final report

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Prepared by: Michael J. D'Occhio

The University of Sydney

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GonaCon™ trial in bull calves 2

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Abstract

This project is a continuation of Project B.AWW.0194.A which 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. In Project B.AWW.0194.A it was found that at 12 months after secondary vaccination Brahman bulls still had suppressed testicular growth. In the current project the monitoring of testicular growth was continued in a subset of control bulls (n = 4) and vaccinated bulls (n = 8). At approximately 21 months after secondary vaccination, five vaccinated bulls continued to have a smaller ($P < 0.01$) testicular diameter (42.0 ± 1.5 mm) compared with control bulls (73.8 ± 3.1 mm) whilst three vaccinated bulls had a testicular diameter (67.0 ± 2.6 mm) approaching that of controls. The latter three vaccinated bulls started to undergo faster testicular growth between approximately 15 and 17 months after secondary vaccination. The project has shown that GonaCon™ can induce an immunocastration condition for up to 18 months in bulls. A proportion of vaccinated bulls have suppressed testicular growth for a longer period.

Executive summary

This project is a continuation of Project B.AWW.0194.A which examined the response of young Brahman bulls to vaccination with the immunocastration vaccine GonaCon™. A detailed background to the use of GonaCon™ in a number of species was provided in Project B.AWW.0194 and Project B.AWW.0194.A. Briefly, the GonaCon™ vaccine targets gonadotrophin releasing hormone (GnRH) which is released from the brain and initiates the reproductive-endocrine cascade responsible for the gametogenic and steroidogenic functions of the gonads, in both males and females. Successful vaccination against GnRH results in the neutralisation of endogenous GnRH and an immunocastration condition. 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.

In Project B.AWW.0194.A, Brahman bulls vaccinated with GonaCon™ had a smaller ($P < 0.0001$) testicular diameter than control bulls at approximately 12 months (356 days) after secondary vaccination (39.7 ± 1.6 mm and 65.0 ± 3.2 mm, respectively).
In the current project, testicular size continued to be monitored in a sub-set of control bulls (n = 4) and vaccinated bulls (n = 8). At approximately 21 months after secondary vaccination, testicular size remained suppressed ($P < 0.01$) in five vaccinated bulls (42.0 ± 1.5 mm) whilst three vaccinated bulls had a testicular diameter (67.0 ± 2.6 mm) that was approaching that of controls (73.8 ± 3.1 mm). The latter three vaccinated bulls started to undergo faster testicular growth between approximately 15 and 17 months after secondary vaccination.

The findings in this project support the suggestion made in Project B.AWW.0194.A that GonaCon™ has potential as a longer-term immunocastration vaccine in bulls.
As noted in B.AWW.0194.A, GonaCon™ incorporates attenuated *Mycobacterium avium* (*M. avium*) which contributes to stimulation of the 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 also applies to the Silirum® vaccine for Bovine Johne's Disease which, it is presumed, has formulation components similar to GonaCon™. A second consideration 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 also is presumed to have formulation components similar to GonaCon™.

Intellectual property, licensing, registration and manufacture are other areas that would need to be looked at in order to commercialise GonaCon™.

Notwithstanding, GonaCon™ certainly warrants further investigation as the suppression of testicular growth in bulls persists for significantly longer than has been reported for commercial and research vaccines (refer Appendix 1, Final Report B.AWW.0194.A; http://www.mla.com.au/Research-and-development/Search-RD-reports/RD-report-details/Animal-Welfare/GonaConTM-trial-in-bull-calves/184).

As endogenous GnRH is identical in males and females, GonaCon™ also has application in heifers and cows.

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1. Background

Gonadotrophin releasing hormone (GnRH) was recognised in the early 1970's as the brain hormone that initiates the reproductive-endocrine cascade that is responsible for maintaining the gametogenic and steroidogenic functions of the gonads. This was soon followed by attempts to block the action of GnRH at the anterior pituitary gland where GnRH stimulates the gonadotrope cells to release the two gonadotrophic hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH together drive gametogenesis and steroidogenesis in the testes and ovaries. If interception of the GnRH signal from the brain to the pituitary gland could be achieved, then this would lead to the cessation of steroid production, the suppression of aggressive and reproductive behaviour, and infertility. In bulls, effective neutralisation of GnRH would additionally result in animals with growth, carcase and meat characteristics similar to steers.

