Immunocastration and its application in ram lambs

By

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DECLARATION

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SUMMARY

In South Africa, Dohne Merino sheep are farmed extensively for wool and meat production. Ram lambs are typically castrated to enable easier management of mixed-sex flocks by manipulating reproduction and associated behaviour, while promoting fattening and improving meat quality. Due to various welfare concerns regarding the pain associated with castration and healing complications, the practice is under scrutiny, which necessitates producers to investigate alternatives to physical castration. International pork and beef industries have successfully applied immunocastration in commercial enterprises; however, no formal recommendations regarding its commercial application in the lamb industry are available. Improvac® was administered at two doses during the interval from weaning until slaughter and was sufficient in supressing testes growth and functioning in Dohne Merino ram lambs. Varying the interval between the two doses (two, three or four weeks) and the interval between second vaccination and slaughter (four or six weeks) did not influence growth performance or slaughter performance of ram lambs after weaning. The primary vaccine dose decreased serum testosterone concentrations, which resulted in a decrease in scrotal circumference, semen quality and seminiferous tubule size for all immunocastration vaccination schedules. Testes cut surface colour was redder and more yellow in immunocastrated than in intact rams, providing a possible on-line detection method for vaccination success after slaughter to ensure correct sex classification. An inter-vaccination period of two weeks and a six-week interval after second vaccination until slaughter, had the most pronounced and sustained effect on reproductive capacity of the rams, as well as the lowest frequency of injection site reactions. The recommended injection protocol for the commercial application of Improvac® in lambs

involves subcutaneous administration on the flat surface of the shoulder area, and alternating shoulder sides per dose. Immunocastration resulted in improved welfare of ram lambs, when compared to rams castrated using the Burdizzo-method. The latter method resulted in increased serum cortisol concentrations, abnormal behaviours in response to pain experienced during the procedure, despite the use of pain mitigation, and tissue necrosis. Minor differences in offal yields were observed in immunocastrated lambs but no influence on carcass cutting yield or meat quality, other than cut surface colour, was reported. Thus, immunocastration can be considered a feasible alternative to physical castration in the commercial production of Dohne Merino ram lambs. However, further investigation is recommended into the application of immunocastration within a feedlot environment to further elucidate the effects on feed intake, nutrient requirements, differential fat deposition and behaviour in Dohne Merinos and other important meat-producing breeds in South Africa.

OPSOMMING

In Suid-Afrika word daar ekstensief met Dohne Merino skape geboer vir wol- en vleisproduksie. Ramlammers word tipies gekastreer om die bestuur van gemengde-geslags troppe te vergemaklik deur voortplanting en gepaardgaande gedrag te manipuleer, terwyl die bevordering van vetheid en vleiskwaliteit ook verbeter word. Weens verskeie welsynsbekommernisse, rakende die pyn wat verband hou met kastrasie en genesingskomplikasies, word die praktyk onder die loep geneem, wat produsente noodsaak om alternatiewe vir fisiese kastrasie te ondersoek. Internasionale vark- en bees industrieë het immunokastrasie al suksesvol in kommersiële ondernemings toegepas; daar is egter geen formele aanbevelings beskikbaar aangaande die kommersiële toepassing daarvan in die lamsbedryf nie. Twee doserings van Improvac® was toegedien tydens die interval van speen tot slag en was voldoende om testesgroei en -funksionering van Dohne Merino ramlammers te onderdruk. Wisseling van die interval tussen die twee dosisse (twee, drie of vier weke) en die interval tussen die tweede inenting en slagting (vier of ses weke), het nie die groeiprestasie of slagprestasie van ramlammers beïnvloed nadat dit gespeen is nie. Die primêre entstof dosis het serum testosteroonvlakke verminder, wat gelei het tot 'n afname in skrotale omtrek, semengehalte en seminifereuse buisie grootte vir alle immunokastrasie-inentingskedules. Die testis se gesnyde oppervlakkleur was rooier en meer geel vir die immunokastreerde ramme in vergelyking met die ongeskonde ramme, wat 'n moontlike vroeë deteksie metode bied om suksesvolle inenting na slagting te bepaal indien die inenting sukses bevraagteken word. 'n Inter-inentingsperiode van twee weke en 'n ses-week interval na die tweede inenting tot slag, het die grootste en mees volgehoue uitwerking op die voortplantingskapasiteit van die ramme, asook die laagste frekwensie van inspuitingsreaksies, gehad. Die aanbevole inspuiting protokol

vir die kommersiële toediening van Improvac® vir lammers behels onderhuidse toediening op die plat oppervlak van die skouer area met die afwisseling van skouerkante per dosis. Immunokastrasie het gelei tot ‘n verbeterde welsyn van ramlammers in vergelyking met ramme wat met die Burdizzo-metode gekastreer is. Laasgenoemde metode het gelei tot verhoogde serum kortisolvlakke en abnormale gedrag in reaksie op pyn wat tydens die prosedure ervaar is, ten spyte van die gebruik van pynbeperking en weefselnekrose. Geringe verskille in afval opbrengste is waargeneem vir immunokastreerde lammers, maar geen invloed op karkas opsny opbrengs of vleiskwaliteit, behalwe gesnyde oppervlakkleur, is gerapporteer nie. Daarom kan immunokastrasie beskou word as 'n haalbare alternatief vir fisiese kastrasie tydens die kommersiële produksie van Dohne Merino ramlammers. Verdere ondersoek word egter aanbeveel vir die toepassing van immunokastrasie binne 'n voerkraalomgewing om die effekte op voerinname, voedingstof vereistes, differensiële vetdeposisie en die gedrag van Dohne Merinos en ander belangrike vleisproduserende skaaprasse in Suid-Afrika verder te verhelder.

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LIST OF ABBREVIATIONS

GnRH/ GnRF/ LHRH	Gonadotropin-releasing hormone / Gonadotropin-releasing factor/ Luteinizing-releasing hormone
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
GH	Growth hormone
IGF-1	Insulin-like growth factor-1
HPG	Hypothalamic-pituitary-gonadal
ADG	Average daily gain
HCW	Hot carcass weight
KLH	Keyhole limpet hemocyanin
FCA	Freund's complete adjuvant
UPC^2-MS/MS	Ultra-performance convergence chromatography tandem mass spectrometry
GIT	Gastrointestinal tract
VEPAC	Variance estimation, precision and comparison
REML	Restricted maximum likelihood
LSD	Least significant difference
SE(M)	Standard error (of the mean)
SD	Standard deviation
LSMeans	Least Squares Means
ANOVA	Analysis of Variance
IC	Immunocastrated
IC2/3/4	Immunocastrated with a two/three/four-week interval between doses
ICS4/6	Immunocastrated with second dose four/six weeks before slaughter
R	Intact/entire male ram
B	Burdizzo-castrated lamb
EC	Elastrator-castrated lambs
MTBE	Tert-Methyl Butyl Ether

DHEA	Dehydroepiandrosterone
A4	Androstenedione
T	Testosterone
C	Cortisol
11OHA4	11β-hydroxyandrostenedione
PROG	Progesterone
5α-dione	5α-androstanedione
DHT	5α-dihydrotestosterone
11KDHT	11-ketodihydrostestosterone
D	Day
W	Week
RCF	Relative centrifugal force
RPM	Revolutions per minute
FQWS	Forequarter walking score
HQWS	Hindquarter walking score
P	Palpation
HQ	Hindquarter
LT	*Longissimus thoracis* muscle
LTL	*Longissimus thoracis et lumborum* muscle
pH_{24}	pH at 24 hours *post-mortem*
WBSF	Warner-Bratzler shear force

LIST OF FIGURES

LIST OF TABLES

NOTES

This thesis is presented in the format prescribed by the Department of Animal Sciences, Stellenbosch University (SU). The format of the thesis is such that each chapter has either been published or prepared for publishing as a journal article. As each chapter has been written as an individual entity, some repetition between chapters is unavoidable. The research chapters are prefaced by a summary of research performed, general introduction of the topic, culminating in a general discussion and conclusion of the project. Language, style and referencing are in accordance with specifications of the journal Small Ruminant Research. The opinions expressed, and conclusions arrived at in this study are those of the author and are not necessarily to be attributed to the NRF or the Department of Animal Sciences, SU.

