www.daff.qld.gov.au/__data/assets/pdf_file/0006/49965/bovine-johnes-disease-information-pack.pdf

http://www.mla.com.au/News-and-resources/Industry-news/Speeding-up-Johnes-disease-diagnosis2

A second issue is that the AdjuVac™ adjuvant component of GonaCon™ has the potential to cause site reactions. Site reactions have been observed with the Ovine Johne's Disease vaccine in sheep (Gudair™) that is also presumed to have formulation components similar to GonaCon™.

The TB cross-reactivity and potential site reactions are relevant issues. These need to be addressed and balanced against the important animal welfare and management gains that would be achieved by a practical alternative to castration of bulls (and spaying of heifers and cows).

Vaccination with GonaCon™ could be readily incorporated into the current management of young bulls. Bulls would be given a primary vaccination at the time of branding and secondary vaccination at weaning. A vaccine that maintained an immunocastration response for 12 to 18 months would have broad industry application, including female cattle. Vaccination against GnRH could be used in bulls at later ages depending on the production system and management (6). Potential target applications could be during backgrounding and in feedlots.

As noted above, immunocastration leads to a marked suppression of circulating concentrations of gonadal steroids (primarily testosterone) in bulls (6). As gonadal steroids have anabolic actions it might be anticipated that vaccinated bulls would have reduced growth performance compared with unvaccinated bulls. The rate of live weight gain for control (0.68 ± 0.04 kg/day) and vaccinated (0.65 ± 0.01 kg/day) bulls in the present project did not differ. The live weight gains in the present project could be regarded as relatively low but were not entirely atypical for extensively managed cattle. A previous study in Brahman bulls maintained on natural subtropical pastures also found no differences in live weight gain between control bulls and bulls vaccinated against GnRH (6). It is highly likely that the availability and quality of feed determine whether any differences in growth potential between control and vaccinated bulls are expressed. In this regard, differences in growth performance were observed under feedlot conditions between control bulls and bulls vaccinated against GnRH (2), and also control and vaccinated heifers (1) (Appendix 1). The general observation has been that the growth performance of bulls vaccinated against GnRH is intermediate between that of entire bulls and steers (2) Appendix 1. Further studies are warranted in larger numbers of extensively managed animals to determine whether there are differences in growth performance between entire bulls, bulls vaccinated against GnRH and steers.

GnRH is the same in males and females and has the same biological function. Therefore, GonaCon™ also has potential as an alternative to spaying in heifers and cows.

6. Conclusion

The vaccination of young bulls with GonaCon™ was associated with a high and sustained immune response and the suppression of testicular growth longer-term. The response to GonaCon™ was relatively uniform amongst bulls. The immune and testicular responses were of substantially longer duration than previously reported after the vaccination of cattle against GnRH. The current project has shown that GonaCon™ has potential as a practical immunocastration vaccine in bulls. As GnRH is the same in males and females, and has the same biological function, GonaCon™ also has potential as an alternative to spaying in heifers and cows.

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