Vaccination has been explored for over 35 years in cattle as a strategy to neutralise endogenous GnRH (Final Report, MLA Project B.AWW.0194.A). One of the major issues remaining to be resolved is the relatively short duration of the immunocastration response (26 to 30 weeks) after vaccination against GnRH (Appendix 1, Final Report, MLA Project B.AWW.0194.A). This is largely due to the inability of commercial and research GnRH vaccines to maintain adequate levels of anti-GnRH antibodies in circulation (1-6, 8-12, 14-17, 34-44) (Final Report, MLA Project B.AWW.0194.A). Anti-GnRH antibody titres can be maintained to some degree by administering repeated vaccinations (6, 29) (Final Report, MLA Project B.AWW.0194.A). However, this is impractical and particularly in extensive beef production systems.

The GonaCon™ vaccine was shown to induce sustained anti-GnRH antibodies, and an immunocastration condition, for a relatively long period (up to 2 to 3 years) in males and females of avian and mammalian species (7, 22-27, 31, 33, 45). This was reviewed in the Final Reports for MLA Project B.AWW.0194: http://www.mla.com.au/Research-and-development/Final-report-details? Projectid =15388 and Project B.AWW.0194.A (http://www.mla.com.au/Research-and-development/Search-RD-reports/RD-report-details/Animal-Welfare/GonaConTM-trial-in-bull-calves/184).

A further overview of GonaCon™ is available at 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 attenuated *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, *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 and the GonaCon™ site above).

A single vaccination with GonaCon™ did not induce a significant response in heifers sourced from Queensland (Johne's Disease not endemic) but a response was elicited with primary and secondary vaccinations (Final Report MLA Project B.AWW.0194). The duration of the response to GonaCon™ was extended when higher doses of the vaccine were used in young bulls (Final Report MLA Project B.AWW.0194.A). In the latter project, bulls vaccinated with GonaCon™ had suppressed testicular growth at approximately 12 months after secondary vaccination (see Bibliography, Invited Conference Paper 1).

It was of interest to ascertain the duration of the immunocastration response in bulls vaccinated in Project B.AWW.0194.A. The present project therefore continued to monitor testicular growth in a sub-set of these bulls.

As noted in Project B.AWW.0194.A, a two vaccination schedule with GonaCon™ could be incorporated into the existing management of young cattle at branding (primary vaccination) and weaning (secondary vaccination).

2. Project objective

The objective of this project was:

1. To ascertain the period of suppression of testicular growth in young bulls treated with the immunocontraceptive vaccine GonaCon™.

3. Methodology

Approvals

Animal ethics

The project was approved by Research Integrity, Animal Ethics Committee, The University of Sydney (Project number 652).

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, Queensland breeder (Final Report MLA Project B.AWW.0194.A). For this project, the bulls were maintained on standard temperate pastures at John B. Pye Farm, The University of Sydney.

Treatment

Vaccination with GonaCon™ and the procedure to measure testicular diameter are described in the Final Report for MLA Project B.AWW.0194.A (http://www.mla.com.au/Research-and-development/Search-RD-reports/RD-report-details/Animal-Welfare/GonaConTM-trial-in-bull-calves/184).

In Project B.AWW.0194.A, testicular diameter was determined up to Day 411. In the current project, testicular diameter was ascertained on Days 484, 512, 643, 574, 595, 628 and 677. These are respectively reported as Days 0, 28, 59, 90, 111, 144 and 193. On Day 770, one control bull and seven vaccinated bulls were slaughtered and the testes recovered and weighed.

GonaCon™ vaccine

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

Vaccination was by intramuscular injection at the rump site.

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

Live weight

Results for live weight are shown in Figures 1 and 2 and the data are summarised in Table 1. There was a significant ($P < 0.001$) effect of day on live weight but there was no effect of treatment on live weight.