Results from this dissertation that have been published in the following journals:

- Needham, T., Lambrechts, H., Hoffman, L. 2016. The influence of vaccination interval on growth, carcass traits and testicle parameters of immunocastrated ram lambs. Small Rum. Res. 145, 53-57.
- Needham, T., Lambrechts, H., Hoffman, L. 2017. Castration of male livestock and the potential of immunocastration to improve animal welfare and production traits: Invited Review. S. Afr. J. Anim. Sci. 47, 731-742.
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LIST OF CONTENTS

CHAPTER 1
General Introduction

1.1 Background

The physical castration of male livestock has raised ethical and animal welfare concerns amongst consumers, placing pressure on the livestock industry to address the production practice. Physical castration has been used to assist in the management of mixed-sex flocks and to prevent the various carcass and meat quality issues associated with intact males (Cronin et al., 2003; Price et al., 2003). The European Union decided to voluntarily ban the physical castration of piglets without anaesthesia from 2018 onwards (Font-i-Furnols et al., 2012), and it is expected that similar regulations will be implemented in the lamb and beef industries. Standard production practices involve the castration of ram lambs shortly after birth, when handling is easy and wound-healing complication risks are fewer (Baird & Wolfe, 1988).

However, there are not only direct cost involved with physical castration, such as the castration product or equipment, preventative measures of infection, labour, and potential veterinary expenses should pain mitigation be required; but also, indirect costs associated with production loss and animal welfare. Physical castration decreases the growth of rams (Sales, 2014), not only due to the decrease in anabolic steroid production, but also due to acute and chronic pain despite the use of anaesthetics (Melches et al., 2007), which contributes to an increase in morbidity and mortalities amongst young animals. Costs related to animal welfare issues include pain, handling stress and, in some cases, infection and abscessing. Infections and abscesses require additional veterinary intervention, and result in production losses which all contribute to a loss in potential income.

Legislation regarding the castration of ram lambs varies between countries, with no readily available regulation within South Africa. As a result, lambs are often castrated at an older age without pain mitigation. This may be preferable for various management reasons,

such as preventing the stress and subsequent interruption in growth during the critical period shortly after birth. It may also be necessary to keep rams intact until the selection of breeding and replacement animals after which unselected rams are finished for slaughter. Although new regulations could motivate the use of pain mitigation with castration, on-farm application may be sub-optimal, including factors such as identifying products which are effective, long-acting and food-safe, and applying them at the correct dose to animals recovering in a flock environment.

There is thus a need to identify and develop alternative strategies to both physical castration and intact male production that will ensure efficient growth and result in optimum carcass and meat quality, without compromising animal welfare. Immunocastration is considered as an alternative to physical castration techniques and its application has been extensively studied in commercial pork production. Immunocastration involves injecting the animal with a vaccine containing a synthetic gonadotropin-releasing hormone (GnRH) analogue which stimulates GnRH-antibody production, blocking the stimulatory action of GnRH and compromising testes functioning. Immunological castration presents an opportunity to mitigate or eliminate many of the welfare-issues associated with physical castration and recovery in rams.

1.2 Motivation of research

Various immunocastrating vaccines have been formulated and investigated for research purposes in sheep, but to date no commercialized product is registered for use in ram lambs (Parthasarathy et al., 2002; Ülker et al., 2002; Oatley et al., 2005; Karakus et al., 2013). Variation in the composition and design of vaccines manufactured on a small scale for research purposes, as well as variation in the response experienced with different types of adjuvants,

complicates and limits the extrapolation and comparison of available results. Literature on the immunocastration of sheep is scarce, with the study of Janett et al. (2003) being the only readily available literature examining the immunocastration of ram lambs using Improvac®.

To determine the potential of a commercial vaccine to be used as an immunocastration treatment in ram lambs, it is necessary to study the effect on the endocrine profile, gonad integrity, growth performance, carcass traits and meat quality of a commercial breed in a controlled environment. The optimal vaccination schedule(s) regarding the intervals between the first and second/booster immunocastration vaccinations, the timing of second vaccination with regards to slaughter, and standard operating procedures for vaccination, need to be developed for immunocastration in ram lambs. A study of this nature and scope will elucidate whether it is possible to use such a vaccine for immunocastration purposes in ram lambs while sustaining growth rate without compromising meat quality and animal welfare.

1.3 Research question, aims and objectives

The research question for this project is as follows: what is the optimal immunocastration vaccination schedule for sheep with regards to their growth performance, reproductive functioning, carcass traits and meat quality? The aim of this thesis was to design an immunocastration vaccination schedule for a popular dual-purpose (wool and meat) South African sheep breed, the Dohne Merino. The specific objectives were:

1. To establish whether the interval between successive immunocastration vaccine administrations influences growth, serum testosterone concentrations, and testicle growth, morphology and function.

2. To elucidate whether extending the interval between second vaccination and slaughter influences the carcass cutting yield, backfat thickness and meat quality of immunocastrated rams while comparing their performance to both physical castrates and intact rams.
3. To determine potential side effects of vaccination, such as localized reaction at the respective injection sites and raised body temperature.

1.4 Significance of research

The value of immunocastration needs to be evaluated from various perspectives, including the producer (growth), the abattoir (carcass traits) and the consumer (meat quality). Findings will ultimately contribute to improving our understanding of the effects of immunocastration of sheep on the steroid hormone profile, growth and meat quality as well as produce results that can assist with the formulation of protocols for incorporation of immunocastration in commercial production programs. These immunocastration protocols may be applied for producing lamb meat under conditions that restrict the use of physical castration or as an alternative to entire ram production. The results from this study will hopefully allow castrated animals to be produced in an economical but also ethical fashion by mitigating the discomfort of physical castration. The information generated within this study may thus allow lamb producers to consider the commercial use of immunocastration and will contribute to the existing body of literature on immunocastration in sheep.

1.5 Brief chapter overview

The research chapters and outlay of this thesis are summarized in Table 1.1. The controversial topic of physical castration and its welfare implications are discussed in Chapter 2, comparing the research done over various livestock species in terms of immunocastration while

highlighting the need for further research in rams. The sexual development of Dohne Merino lambs after puberty is described and quantified in Chapter 3, as a standard for subsequent research. The influence of varying periods between first and second immunocastration vaccinations on the growth and slaughter performance of weaned, peri-pubertal Dohne Merino ram lambs, and hormone profile and spermatogenic activity within the testes was evaluated in Chapter 4 and 5, respectively. The effects of extending the interval between second immunocastration vaccination and slaughter on pubertal Dohne Merino growth performance, testes development and behaviour was assessed in Chapter 6 relative to both physically castrated and intact rams. Chapter 7 examines the effects of this extended interval before slaughter on the suppression of testosterone and timing of castration effect on semen quality with the effect on carcass cutting yield and meat quality discussed within Chapter 8. The commercial application of immunocastration in pre-weaned lambs was investigated as a technical note within Chapter 9 as a base study for the commercial application of the immunocastration protocol. The overall findings and implications of this project are concluded in Chapter 10.

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Table 1.1 The trial outlay for the various research chapters including animal numbers (n), approximate age at the start of the trial, weight (± SD) at the start and end of the trial, the total trial duration and the treatments investigated.

