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Technical note

Application of immunocastration in a commercial Dohne Merino ram flock before weaning

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Abstract

The influence of pre-weaning immunocastration on the growth, incidences of reaction to vaccination, serum testosterone concentration and slaughter performance of ram lambs was determined and compared to that of physically castrated lambs. Immunocastration was performed using two doses of 2 mL Improvac® administered subcutaneously in the shoulder, alternating sides per vaccination, using a Sterimatic® needle guard system. The first vaccination was administered to 50 lambs with an average weight (± SD) of 16.0 ± 3.05 kg, and the second vaccination was carried out six weeks later, when the lambs weighed on average (± SD) 20.5 ± 4.11 kg. An additional 50 lambs were physically castrated using elastrator bands at the same time as the primary vaccination given to the immunocastrates. Data were collected during four sessions over the 25-week period, at time points which fitted into the normal management activities of the commercial operation (Weeks 1, 6, 16 and at slaughter). During these sessions, all lambs were weighed, and blood samples were collected from immunocastrates. The immunocastration injection site was also scored for adverse reactions. Immunocastration was successful in preventing testosterone secretion for the duration of the trial and no differences were reported regarding weight gain or slaughter performance between the treatments. The Sterimatic® and Stericap® system, as used in this study, proved to be an easy-to-use and safe system for the commercial administration of Improvac®, with no adverse reactions to vaccinations recorded at the injection sites. Pre-weaning immunocastration in lambs is thus possible, and the growth rate, carcass weight and carcass fatness of immunocastrates are similar to that of elastrator-castrated lambs.

Keywords: Carcass, GnRH, immunocastrate, Improvac, sheep
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Introduction

In South Africa, ram lambs are castrated so that mixed-sex flocks may be maintained in extensive production systems, without the concerns of aggressive behaviour and unwanted, uncontrolled breeding among slaughter lambs. However, physical castration causes pain despite the use of pain mitigation (Melches *et al.*, 2007) and thus immunocastration has been considered as an alternative. The effects of post-weaning immunocastration in Dohne Merino ram lambs have been investigated (Needham *et al.*, 2016); however, the potential of the research-based protocol to be applied at industry level needs to be validated. Additionally, some producers may prefer to immunocastrate lambs shortly after birth. However, the effects of early immunocastration using Improvac® in ram lambs have not yet been compared to elastrator-castrated lambs, with the latter being the preferred physical castration method in the industry for young lambs. The potential of a safe needle guard and high hygiene system that can be used commercially to immunocastrate lambs also needs to be identified to ensure user safety and prevent or minimise infection at injection sites. Thus, the aim of this study was to implement the vaccination protocol developed by Needham (2018) on pre-

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weaned lambs in a commercial production system using a safety vaccinator system (Sterimatic®) and compare their growth and slaughter performance to elastrator-band castrated lambs under the same production conditions.

Materials and Methods

Ethical clearance was obtained from the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00073). One hundred Dohne Merino ram lambs (average live weight ± SD: 16.0 ± 3.05 kg; ~ 1 month old) were randomly selected from an extensively farmed commercial flock in the Bredasdorp region (Western Cape, South Africa). The ram lambs were randomly allocated to two treatment groups: immunocastration (IC), and castration by using the elastrator-band method (EC). As the trial was performed under commercial production conditions, the system used did not allow for intact rams to be maintained as a separate flock. The production enterprise consisted of a commercial flock of Dohne Merino for meat and wool production. An extended vaccination schedule had to be developed that could accommodate a monthly management programme of the farm. A six-week vaccination interval was therefore chosen to coincide with dosing for internal parasites and vaccinating for enterotoxaemia (pulpy kidney).

Fifty lambs were injected subcutaneously with 2 mL Improvac® on the shoulder, alternating sides of the body for each vaccination, using a Sterimatic® needle guard system (Sterimatic Worldwide Ltd, UK) fitted with a Stericap® (Sterimatic Worldwide Ltd, UK). The Stericap® consists of a sealed plastic container that covers a piece of foam soaked in 2.5% glutaraldehyde and 5% Bardac 22 (didecyl dimethyl ammonium chloride). Coinciding with the administration of the primary vaccination dose, another 50 ram lambs were fitted with elastrator rubber rings over the scrotum as per standard farm protocol. Care was taken to ensure that the testes were below the ring before the applicator was removed.