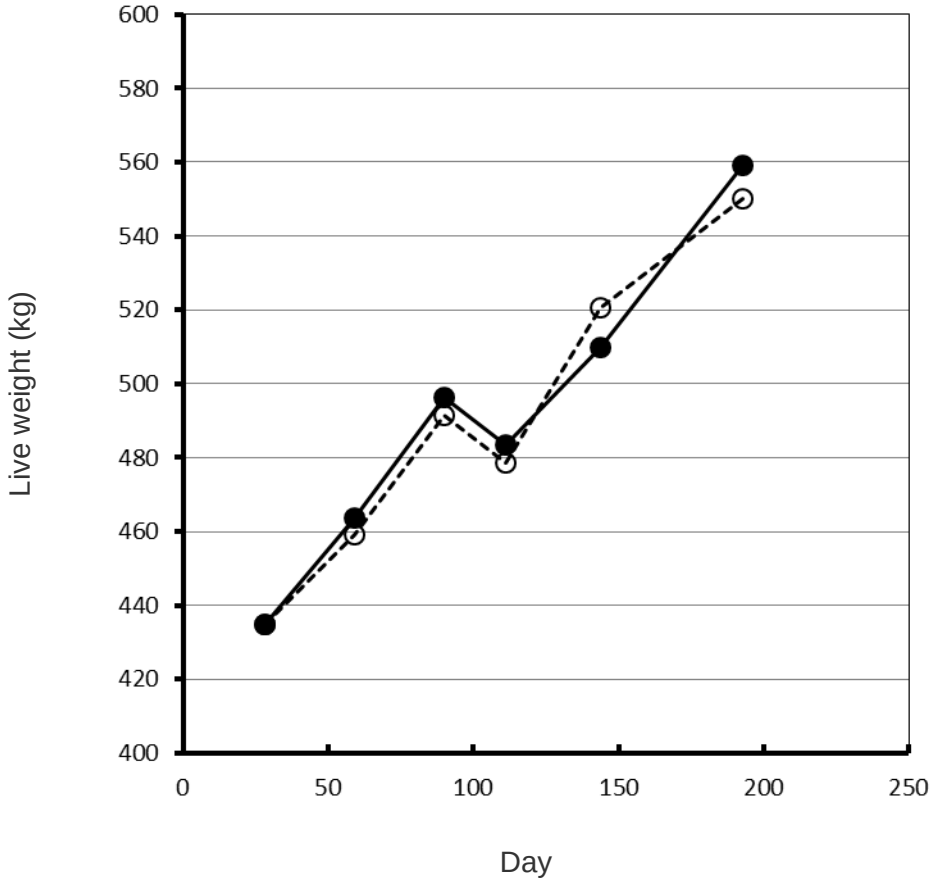

Figure 1. Longitudinal changes in live weight for control Brahman bulls (○) and bulls vaccinated with GonaCon™ (●). Results are average live weight.

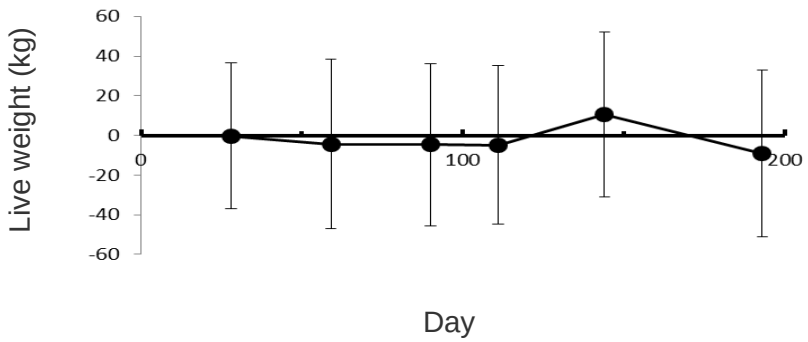

Figure 2. The longitudinal difference in live weight between control Brahman bulls and bulls vaccinated with GonaCon™. The results are presented as the difference and 95% confidence intervals.

Table 1. Live weight for control Brahman bulls and bulls vaccinated with GonaCon™. Results are means ± SEM.