Chapter	Title	n	Age at start of trial	Weight (start-end)	Trial duration	Treatments
3	Description of the reproductive development, growth and slaughter performance of Dohne Merino rams	24	16 months	65.0 ± 4.64 to 76.6 ± 4.58 kg	9 weeks	Intact rams
4	The influence of vaccination administration interval on growth, scrotal circumference and carcass traits of immunocastrated ram lambs	40	5.5 months	35.0 ± 2.18 to 48.8 ± 4.56 kg	9 weeks	Four treatments (10 lambs/ treatment). Three immunocastrated groups: 2, 3 & 4-week intervals between two vaccinations, the second given 4 weeks before slaughter. Intact ram control group
5	Influence of immunocastration vaccine administration interval on serum androgen concentrations and spermatogenic activity in ram lambs.					
6	Growth, scrotal circumference and behaviour of immunocastrated lambs, as influenced by administration interval between second vaccination and slaughter	40	6.5 months	45.4 ± 3.68 to 52.6 ± 4.73 kg	8 weeks	Four treatments (10 lambs/ treatment). Two immunocastrated groups: 2-week interval between vaccinations, the second given 4 or 6 weeks before slaughter. Burdizzo-lamb and intact ram groups.
7	Changes in reproductive capacity of immunocastrated ram lambs after extending the administration interval between second vaccination and slaughter					
8	Influence of extending the interval between second vaccination and slaughter on carcass traits, cutting yields and meat quality of immunologically castrated lambs					
9	Immunocastration of a commercial Dohne Merino ram flock before weaning: a technical note	100	1 month	16.0 ± 3.05 to 49.1 ± 4.85 kg	25 weeks	Two treatments (50 lambs/ treatment). Immunocastrated at Week 1 and Week 6. Elastrator castrated at Week 1.

CHAPTER 2

Castration of male livestock and the potential of immunocastration to improve animal welfare and production traits: a review[1]

Abstract

Growing consumer awareness about animal welfare has led to the assessment of the impact of common farming practices, such as physical castration, on animal well-being under production conditions. Physical castration is used in livestock industries to prevent indiscriminate breeding, control aggression, and improve meat and carcass quality. In terms of animal welfare, physical castration causes pain, decreased growth performance, infection, and mortality. An alternative approach to castration is thus warranted that will ensure optimal growth without compromising the castrated animal's wellbeing. Immunocastration has proved to be an effective method of suppressing the development and functioning of the reproductive system in various domesticated and wildlife species. The effect of immunocastration on production performance is well-documented for both swine and cattle. Although ram lambs used for meat production are often physically castrated, information regarding the potential application of immunocastration in sheep is limited. However, immunocastration may potentially improve the welfare, performance, and meat quality of ram lambs used in commercial meat production systems. The purpose of this review is to compare the application and the effects of immunocastration on male livestock to highlight and motivate the need for further research into its use on ram lambs.

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[1]*Needham, T., Lambrechts, H., Hoffman, L. 2017. Castration of male livestock and the potential of immunocastration to improve animal welfare and production traits: Invited Review. S. Afr. J. Anim. Sci. 47 (6), 731-742*

2.1 Introduction

Consumer awareness about the welfare of production animals has increased recently, with the physical castration of livestock receiving considerable attention. Recently, the Global Meat News' State of the Industry Survey Report (2015) stated that, "eighty-three percent of meat industry professionals strongly agreed that the global meat industry must put more emphasis on animal welfare and impose tougher regulations". This point of view was resonated in the European Union's decision to voluntarily ban the physical castration of piglets without anaesthesia from 2018 (Font-i-Furnols et al., 2012). Males used for lamb and beef production are often castrated without pain mitigation to promote fattening and assist with management. Additionally, it is expected that the banning of physical castration may soon be enforced in the mutton and beef industries.

Although intact males have a faster growth rate and superior feed efficiency than castrates (Field, 1971; Pauly et al., 2009; Sales, 2014), various management and welfare issues exist regarding the raising of intact males (Cronin et al., 2003; Price et al., 2003). Heavy or more physiologically developed intact male carcasses are also penalized at the abattoir due to various meat quality issues. These issues include boar taint in heavy boars and the tendency for older bulls to develop dark, firm and dry meat. Thus, boars over 100 kg (approximately 22 weeks old) and bulls or rams with one or more permanent incisors are marked "MD" and receive a lower price per kg in South Africa (SAMIC, 2006). There is thus a need to formulate alternative management practices that will ensure efficient growth of male animals that will result in optimum carcass and meat quality, without having to compromise animal welfare.

Immunological castration, also known as immunocastration, has shown promise in this regard, and to date has been successfully applied in the international and local pork industry (Needham et al., 2016). Immunocastration, resulting from the administration of a vaccine designed to block the action of gonadotropin-releasing hormone from the hypothalamus,

ultimately disrupts the normal functioning of the testes, resulting in a suppression of spermatogenesis and testosterone production. Various immunocastration vaccines have been formulated and tested in sheep (Parthasarathy et al., 2002; Ülker et al., 2002; Oatley et al., 2005; Karakuş et al., 2013); however, to date, no formal commercialized product is available for use in sheep.

For the purpose of this review, the production of both intact and castrated meat-producing livestock species will be discussed, along with the associated concerns of farming intact or castrated males. The technique of immunocastration will be explained, and its application in various livestock and wildlife species highlighted. Due to the variation in effects seen with small-scale vaccines manufactured for research purposes, research using commercially available products such as Bopriva® (cattle) and Improvac® (pigs) will receive particular emphasis. The review will focus on the influence of immunocastration on meat production, indicating how immunocastration can potentially assist in addressing the welfare issues raised with physical castration, while highlighting the potential beneficial influence on production parameters, carcass traits, and meat quality in lambs.

2.2 Production and management of male livestock

The production of intact males poses various handling, management, and carcass and meat quality issues (Seideman et al., 1982; Cronin et al., 2003; Price et al., 2003). Handling intact male animals can be dangerous to both the handler and the animal, and thus castration at an early age is usually preferred. Post-pubertal bulls are especially difficult to manage from a human safety aspect due to their size and can cause damage to pasture and infrastructure (Seideman et al., 1982; Aasen, 2000). Management issues include having to either maintain intact males in isolation or at low stocking densities to either prevent or minimise the incidence and associated effects of aggressive and sexual behaviour (Aasen, 2000). In addition to this,

males are often kept isolated from females to prevent indiscriminate breeding, as with extensive cattle production (Winter, 1996). The need to isolate intact males can complicate management further if a breeding enterprise is being maintained in conjunction with slaughter animal production where indiscriminate breeding can occur, should cull males indivertibly enter the breeding female herd.

Intact male livestock may be the preferred choice compared to castrates and females, due to their superior growth, leanness of carcass, and feed efficiency (Gispert et al., 2010; Sales, 2014). These production traits can largely be ascribed to the effect of male androgens, such as testosterone, that promote lean muscle growth (Snochowski et al., 1981). The impact of testosterone on fat deposition can either be viewed as a positive or negative, depending on the market demands. For example, currently in the South African pork market, lean carcasses are favoured, and thus intact males are preferred while the risk of boar taint is often not considered. In the production of lamb and beef, emphasis is on the finishing of livestock with a certain degree of fatness, with an economic value generally placed on carcasses with a medium fat covering. Currently, beef and sheep carcasses in the A2 (1.0 – 4.0 mm backfat) and A3 (4.1 – 7.0 mm backfat) grading categories, according to SAMIC (2006), fetch the highest price per kg in the South African red meat market (RPO, 2017). Sheep and cattle carcasses from lean intact males generally score lower on fat covering when slaughtered at the same age as castrated male or female animals. Intact male animals are thus maintained longer in the finishing phase to deposit more fat, which ultimately negates the benefit of their superior feed efficiency. Therefore, a balance needs to be achieved where the most economical carcass is produced. Furthermore, the value of intact male carcasses can be decreased due to bruising and lesions from aggressive and sexual behaviour, which is a problem seen particularly in feedlot systems (Seideman et al., 1982).