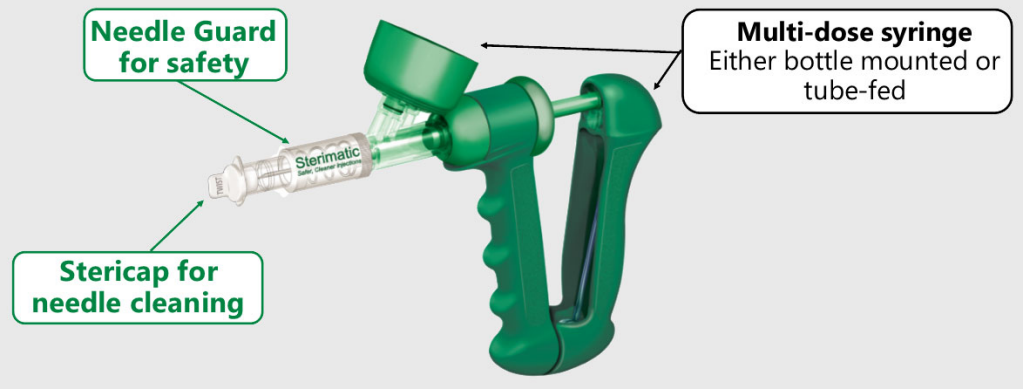

Figure 1 Illustration of the Sterimatic® safety needle guard system fitted with a Stericap® and mounted to a multi-dose vaccinator

The vaccinator is fitted with a 25 mm metal hub needle, resulting in a 12 mm deep subcutaneous injection through the needle guard and Stericap®. This system was used to immunocastrate 50 commercial Dohne Merino ram lambs, providing improved user safety from accidental needle sticks and improved hygiene through needle disinfection within the Stericap® (http://www.fwi.co.uk/advertisement/sterimatic-how-to-guide-for-injecting-cattle-and-sheep.htm)

To minimise disruption of routine handling activities, data recording was simplified and timed to coincide with farm management activities. Lambs were weighed at first injection/elastrator-band castration at Week 1 (W1), at second vaccination (W6), and 70 days later (W16). Ten lambs per treatment were randomly selected for the collection of blood samples. Blood was taken from these (20) lambs during the first, sixth, and sixteenth week of the trial (W1, W6, and W16) to coincide with the first immunocastration vaccination and physical castration in Week 1, second vaccination in Week 6, and during Week 16 when animals were handled for routine management purposes. The first blood sampling was performed prior to castration and represented the baseline/initial testosterone concentration of each animal (W1). Testosterone was extracted by adding 1.5 mL of UHPLC-grade tert-Methyl Butyl Ether (Sigma-Aldrich, Steinheim, Germany) to 500 µL of serum. An internal standard of testosterone-1, 2-d2 was included at 1.5 ng per 500 µL of serum sample. The samples were then vortexed at 1000 RPM for 10 min and frozen at -80 ºC for 60 min. The non-polar phase was transferred and dried under nitrogen gas at 55 ºC before the extract was reconstituted in 50 µL of 50% methanol (ROMIL, Cambridge, England) and stored at -20 ºC until analysed. A standard curve was established for testosterone-1, 2-d2 (in 50 % methanol) and the sample testosterone concentrations were

established using ultra-performance convergence chromatography tandem mass spectrometry (Acquity UPC^2-MS/MS) fitted with an Acquity UPC^2 BEH 2-EP column (3 mm x 100 mm; 1.7 µm particle size; Waters Corporation, USA), under the conditions discussed in detail by Quanson *et al.* (2016). Analysis of raw data was performed using MassLynx^{TM} software (Waters Corporation, USA).

Injection sites were monitored at W6 and W16 and scored according to Needham (2018), summarized in Table 1. The injection site was either scored as normal (0), mild (1), moderate (2), major (3) or severe (5). Testes palpation was performed at W16 to determine the presence or absence of developed and functional testes. Average daily gains (ADG; g/day) were calculated for the periods from W1 to W6, from W6 to W16, from W16 to W25, as well as for the entire period from W1 to W25.

At W25 (~ 7.5 months old), live weight was measured before transport to the abattoir, where the lambs remained in lairage overnight. Slaughtering was in accordance with commercial standards. Hot carcass weight was recorded, and carcass fatness commercially graded according to SAMIC (2006) as follows: 0 (no fat on the carcass), 1 (very lean; 0 – 0.9 mm), 2 (lean; 1.0 – 4.0 mm), 3 (medium; 4.1 – 7.0 mm), 4 (fat; 7.1 – 9.0 mm), 5 (slightly fat; 9.1 – 11 mm), and 6 (excessively overfat; > 11.0 mm). Testes were collected on the slaughter line, trimmed of excess tissue and epididymides, and weighed.