Day	Live weight(kg)		P value
	Control (n= 4)	GonaCon™ (n = 8)	
28	435 ± 15	435 ± 11	P > 0.05
59	459 ± 17	464 ± 12	P > 0.05
90	492 ± 17	496 ± 12	P > 0.05
111	479 ± 16	484 ± 11	P > 0.05
144	520 ± 17	510 ± 12	P > 0.05
193	550 ± 13	559 ± 13	P > 0.05

Testicular size

Results for testicular size are shown in Figures 3 and 4 and the data are summarised in Table 2. There were significant (P < 0.0001) effects of day, group, and day x group on testicular size. Testicular size for control bulls remained relatively constant to Day 193 (73.8 ± 3.1 mm). Bulls vaccinated with GonaCon™ showed a progressive increase in testicular size from Day 0 (36.8 ± 1.5 mm) to Day 193 (51.4 ± 4.7 mm). The increase in testicular size for bulls vaccinated with GonaCon™ was due to testicular growth in 3 bulls (67.0 ± 2.6 mm) while 5 vaccinated bulls continued to have suppressed testicular growth on Day 193 (42.0 ± 1.5 mm).

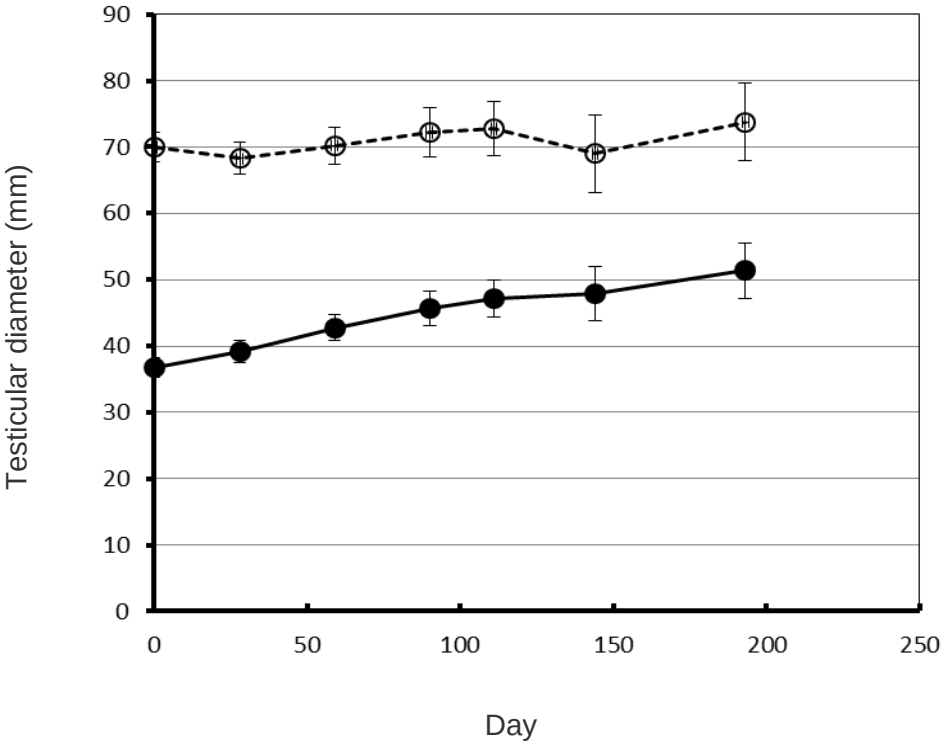

Figure 3. Longitudinal changes in testicular diameter for control bulls (○) and bulls vaccinated with GonaCon™ (●). Results are means ± SEM.