2.2.1 Motivation for castration

Castration is not limited to use in livestock species, but also finds application in companion animals, horses maintained for recreational purposes, and, more recently, wildlife (De Nys et al., 2010). The typical purpose of castration is to ease handling and management by controlling sexual and aggressive behaviour. Another important management use of castration is the prevention of unwanted pregnancies; whether it be in mixed-sex herds intended for slaughter, the accidental breeding of stud animals by those males not intended for breeding purpose, or population control (Ladd et al., 1994). The control of indiscriminate breeding becomes particularly important in the livestock industry when prolonged finishing of slaughter animals is required, such as in extensive systems, where male animals may reach puberty prior to slaughter (Amatayakul-Chantler et al., 2013). This is typically experienced when free-range animals are the end-product.

The use of castration as a tool to improve meat quality is important in livestock species such as pigs, sheep, and cattle. Castrating male livestock decreases the anabolic potential of the animal and results in the increased deposition of fat. As mentioned, this can be beneficial in the sheep and cattle industries. However, the degree of fatness of a carcass not only influences the value of the carcass, but also the meat quality of the carcass, as fatness influences juiciness, which in turn is related to the tenderness of the meat. A meta-analysis of literature using bloodless castration techniques summarized that castrated rams had decreased weight gain, feed efficiency, and leanness; however, tenderness was improved (Sales, 2014).

Not only do male steroid hormones contribute to behavioural issues, it can contribute to unwanted flavours and aromas. An example of this is an objectionable sensory quality of pork from intact male pigs, known as boar taint (Patterson, 1968). The testes of boars produce a pheromone known as androstenone, which is stored within the salivary glands and released during pre-copulatory activities (Bonneau et al., 1982). However, androstenone is also

lipophilic and is thus deposited within the adipose tissue. An unpleasant smell and taste of urine or sweat is described by those sensitive to androstenone when the pork of an entire male pig is cooked and androstenone is present in the tissue, which results in consumer acceptability issues (Font-i-Furnols, 2008). The meat from intact male goats is also perceived to have an unpleasant aroma and flavour termed "buck odour" and castration has shown to improve the palatability of the resultant meat (Zamiri et al., 2012), which indicates the possible involvement of male steroid hormones produced by the testes.

2.2.2 Concerns with castration

Various methods can be used to physically castrate livestock. The testicles can be excised surgically from the scrotum or by placing a rubber ring/ band around the neck of the scrotum, with the latter method resulting in ischaemia and sloughing of the testicles and scrotum due to necrosis (Winter, 1996). Alternatively, a portion of the spermatic chord, blood vessels, nerves, and scrotal tissue can be destroyed using the closed crushing/ Burdizzo technique, which also leads to testicle atrophy due to ischaemia; however, with this method the testicles remain intact. Surgical castration is generally favoured in swine; however, depending on the country's legislation, it may or may not stipulate the use of pain mitigation. Generally, band castration, as well as Burdizzo castration, tends to be favoured in rams and bulls; however, surgical castration is also used. Again, the use of pain mitigation may or may not be mandatory in these practices (Melches et al., 2007). Local anaesthesia can be used to reduce the pain experienced with castration; however, this does have cost implications and not all legislation requires it to be used during castration.

To quantify the pain response to castration, factors such as cortisol concentrations, behaviour, and posture are often taken into consideration, with both the short and long-term responses investigated to fully describe the effects of castration on the welfare of male animals.

Rams surgically castrated under sedation with local anaesthesia indicated more immediate and frequent pain responses, resulting in a decreased feed intake and weight loss when compared to rams castrated by using the band and Burdizzo castration methods where local anaesthesia was also used (Melches et al., 2007). Although Burdizzo castration resulted in a more pronounced immediate pain response when compared to elastrator-band castration, wound healing occurred faster and with fewer complications after Burdizzo castration. Surgical and band castration resulted in a compromised recovering ability of the animals, with purulent secretion, infection, and abscesses the most common problems occurring. Although band castration showed less immediate pain responses, the castrated rams exhibited an increased level, and prolonged period, of pain due to an extended healing period of 12.3 ± 11.5 days, with the sloughing of the testes occurring at 35 ± 6.9 days. Rams surgically castrated took on average 4.9 ± 4.3 days to heal, and the Burdizzo-castrated rams on average 1.3 ± 1.0 days. Even though all treatments received pain relief, pain was however still experienced by the rams, and it can be assumed that the animals' wellbeing was not improved (Melches et al., 2007).

According to Baird and Wolfe (1998), ram lambs are typically castrated shortly after birth since animal handling is easier and fewer post-operative complications are experienced. However, it may be more favourable to castrate rams at a later age, typically found with slower growing rams who did not reach slaughter weight prior to puberty, or rams culled from breeding stock after selection based on performance testing.

Currently, legislation in certain countries dictates as to when certain castration techniques can be applied; however, some regulations do not adequately describe when veterinary intervention and pain mitigation is required. According to welfare codes under Mutilations Regulations (Permitted procedures, England) (2007), ram lambs in the United Kingdom must be castrated using rubber rings before seven days of age, but before 12 weeks of age using other techniques, with pain mitigation required after three months of age. Canadian

regulations also stipulate that rams be castrated after colostrum intake, but before seven days of age with pain mitigation being required on rams older than three months of age (NFACC, 2013). The Australian Model Code of Practice (2006) for sheep states that rams need to be castrated within 12 weeks after birth and that anaesthesia must be used if castration needs to be done after six months of age. Similarly, New Zealand requires castration to be done as early as possible, but pain mitigation is only required for rams older than six months of age (NAWAC, 2010). However, no formal or readily available guidelines exist for the physical castration of rams in South Africa.

Other factors that should be considered with physical castration, is the risk of morbidity and mortalities, as well as various labour-related issues. Firstly, persons performing the technique need to skilled to do so, using appropriate hygienic methods and well-maintained tools. Furthermore, if local anaesthesia is used, this frequently requires the administration by a registered veterinarian, which has additional time and cost implications. One also needs to consider the stress of herding, handling, and restraining the animals to perform these techniques at an appropriate age using appropriate facilities. Post-castration inspections need to be performed regularly, which requires skilled labour, increased handling and time. The importance of technique choice, pain mitigation use, recovery, and monitoring of castrated lambs is highlighted by Melches et al. (2007). Therefore, physical castration can be a time-consuming process where skilled labour is required, and the welfare of the animals can be compromised.

Physical castration of livestock may also increase the input costs per animal as well as the loss in yield per animal due to poorer feed efficiency and growth rates (Sales, 2014). Direct costs include the cost of the castration procedure itself, pain mitigation (should it be chosen or required to be used), the prevention of infection and the treatment of complications. Further financial losses are incurred when the animal experiences morbidity, decreasing growth

performance in response to both pain and lack of anabolic hormones, and when mortalities arise resulting from infection or complications. Complications and the associated financial implications regarding physical castration can be prevented using immunocastration. Amatayakul-Chantler et al. (2013) reported that adverse issues were experienced in 8 % of surgically castrated bulls raised on pasture, while those bulls immunocastrated experienced no complications related to the castration procedure. It is for these aforementioned reasons that alternatives to physical castration be investigated, while still controlling the problems associated with intact male production and preventing the welfare issues associated with both intact male and castrate production.

2.3 Immunocastration and its application

Active immunization against gonadotropin-releasing hormone has shown potential in replacing physical castration and has been successfully implemented as part of management programs in the pork industry. D'Occhio (1993) reviewed the reproductive consequences of fertility control using immunological suppression of various reproductive hormones. Furthermore, Thompson (2000) reviewed the technical aspects of the immunocastration in various livestock species which may be consulted for further information on the development of commercial vaccines. The principle of immunocastration centres on the "blocking" of the action of the gonadotropin-releasing hormone. Gonadotropin-releasing hormone, also known as Luteinizing hormone-releasing hormone (LHRH), is transported by the hypophysial portal systems to the anterior pituitary. The short exposure time of the GnRH to the blood system during transport from the hypothalamus to the pituitary gland is the only opportunity for exposure of GnRH to circulating antibodies. If antibodies specific to GnRH are exposed to GnRH during this stage, GnRH will bind to GnRH-specific antibodies that essentially "neutralizes" the hormone, either by

preventing diffusion through the capillaries or by occupying the binding site on GnRH which prevents it binding to the anterior pituitary.