Table 1 The injection site scoring system developed by Needham (2018) and used to describe the reaction to immunocastration vaccination of commercial extensively produced Dohne Merino ram lambs

Score	Degree of reaction	Edema	Erythema	Induration	Contusion	Exudate
0	Normal	Slight; ≤ 0.5 cm diameter	Very slight; barely perceptible	None	Slight petechiae	None
1	Mild	Mild; palpable; ≤ 1cm diameter	Mild but well defined	Mild, palpable, ≤ 1cm diameter	Mild petechiae or slight purpura formation	None
2	Moderate	Considerable; > 1 cm diameter	Moderate	Moderate; > 1 cm diameter	Purpura	Serous
3	Major	Palpable focal edema	Severe; beet-redness	Eschar formation; crepitus	Ecchymosis	Sero-sanguineous
4	Severe	Severe diffuse edema	Severe; beet-redness	Hardened tissue broken open	Severe bruising	Purulent

Primary and secondary vaccinations were administered 6 weeks apart

An 8% mortality rate was recorded after W1 after tail docking due to spinal infection as confirmed after necropsy, with seven mortalities within the immunocastrated group and three mortalities within the physically castrated group. Two immunocastrated lambs had only one testis present at the time of slaughter, weighing 7.74 and 6.17 g, respectively, and were thus omitted from the determination of the mean paired testes weights for the immunocastrated lambs. The respective data for those animals were therefore excluded from the trial and statistical analysis. Statistical analysis was performed following the Variance Estimation, Precision and Comparison procedure in STATISTICA 13 (Dell Inc., 2016), and the restricted maximum likelihood method was used to determine treatment differences over the study period for live weight and serum testosterone concentrations. The grouping variables were animal, treatment, day; fixed effects were treatment, day and treatment*day, and lamb was considered a random effect. The ADG data and slaughter data were analysed using ANOVA. Fisher's LSD was the chosen post-hoc test and differences between means were reported at a significance level of 5%.

Results and Discussion

The results for the trial are summarized in Table 2. Immunocastration with Improvac® has been previously successful in suppressing testosterone secretion for up to 12 weeks after second vaccination in

Table 2 The average (± SE) serum testosterone concentration, weight, growth rate and slaughter recorded for extensively farmed Dohne Merino lambs castrated by means of the elastrator-band method or immunocastration

Parameters	Elastrator-castrated				Immunocastrated			
	Week 1	Week 6	Week 16	Week 25	Week 1	Week 6	Week 16	Week 25
Growth performance								
Live weight, kg	16.9 ± 0.59	20.5 ± 0.59	35.2 ± 0.59	48.2 ± 0.61	16.7 ± 0.61	20.5 ± 0.61	36.1 ± 0.61	49.9 ± 0.64
ADG periods, g/day	83.5 ± 7.94	209.9 ± 7.94	212.3 ± 7.24		90.0 ± 8.30	222.08 ± 8.30	221.1 ± 7.57	
ADG overall, g/day	179.6[b] ± 3.50				190.0[a] ± 3.65			
Vaccination success								
Testes weight, g	-				14.3 ± 1.25			
Testosterone, ng/mL	-				0.076[b] ± 0.087	0.052[b] ± 0.092	0.042[b] ± 0.092	-
Reaction score, 0-5	-				0	0	0	-
Slaughter performance								
Hot carcass weight, kg	23.2 ± 0.43				23.8 ± 0.40			
Dressing percentage	48.0 ± 0.31				47.7 ± 0.33			
Offal percentage	52.0 ± 0.36				52.26 ± 0.27			

[a, b] LSMeans with different superscripts within rows are significantly different ($P \leq 0.05$)
ADG = average daily gain

lambs weighing 20 kg at primary vaccination (Janett *et al.*, 2003). Similarly, in the present study, serum testosterone levels in IC lambs did not increase for 16 weeks after primary vaccination. The expected serum testosterone concentration of lambs at this weight should be approximately (± SD) 2.6 ± 2.56 ng/mL (live weight of 35.6 ± 4.74 kg; Needham, 2018). The borderline, non-detectable serum testosterone concentrations in the lambs in the current study can potentially be of adrenal gland origin, but were deemed negligible. These levels of testosterone are below the concentrations required for mounting (0.32 to 0.65 ± 0.01 ng/mL) and copulation (1.26 ± 0.13 ng/mL) behaviour in rams (D'Occhio & Brooks, 1982). This suppression of testosterone production during physiological development is likely to have a permanent effect on testis function. Testes were present at W16 in IC lambs; however, they were barely palpable within the scrotum and extremely small compared to the average scrotal circumference ± SD (30.3 ± 1.48 cm) and trimmed paired testes weights (258.5 ± 61.92 g) for intact rams at a similar live/slaughter weight of 50.2 ± 3.66 kg (Needham *et al.*, 2016).