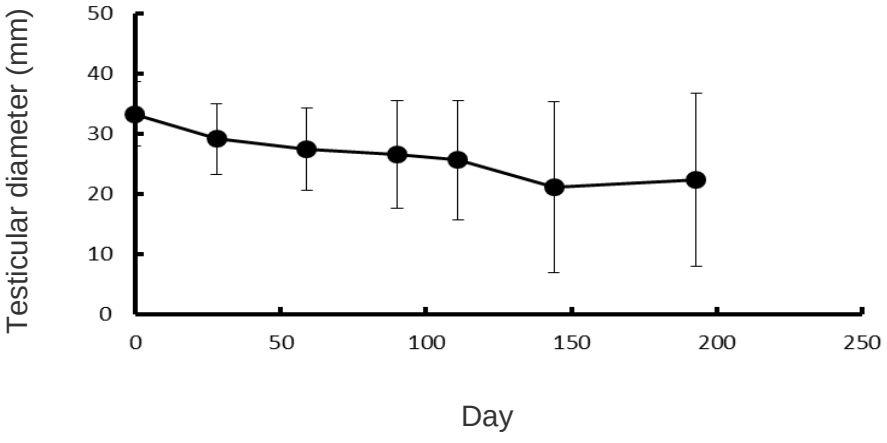

Figure 4. Longitudinal trend for the difference in testicular size between control bulls and bulls vaccinated against GonaCon™. Results are the difference between control and vaccinated bulls and 95% confidence intervals

Table 2. Testicular size for control Brahman bulls and bulls vaccinated with GonaCon™. Results are means ± SEM.

Day	Testicular size (mm)		P value
	Control (n = 4)	GonaCon™ (n = 8)	
0	70.0 ± 2.2	36.8 ± 1.5	P < 0.001
28	68.3 ± 2.4	39.1 ± 1.7	P < 0.001
59	70.3 ± 2.8	42.8 ± 2.0	P < 0.001
90	72.3 ± 3.7	45.6 ± 2.6	P < 0.001
111	72.8 ± 4.0	47.1 ± 2.8	P < 0.001
144	69.0 ± 5.8	47.9 ± 4.1	P < 0.01
193	73.8 ± 3.1	51.4 ± 4.7	P < 0.01

The relationship of testicular size in bulls vaccinated with GonaConTM to that of control bulls is shown in Table 3. Vaccination was associated with the arrestment of testicular growth whilst the testes in control bulls continued to grow. This meant that progressively fewer vaccinated bulls had a testicular size similar to that of control bulls (0 to 6 months after 2^0 vaccination).

Table 3. Testicular features of bulls vaccinated with GonaCon™.

Time after 2^0 vaccination (months, approx.)	Proportion of GonaCon™ bulls with testicular growth ≥ 50% of the growth in control bulls	Testicular size of GonaCon™ bulls compared with control bulls	
		Within 5 mm of the testicular diameter of control bulls	Within 10 mm of the testicular diameter of control bulls
Project B.AWW.0194.A			
0	-	19/20	20/20
1	0/20	11/20	20/20
2	1/20	8/20	18/20
3	1/20	1/20	17/20
4	2/20	2/20	14/20
5	2/20	1/20	10/20
6	2/20	1/20	3/20
8	2/20	0/20	2/20
9	2/20	0/20	1/20
10	1/20	0/20	1/20
11	3/20	0/20	1/20
12	4/20	0/20	1/20
Project B.AWW.0241			
14	0/8	0/8	0/8
15	1/8	0/8	0/8
16	1/8	0/8	0/8
17	2/8	0/8	0/8
18	3/8	0/8	0/8
19	3/8	0/8	2/8
21	3/8	2/8	2/8

Bulls vaccinated with GonaCon™ that continued to have suppressed testicular growth had a markedly reduced testicular size and weight at Day 715 after secondary vaccination (Figure 5, Table 4). The epididymis is an androgen-dependent organ and showed a size and weight in proportion to the testis (Table 4).

5 23 24 8

Figure 5. Testes of a representative control bull (5) and bulls vaccinated with GonaCon™ (8, 23, 24). Testes were obtained on Day 715 after secondary vaccination of GonaCon™ bulls (vaccinations on Day 0 and Day 55).

Table 4. Average weight of the testis and epididymis for a representative control bull and bulls vaccinated with GonaCon™. Testes were obtained on Day 715 after secondary vaccination of GonaCon™ bulls (vaccinations on Day 0 and Day 55).