The GnRH itself is too small to be immunogenic, and various techniques have been used to "fool" the animal's system into recognizing GnRH as foreign. Such techniques typically involve conjugating GnRH to a large, non-self or foreign protein using various sites and methods of conjugation. Numerous techniques have shown success in producing sufficient GnRH-antibody titres; however, the application of such vaccines have been limited regarding commercial use. The type of adjuvant used, and the number of immunizations required to elicit a response, are two constraining factors that hampers the use of immunovaccines. The variation in the composition and design of vaccines manufactured on small scale for research purposes, as well as variation in the response experienced with different types of adjuvants complicates and limits the extrapolation and comparison from available results. Commercial vaccines have been designed to minimise adverse reactions to the adjuvant in the relative species as well as to minimise frequency of vaccinations. However, other factors also influence the results seen from immunocastration, such as the interval between vaccinations, the timing of the second vaccination relative to slaughter, the age of the animal, the duration of the study, as well as the nutritional strategy applied. These factors thus need to be considered when comparing results. Commercially available vaccines include Bopriva® (Zoetis™) for cattle and Improvac® (Zoetis™) for swine. Previously, Vaxstrate (Arthur Webster Pty Ltd, Castle Hill, N.S.W.) was available for use in heifers but has been discontinued.

Immunocastration may be used commercially to improve the on-farm welfare of male livestock species by circumventing many of the concerns with physical castration methods. A major benefit of immunocastration is preventing the pain associated with the castration procedures and the risk of wound infection. Thus, the incidences of morbidity and mortalities due to wound healing complications on-farm may be reduced. Immunocastration still requires

the animal to be handled; however, producers are not constrained to the short timeframe in which physical castration is recommended to be performed after birth. Producers can wait until later in the growth of the animal to handle them, potentially integrating the vaccination schedule with other routine vaccinations or dosing. The early handling and separation of lambs from their mothers for castration and tail docking procedures may be rendered unnecessary, decreasing the stress of such an exercise on both lambs and ewes. Due to the decreased risk of infection and removing the risk of wound healing complications, this may decrease the need to further handle the animals to treat them for such infections. The administration procedure of the immunocastration vaccine is simple in comparison to physical castration methods and does not require the presence of a veterinarian.

With regards to improving the animal welfare of intact male livestock on-farm, using immunocastration will provide the same welfare benefits as physical castration in terms of fertility and behavioural control. Control of aggressive behaviour decreases fighting-related injuries as well as carcass bruising in male animals. The prevention of pregnancies in a mixed-sex flock is also essential in maintaining the welfare of those female animals intended for slaughter. It has also been suggested that immunocastration may have a positive effect on the immune system by improving splenic immune markers and immune cytokines (Han et al., 2016). Thus, the animal's immune status and well-being may actually benefit from immunocastration. However, further investigation into these benefits on a commercial scale is yet to be quantified. An economic comparison looking into the indirect benefits of improved animal welfare from immunocastration on production parameters needs to be considered.

2.3.1 Swine

Immunocastration with Improvac® has been used in swine to prevent boar taint while improving growth and carcass performance in comparison to physical castrates. The

vaccination schedule for Improvac® has been designed to minimise the impact on fat deposition and feed efficiency while allowing sufficient clearance time for compounds associated with boar taint (Claus et al., 2007). Following the recommended vaccination schedule, the primary vaccination appears to have no significant effect on the testosterone production in swine; with antibodies against GnRH increasing within three to five days after the booster vaccination and peaking four to six days after the booster vaccination (Claus et al., 2007). Subsequently, Luteinizing hormone and testosterone concentrations decline almost simultaneously within four to eight days and five to ten days, respectively, after the booster injection, and remain stable for approximately 44 days after the booster (Claus et al., 2007). It has been demonstrated that immunocastrated swine are unique with regards to their hormone profile such as oestradiol, growth hormone (GH) and insulin-like growth factor 1 (IGF-1). Although immunocastrated pigs have low concentrations of oestradiol compared to surgical castrates, their GH concentrations are high and comparable to that of intact boars (Brunius et al., 2011; Bauer et al., 2009). As a result, IGF-1 concentrations in immunocastrates are intermediate to those of intact and surgically castrated males (Brunius et al., 2011). The IGF-1 concentrations in immunocastrates were shown to gradually decrease five days after the booster and stabilising within six to ten days (Claus et al., 2007). Thus, immunocastrates could have a higher anabolic lean growth potential than surgical castrates even though testosterone production is compromised, provided their nutritional needs are met. This potential change in anabolic growth potential and nutritional requirements was investigated by increasing dietary lysine in the diets of immunocastrates, showing that their cutting yields improved and backfat thickness decreased in comparison to surgical castrates (Boler et al., 2011). Responses to varying dietary protein content also differed between immunocastrates and intact males with regards to backfat deposition, also indicating a possible difference in anabolic growth potential and nutritional requirements between immunocastrates and intact boars (Needham & Hoffman, 2015a).

However, the addition of ractopamine hydrochloride at 10 mg/kg to the diet of immunocastrates can improve the lean meat yield and carcass traits (Needham & Hoffman, 2015b).

In group-housing based trials, immunocastrated swine performed better than intact males, most likely due to their decreased activity including aggressive and sexual behaviour (Dunshea et al., 2001). These immunocastrates were also leaner and had a more improved feed efficiency than surgical castrates in the trial, even though feed intake has been shown to increase in immunocastrated pigs compared to intact boars. This increase in feed intake could be a combination of increased feeding activity due to decreased aggressive and sexual behaviour as well as the absence of the appetitive suppression effect of androgens. Although dietary protein level did not influence feed intake *per se* in individually housed immunocastrates, immunocastrates experienced improved feed efficiency when dietary protein content was increased (Needham et al., 2016a). The extent to which the increase in feed intake seen in immunocastrates influences fat deposition largely depends on the period between the booster and slaughter, as the increase in feed intake is seen approximately two weeks after the booster vaccination regardless of its timing (Lealiifano et al., 2011). Likewise, the effects of immunocastration on carcass traits and yields depend on the vaccination schedule used and the age of the pigs when it was applied; which also appears to be the case in other livestock species.

2.3.2 Cattle

In bulls, immunocastration, with Bopriva® has been successful in decreasing testosterone concentrations to those comparable to physical castrates within 14 days after the booster (Amatayakul-Chantler et al., 2013) and thereby controlling aggressive and sexual behaviour (Huxsoll et al., 1998) as well as decreasing overall activity (Janett et al., 2012). The use of Bopriva® in cattle has shown consistent immune responses in individuals, which is not always

shown to be experienced with small-scale vaccines manufactured for research purposes (Thompson, 2000).

Steers are preferred in beef enterprises for it is easier to manage their behaviour, have an improved fat deposition and thus carcass grading, as well as meat quality. However, as with other livestock species, the castrated steers grow slower and are less feed efficient, when compared to intact males. The fattening period of cattle is much longer relative to that of, for example, sheep or pigs and thus the possibility of the animals reaching puberty prior to slaughter becomes more of a concern, particularly when cattle are finished on pasture. Such an example of this is the large beef production industry in Brazil, Namibia, and Botswana that finish cattle on pasture until they are 30 to 36 months of age. In Brazil, the industry tends to practise late castration at 18 to 24 months of age to take advantages of male steroid hormones and the associated growth and feed efficiency as much as possible. Bulls in extensive systems can be destructive with regards to infrastructure as well as pasture and thus need to be managed carefully. For instance, bulls cannot be grazed near cows as the risk of them breaking through fences to get to the females is high and can cause indiscriminate breeding. Not only is this a welfare issue in slaughter cows, it also presents a huge threat to heifers not ready to be mated. A further issue associated with castration in extensive production units in tropical areas such as Brazil include the risk of infection of the healing wound by the screw worm fly, *Cochliomyiahominivorax*, should preventative and therapeutic treatment fail, which also require extra input costs (Muniz et al., 1995).