Administering the first vaccination prior to weaning resulted in average testes weights (± SD) of 14.3 ± 7.61 g and was indicative of the successful interruption of testis development and functioning prior to puberty (Table 1). Immunocastration was thus successful in interrupting testis growth for a total of 25 weeks after the primary vaccination, and 19 weeks after the secondary vaccination. The duration of immunocastration effect after secondary vaccination within this trial was two months longer than when lambs were injected with their primary dose at two months of age (Janett *et al*., 2003).

No reactions were evident at the injection site after immunocastration on the days of measurement, indicating that the location and hygiene system were appropriate for use under commercial conditions. The Sterimatic® and Stericap® system fitted to a multi-dose injector provided user safety and was easy to use resulting in immunocastration taking a similar amount of time to perform as elastrator-band castration. Thus, the commercial enterprise was satisfied with the immunocastration technique as it resulted in no deviation from the typical handling. Furthermore, the immunocastration technique is not a new skill as subcutaneous injections were routinely used for the administration of other medications.

Although the immunocastrated lambs had a higher overall ADG (P <0.05), no differences were observed in live weight and hot carcass weight (HCW) between the two castration methods (Table 1). The lack of differences in animal and carcass weights is likely due to the successful suppression of testosterone from W1 to W16 by both treatments. However, the small differences in ADG may be amplified should a larger sample number be incorporated and factors such as parity number and birth status be considered. Furthermore, grazing was considered relatively poor during the trial, and although it represents accurate commercial challenges of lamb production, it is a limiting factor regarding the interpretation of the scientific results. Providing a higher plane of nutrition during a feedlot finishing phase may influence the growth of the immunocastrated and elastrator-castrated lambs, as poor nutrition may suppress sex differences (Prescott, 1969). It was expected that the chronic pain that physically castrated lambs experience after elastrator-band castration (Melches *et al.*, 2007), would suppress growth rate when compared to immunocastrated lambs. Despite the general conclusion that physical castration decreases ADG (Sales, 2014), no differences were observed in the growth rate of physically castrated, immunocastrated and intact lambs by Needham *et al.* (2018a). Previous studies have also reported no differences in growth rate between physically castrated and immunocastrated ram lambs (Kiyma *et al.*, 2000; Ülker *et al*., 2002; Ülker *et al*., 2003). However, despite the lack of effect on live weight, physical castration of lambs causes pain, and based on the conclusions by Melches *et al.* (2007) and Needham (2018) immunocastration should thus be used as an alternative to improve the welfare of ram lambs.

No differences were recorded for slaughter performance (HCW, dressing and offal percentages) and grading (Table 1). All lamb carcasses were classified as "A" according to the age classification of SAMIC (2006), as they had no permanent incisors present. Both castration treatments produced carcasses with either a "lean" (A2) or "medium" (A3) fat covering, with no differences in the distribution of carcass fatness grading between castration treatments. Of the EC carcasses, 33.3% were classified as A3 and 66.6% as A2, while 32.6% of the IC carcasses were graded A3 and 67.4% were A2. Despite the lack of differences in live weights and HCW, physical castration has been shown to have a negative effect on the welfare of ram lambs (Melches *et al.*, 2007); consumer and producer concern about animal welfare and the enforcement of legislation should be sufficient motivation to abolish this practice. Additionally, in relation to animal welfare and the slaughter of lambs, but not necessarily the aim of this paper, the use of tail docking should be re-examined, considering the high mortality rate resulting from this technique within this commercial trial.

Conclusion

Early immunocastration of male lambs was successful in suppressing testis development and testosterone secretion and thus provides a suitable, welfare-friendly, and commercially viable alternative to elastrator castration. Producers should expect growth and slaughter performances of early immunocastrated

lambs to be equivalent to that of elastrator castrated lambs. The Sterimatic® and Stericap® system improves user safety and efficacy, and can therefore be recommended for the commercial immunocastration of lambs.

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Authors' Contributions

TN conducted the research trial as well as formulated the short communication article as part of her PhD research thesis. HL and LCH were responsible for the supervision, editing and development of the project and article.

Conflicts of Interest Declaration

There are no conflicts of interest.

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