	Bull	Testis weight (g)	Epididymis weight (g)
Control	5	336	24.4
GonaCon™	4	220	20.7
	8	89	9.1
	22	128	15.1
	23	66	3.9
	24	90	10.4
	26	214	18.1
	27	203	22.2

5. Discussion

The aim of the present project was to ascertain the duration of suppressed testicular growth in a sub-set of Brahman bulls vaccinated with the anti-fertility vaccine GonaCon™ in MLA Project B.AWW.0194.A. Testicular growth remained suppressed until around 15 months after secondary vaccination, after which time 3 bulls initiated faster growth between 15 and 17 months. These bulls had a testicular size approaching (but still less) that of control bulls at 21 months. Five other vaccinated bulls continued to have suppressed testicular growth at 21 months after secondary vaccination, at which time the project ended.

The present findings, together with the findings in MLA Project B.AWW.0194.A, have demonstrated that the GonaCon™ vaccine induces a suppression of testicular growth for a substantially longer period than commercial and research anti-GnRH vaccines that are in the public domain (Appendix 1, Final Report Project B.AWW.0194.A). Indeed, the response to GonaCon™ was greater than reported for cattle with vaccines produced by conventional conjugation chemistry (1-6, 8-12, 14-17, 30, 34-44) and bacterial expression systems (40-41). The reason for the greater efficacy of GonaCon™ could be related, at least in part, to the inclusion of *Mycobacterium avium* and AdjuVac™ in GonaCon™ (see also below).

The primary reason for considering an immunocastration vaccine is to replace surgical and other procedures currently used to suppress aggressive and reproductive behaviour in male cattle, and to influence growth, carcase and meat characteristics. We have previously shown that immunocastrated bulls can be assigned a steer carcase type (6). Others have shown that immunocastration is associated with an immature testicular morphology (Supplementary Bibliography 1).

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, Final Report B.AWW.0225

http://www.mla.com.au/Research-and-development/Search-RD-reports/RD-report-details/Animal-Welfare/Review-of-the-alternatives-to-castration-and-spaying-of-ruminants/739).

Mycobacterium avium subsp. *paratuberculosis* is the causative agent for Johne's Disease in cattle. Hence, cattle vaccinated with GonaCon™ could potentially test positive in the caudal-fold tuberculin test for tuberculosis (TB). This issue could also apply to the Silirum® vaccine for Bovine Johne's Disease which, presumably, has 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

Mycopar™ is a second Johne's Disease vaccine approved in the U.S.A. and contains attenuated *Mycobacterium paratuberculosis*.
http://www.bi-vetmedica.com/content/dam/internet/ah/vetmedica/com_EN/MSDS/Mycopar_msds.pdf

The USDA National Wildlife Research Centre has evaluated the addition of AdjuVac™ (contains *M.* avium) to Mycopar™.
http://www.aphis.usda.gov/wildlife_damage/nwrc/research/reproductive_control/adjuvant.shtml

Silirum® is not recommended for use in cattle that are destined for live export given the TB 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 in sheep with the Ovine Johne's Disease vaccine (Gudair™). It is presumed that Gudair™ also has formulation components similar to GonaCon™.

The TB cross-reactivity and potential site reactions are relevant issues. These need to be considered 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 consistently maintained an immunocastration response for 12 to 18 months would have broad industry application in male and female cattle.

6. Conclusion

The present project, which is an extension of Project B.AWW.0194A, has shown that GonaCon™ induces an immunocastration response in bulls that is maintained for a considerably longer period than other commercial and research anti-GnRH vaccines that are in the public domain. Indeed, the duration of the immunocastration response to GonaCon™ suggests that this vaccine has practical application in intensive and extensive cattle production systems. Notwithstanding, the implications of the *Mycobacterium avium* and AdjuVac™ constituents of GonaCon™ need to be considered for a commercial vaccine.

7. Acknowledgements

The project was undertaken in collaboration with Dr Lowell Miller and Dr Douglas Eckery of the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS). Excellent technical input was provided by Ms Darcy S. Orahood (USDA APHIS). Mr Allan Lisle (The University of Queensland) contributed expert statistical advice. The animals were under the professional management of Mr Mark Bauer and Mr Alan Morris (The University of Queensland) and Mr Paul Lipscombe and Ms Jeanette Lipscombe (The University of Sydney).

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