When compared to physically castrated steers, the effects of immunocastration on growth performance, carcass traits and meat quality are generally favourable. Immunocastrated bulls vaccinated twice (20 and 25 months of age) with Bopriva® and raised on pasture have been shown to have greater average daily gains (ADGs), hot carcass weights (HCW) and dressing percentages in comparison to late physical castrates (25 months) (Amatayakul-

Chantler et al., 2013). This improvement in growth performance seen in immunocastrated bulls could be attributed to having a longer exposure time to the male steroid hormones before testosterone concentrations reach those similar to the physical castrates after the booster vaccination, as well as the absence of pain and the associated growth setbacks of physical castration. Furthermore, the immunocastrated cattle showed no negative effects on carcass or meat quality traits with regards to subcutaneous fat thickness, rib eye area, meat colour, fat colour, cooking loss and tenderness (Amatayakul-Chantler et al., 2013).

When used in a feedlot system, immunocastration has been shown to improve carcass traits and beef colour attributes in comparison to surgical castrated and intact bulls, respectively (Miguel et al., 2014). Meat from immunocastrated cattle showed greater redness and lower darkness than intact bulls, which could indicate a possible advantage with regards to retailing. Late castration in feedlot systems is also preferred in late maturing animal breeds, as they tend to take longer to reach the desired carcass weight and fatness. When immunocastration is applied after puberty, carcass traits change from those of intact bulls to long-term castrated bulls (D'Occhio et al., 2001). Thus, the flexibility with regards to when immunocastration is applied can allow the manipulation of carcass traits in bulls of varying maturity types. Positive results have been experienced with regards to applying immunocastration to *Bos indicus* and *Bos indicus* crossbred cattle in both extensive and feedlot systems (Amatayakul-Chantler et al., 2012; Amatayakul-Chantler et al., 2013; Miguel et al., 2014). Immunocastrated bulls appear to have greater development in the hindquarter than steers and bulls, indicated by an increase in the leg perimeter of the hindquarter (Miguel et al., 2014) and thus a higher proportion of economically important cuts. No differences have been reported for carcass length, length of the leg and depth of the chest between these sexes (Ribeiro et al., 2004; Miguel et al., 2014).

Another approach feedlots use to improve the growth performance of steers and late maturing animals is the insertion of growth implants. Growth implants are routinely used in

many feedlot systems; however, their use is limited by bans such as the European Economic Community's decision to prevent hormone-treated meat products from being sold in European nations from January 1989 (FAS, 2015). Immunocastration (Bopriva®) has been successfully applied along with the use of anabolic implants (Component E-S; Elanco™ and Synovex Choice; Zoetis™) in a trial consisting of 1600 animals and 400 bulls per treatment (Amatayakul-Chantler et al., 2012). Although the anabolic potential of the immunocastrates was compromised due to the deficiency in testosterone, immunocastrates with growth implants had greater final body weights than bulls with and without growth implants, as well as immunocastrates without implants. The immunocastrates with growth implants also had heavier hot carcass weights in comparison to bulls and immunocastrates without growth implants. Despite the negative effect on tenderness associated with growth implants, meat tenderness was improved in all immunocastrate treatments in comparison to intact bulls. Thus, Amatayakul-Chantler et al. (2012) concluded that the use of Bopriva® could improve growth performance in combination with implants and meat quality with or without implants.

As mentioned, the effect of immunocastration on growth performance and fat deposition depends on the vaccination interval used and the time between the castration effect and slaughter. This is indicated by the variation in results found in cattle; with no differences in fat thickness seen between steers, bulls and immunocastrates in Nellore and crossbred cattle in feedlot (Miguel et al., 2014), Nellore cattle on pasture (Ribeiro et al., 2004) and *Bos taurus* animals in feedlot (Adams et al., 1993; Adams et al., 1996; Huxsoll et al., 1998). No differences were found in fat thickness between composite bulls and immunocastrates (Cook et al., 2000), as well as between *Bos taurus* immunocastrates and steers (Aïssatet al., 2002) and *Bos indicus* immunocastrates and steers (Amatayakul-Chantler et al., 2013). It is thus important that the timing of vaccination with regards to slaughter is considered in terms of the desired fat deposition, which has a large influence on profitability of a carcass. Not only is fat deposition

important for the grading of beef carcasses, it also has important implications with regards to the eating experience of red meat. Intramuscular fat influences the juiciness and thus tenderness of meat, which is one of the most important sensory qualities of red meat (Mancini & Hunt, 2005).

However, meat colour is one of the first quality attributes a consumer considers when buying meat and thus the impact of immunocastration on meat colour needs to be considered. Immunocastrated cattle have greater L*, a* and b* CIE colour values than bulls, indicating that they have lighter, redder and more yellow meat (Miguel et al., 2014). However, no differences in CIE colour values were observed by Amatayakul-Chantler et al. (2012) and Amatayakul-Chantler et al. (2013) and thus this warrants further investigation. Similarly, the effects of immunocastration on tenderness, measured by instrumental shear force, varies with those indicating it has no effect (Cook et al., 2000; Ribeiro et al., 2004; Amatayakul-Chantler et al. 2013; Miguel et al., 2014) versus Amatayakul-Chantler et al. (2012) who reported that immunocastrates had more tender meat than bulls. However, it is important to note that numerous factors such as the *ante-mortem* stress, the killing process, *post-mortem* interventions, and even the method used to cook the samples prior to evaluation for tenderness can influence the shear force values and thus these too require further investigation.

Another important factor influencing meat quality is the muscle pH *post-mortem*. Muscle pH_{24} was higher in bulls than steers and immunocastrates (Miguel et al., 2014) possibly due to *ante-mortem* stress, as bulls are argued to be more susceptible to stress because of their temperament (Field,1991). This can be supported by the differences seen in activity levels between bulls and immunocastrates (Janett et al., 2012). This increase in pH seen in bulls could explain the lower L* colour values in comparison to immunocastrates. Although pH was influenced, no effects were seen on cooking loss values between immunocastrates, bulls and steers (Miguel et al., 2014; Ribeiro et al., 2004; Amatayakul-Chantler et al., 2013).

Immunocastration has been applied in heifers and cows to prevent unwanted pregnancies and oestrus through suppressing the oestrus cycle (Bell et al., 1997). This suppression of oestrus in immunocastrated females also prevents mounting behaviours and their associated injuries, thus improving their welfare. Immunocastration in females decreases the serum progesterone concentrations, thus decreasing ovarian and uterine weights as well as weight gain (Adams & Adams, 1990). However, this decrease in weight gain could be improved with the use of anabolic growth implants such as Synovex-H (Adams & Adams, 1990). Therefore, immunocastration also has the potential to be an easily applied tool to improve the welfare of heifers and cows used for beef production.

2.3.3 Small ruminants

Immunocastration has been investigated in both sheep and goats; however, no commercial vaccine currently exists for either of these species. However, immunocastration of rams can potentially be used in circumstances where late castration is preferred such as stud breeding programs, where ram lambs are not castrated shortly after birth, but selection of breeding rams occurs after monitoring body weight and wool quality, as in the case of wool or dual-purpose breeds. Immunocastration can thus also potentially be used on cull rams to potentially improve carcass and meat quality.

Vaccines used for immunocastration have either been prepared by the researchers themselves or off-label use of commercial products has been practiced. Such an example is the use of Vaxstrate in goat bucks to control behaviour (Godfrey et al., 1996). Vaxstrate was a conjugated ovalbumin GnRH immunocastration vaccine designed for use in cattle; however, it was discontinued in 1995 because heifers treated with it continued to cycle (Hoskinson et al., 1990). Immunocastration of bucks with Vaxtrate successfully decreased scrotal circumference and testes remained small for more than a year following the first vaccination and thus

indicating a long-term effect. Odour scores associated with seasonal reproductive behaviour and agonistic behaviour was also decreased (Godfrey et al., 1996). Recombinant ovalbumin-GnRH vaccines were used successfully in bucks, decreasing testosterone production, testicular and accessory gland development as well as decreasing seminiferous tubule diameter and basal membrane thickness (Ülker et al., 2009).

Recombinant ovalbumin-GnRH vaccines have also been used in Karakas ram lambs (17 weeks old), successfully interrupting testicular growth with no effects seen on body weight compared to intact rams (Ülker et al., 2002). Immunocastrates had greater chest widths than intact rams but not significantly different from physical castrates with no further differences seen in the various carcass measurements, including dressing percentage and carcass weights, between sexes. Furthermore, no differences were found in wholesale cut weights or in dissected muscle and bone weights between sexes, although, immunocastration and physical castration increased subcutaneous and intramuscular fat weights (Ülker et al., 2002). In conclusion to the study by Ülker et al. (2002), immunocastrates were shown to be intermediate to physical castrates and intact rams in many of the carcass traits studied.

Similar results were found when rams were immunocastrated at 10 weeks of age and entering a feedlot at 27 weeks of age for 70 days, using the same vaccination technique as Ülker et al. (2002) but including a second booster vaccination (3 injections in total) (Ülker et al., 2003). In comparison to intact males, live weights, weight gain, loin eye muscle area, backfat thickness, carcass weights, dressing percentage, offal items, and wholesale cut weights were not affected by immunocastration. Further research into the effects of immunocastration using recombinant fusion proteins on testicular development by Ülker et al. (2005) indicated that all rams immunised at 10 weeks of age produced ejaculates which contained no mature spermatozoa and the testes seminiferous tubule diameter was decreased along with thickening and hyalinization of the basal membrane.

Another method for manufacturing an immunocastration vaccine is the use of GnRH-keyhole limpet hemocyanin (KLH) conjugate emulsified with Freund's Complete Adjuvant (FCA). This vaccination technique was used to vaccinate Western Whiteface rams at 32.6 ± 1 kg, causing decreased testosterone and testicle weights at slaughter with a resultant decrease in mounting frequencies and ejaculations in the growing period (Kiyma et al., 2000). Two adjuvants were also compared for vaccination, indicating that FCA achieved the greatest GnRH antibody titres at slaughter and greatest decrease in sexual behaviours. In contrast to Ülker et al. (2003), immunocastration decreased feed efficiency and rate of gain in comparison to intact rams but did not differ from physical castrates. However, the feeding period required for the immunocastrates to reach a slaughter weight of 58 kg was intermediate to the other two sexes, while yielding more desirable yield grades with less fat and marbling than physical castrates. These results indicated a possible difference in nutrient partitioning for growth and fat deposition between immunocastrates and physical castrates (Kiyma et al., 2000). Immunocastration increased dressing percentages in comparison to intact rams, which could be due to the smaller testes weight in comparison to intact males as well as a decrease in kidney and pelvic fat in comparison to physical castrates (Kiyma et al., 2000).

Passive immunocastration has been performed by vaccinating sheep with KLH, drawing blood samples and isolating the GnRH-KLH antibodies (Parthasarathy et al., 2002). These antibodies where then injected into the rams which formed part of the experiment. Although this was successful in decreasing testosterone, repeated vaccinations were required to elicit a more persistent decrease in testosterone and sexual behaviour. However, for ease of management and cost, it is favourable to have a minimum number of vaccinations required to elicit a desirable immune response along with a flexible vaccination schedule.

Janett et al. (2003) investigated the extra-label use of Improvac® to vaccinate ram lambs by administering two separate doses of 2 mL each, three weeks apart. Improvac® suppressed

testosterone secretion and decreased testicular growth for at least three months after the booster vaccination without significant effects on growth in comparison to intact rams (Janett et al., 2003). Improvac® shows promise in the application of immunocastration in rams to commercial systems; however, the directions of use have been developed for swine and thus warrant further investigation in sheep to establish the optimal use. This includes establishing whether the vaccination schedule is flexible with regards to the period between vaccinations, the timing of the second vaccination with regards to slaughter in order to maintain growth performance, while potentially improving carcass traits and meat quality.

2.3.4 Non-livestock species

Immunocastration has been used in non-livestock species for various reasons, e.g. in dogs and cats as a method of fertility control (Ladd et al., 1994). Immunocastration has been used to study antler growth in red deer stags (Lincoln et al., 1982), showing a varying response to vaccination when antler growth and hardening were considered. The stag with the greatest GnRH antibody titre did not show antler hardening, thus remaining "in velvet" for longer than six months, while the other three stags shed their antler prematurely, consequently growing new antlers. All stags had a reduced testis size and interrupted normal sexual behaviour. Immunocastration has also been used to study the role of melatonin in the seasonal reproductive cycle of stags, as the responses to melatonin are dependent on the presence of GnRH (Lincoln et al., 1984). Immunocastration was used in both stallions and mares (Garza et al., 1988) to suppress reproduction, with the first successful report of its use in stallions (Schanbacher & Pratt, 1985). Immunocastration also showed promise to control aggressive behaviour in wild and captive African elephants by successfully decreasing androgen production (De Nys et al., 2010). Research into immunocastration of primates indicated that an increased GnRH-antibody titre and decreased testosterone production were associated with a reduced prostate weight

(Giri et al., 1991). However, the application of immunocastration in humans has reached beyond fertility control and has been investigated to control prostate and breast cancers, these being dependent on the secretion of gonadal steroid hormones.

2.4 Conclusion

The management practice of immunocastration, applied in several species, has proven to be effective in controlling reproductive behaviour and aggression, with varying influences on growth performance and carcass traits. Immunocastration presents an opportunity to circumvent many of the welfare concerns associated with physical castration. When the sheep industry is compared to the cattle and pork industry, it is evident that extensive research is required to develop protocols for the potential of immunocastration to improve growth and carcass characteristics of sheep, without impacting negatively on animal welfare in production systems. It is also important to assess the potential of the technique from the perspective of the producer, abattoirs, and ultimately the consumer to motivate its use as a welfare-friendly castration technique to improve the sustainability of sheep production systems on all levels of the industry.

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CHAPTER 3

Characterization of the reproductive development, growth and slaughter performance of Dohne Merino rams

Abstract

The Dohne Merino sheep has been developed as a dual-purpose breed to suit the needs of South African farmers, producing both wool and an acceptable lamb carcass. The breed is considered a medium-maturing breed, with rams attaining puberty at a live weight of 40 to 50 kg. The aim of this study was to quantify the post-pubertal sexual development of Dohne Merino ram lambs to establish baseline data for producers selecting or slaughtering older rams as well as future research into the application of immunocastration in rams. Twenty-Four Dohne Merino rams were monitored weekly from 16 months (65.1 ± 0.93 kg) to 18 months of age (76.8 ± 0.93 kg). Body weight, body temperature, scrotal size, scrotal temperature and serum testosterone concentration were determined weekly until slaughter. Slaughter performance and testes tissue development were also assessed. Body weight of post-pubertal rams increased progressively until rams become sexually mature; however, scrotal circumference fluctuated over the nine-week interval of the study (30.7 ± 0.52 to 34.0 ± 0.36 cm). Lambs older than 16 months of age have normal scrotal circumferences and at 18 months, sperm quality characteristics were of acceptable industry standards. Serum testosterone concentrations varied over the nine-week study period (0.84 ± 0.29 to 2.6 ± 0.29 ng/mL). Scrotal surface temperature appeared to be influenced by environmental temperature more so than rectal temperature. Thus, it would be beneficial to standardise the time of day for and season for blood withdrawal for androgen analysis, scrotal circumference and temperature measurements to obtain more accurate data in future research. Testis cut surface colour values were 64.1 ± 0.22, 1.4 ± 0.13 and 12.4 ± 0.16 for L, a, and b* CIE values, respectively. At approximately 18 months of age, seminiferous tubule circumference and epithelium thickness averages 980.3 ± 21.57 µm and 64.4 ± 1.81 µm, respectively. These colour values and seminiferous tubule parameters can be considered as representative for normal testicular function at the onset of sexually maturity in Dohne Merino rams.

3.1 Introduction

Dohne Merino sheep is a South African composite dual-purpose breed developed in the 1950's from South African Merino ewe and German Mutton Merino ram crosses, aimed to cope with the harsh sourveld region (Snyman, 2014). The medium to large-framed breed has an average ram weight of 5.2 kg, 29.9 kg, 60.3 kg and 80 - 100 kg at birth, weaning (100 days), 12 months of age and mature adult weight, respectively. Ewe weights are on average 4.9 kg, 31.1 kg, 48.6 kg and 66.0 kg at birth, weaning, yearling and mature weight, respectively (Snyman, 2014).

The breed is considered as a medium maturity type, which implies that animals can be marketed at a later age without excessive fat deposition, when compared to an early maturing breed such as the Dormer (Cloete et al., 2012). Typically, Dohne Merinos reach a South African market weight of approximately 40 kg at four to six months of age but may be maintained for longer in the case of the primary product being wool. Rams may also be kept longer in stud enterprises where final genetic selection may take place from weaning up until two years of age, where inferior rams are slaughtered. However, rams attain puberty at approximately 50 to 60 % of their mature live weight. Puberty signals the initiation of display of reproduction-associated behaviour (e.g. territorial aggression) and a more synchronized interaction between the hypothalamus, pituitary and testes. Generally, rams reach spermatogenic maturity at approximately 18 to 20 months of age (Mandiki et al., 1998). Rams intended for slaughter are thus castrated to prevent indiscriminate breeding and to control undesired behaviours such as aggression, thereby aiding in the management of mixed-sex flocks, particularly in extensive production systems when rams may enter puberty before slaughter.

Physical castration of ram lambs can be performed using a range of techniques, including surgical testes removal, closed-crushing of spermatic chords and scrotal sloughing due to rubber-band application. However, these techniques have been shown to cause pain, stress and decreased growth, regardless of pain mitigation strategies (Melches et al., 2007).

Furthermore, legislation regarding lamb age, castration technique, use of pain mitigation and veterinary intervention when castrating lambs varies between countries, with no readily available regulations in South Africa. The lack of formal legislation surrounding castration of livestock has caused concern amongst consumers, resulting in the intention to enforce tougher regulations on lamb producers (Font-i-Furnols et al., 2012).

Immunocastration has been considered as an alternative to physical castration, and research is limited to studies on lambs using self-manufactured vaccines in breeds such as the Western Whiteface (Kiyma et al., 2000), Karakus (Ülker et al., 2002; Ülker et al., 2009) and Tibetan rams (Han et al., 2016). Little research has been done on the application of commercially available vaccines, such as Improvac® (Zoetis™), on ram lamb performance (Janett et al., 2003), with no available research conducted on Dohne Merinos.

It would be beneficial to establish immunocastration vaccination protocols and intervals as an alternative to physical castration as the Dohne Merino is an important sheep breed in South Africa (Cloete et al., 2012). To achieve a commercially effective immunocastration vaccination schedule, the vaccine needs to demonstrate the ability to inhibit and ultimately prevent communication between the hypothalamic-pituitary-gonadal (HPG) axis, which in turn will suppress functioning of the testes and associated sex glands in the rams. Vaccination success can be assessed, amongst other techniques, by determining the serum testosterone concentrations, which ultimately provides an indication of testicular functioning.

To develop immunocastration protocols, it is important to be able to quantify the influence of the vaccine on the reproductive development of Dohne Merino rams. The reproductive development of Dohne Merino rams has been described from three to 14 months of age, however, this study did not include the interval from the end of puberty to the time that rams became fully sexually mature (Schoeman & Combrink, 1987). Factors such as live weight, testes size and serum testosterone concentrations of post-pubertal Dohne Merino rams

reaching sexual maturity thus need to be quantified. Furthermore, body temperature may influence ram fertility, and thus baseline data needs to be established for sexually developing Dohne Merino rams. Therefore, the aim of this study was to describe the growth and reproductive development of young Dohne Merino rams after puberty, as they reach mature weight and sexual maturity.

3.2 Materials and Methods

Ethical clearance was obtained from the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00073) and animal husbandry was in accordance specifications of the South African National Standards 10386: 2008.

3.2.1 Experimental animals and husbandry

Twenty-Four Dohne Merino ram lambs were sourced from the Stellenbosch University stud flock at 16 months of age (65.1 ± 0.93 kg). The rams were transported to Welgevallen Experimental Facilities where they remained for the duration of the data collection period (July to September during the winter-spring season). Upon arrival, each ram was weighed and ear-tagged with an individual number. All rams were dosed for internal parasites using Ivomec® (Merial, Australia) and vaccinated against bluetongue and pulpy kidney. The rams were maintained in a single flock and grazed kikuyu pasture during the day, with *ad libitum* access to water. At approximately 4pm on each day of the trial period, the rams were moved into the research sheep shed facility and housed overnight to prevent stock-theft. Rams had *ad libitum* access to lucerne and water in the sheep shed.

Each sheep was weighed weekly using a livestock scale (Model SI2963, Scales Incorporated, South Africa) accurate to 200 g. Body temperature, scrotal circumference, scrotal surface temperature and blood samples were recorded weekly, starting at 9am. Body temperature was recorded on both the skin surface (Alla France infrared thermometer,

accuracy: ± 2 °C), and measured rectally for the first four weeks of the study (Digit clinical thermometer, ACT2020/ACTHERM, Wellkang, range: 32 - 43.9 °C). For measuring skin surface temperature, wool was clipped from a square area on the shoulder blade so that the infrared thermometer could be placed flush with the skin. Scrotal circumference was measured around the widest circumference of the testes, using a flexible plastic tape measure (Foote, 1969). Scrotal skin surface temperature was measured by parting the wool on the left testis and placing the infrared thermometer onto the skin surface (caudal) in the middle of the testis.

3.2.2. Blood collection & testosterone analysis

Blood was collected weekly, prior to other measurements, at 9am, from the jugular vein of each ram using an 18-gauge vacutainer needle and 6 mL Z Serum Clot Activator Vacuettes® (Greiner Bio-One International, Austria). After remaining at room temperature for 45 minutes, blood samples were centrifuged at 1500 RCF for 15 minutes at 4 °C. Serum was removed and aliquoted into two 2 mL graduated microtubules before storage at -20 °C until analysis. Testosterone was extracted using a liquid-liquid extraction (Quanson et al., 2016). Firstly, 50 µL of deionized water containing the internal standard of 1.5 ng testosterone-1, 2-d2 (Cambridge Isotope Laboratories, Andover, USA) was added to 500 µL of the collected serum samples. Subsequently 1.5 mL of UHPLC-grade tert-Methyl Butyl Ether (Sigma-Aldrich, Steinheim, Germany) was added to the serum and vortexed at 1000 RPM for 10 minutes. The samples were then frozen at -80 °C for 60 minutes after which the non-frozen, non-polar phase was transferred and evaporated under nitrogen gas at 55 °C. Samples were reconstituted using 50 µL of 50 % methanol (ROMIL, Cambridge, England), vortexed and transferred into vials to be stored at -20 °C until analysis.

Standard curves were established for testosterone using testosterone-1, 2-d2 in 50 % methanol at the following concentrations: 0, 0.05, 0.10, 0.25, 1.00, 10.00, 50.00 and 250.00

ng/mL. Testosterone concentration was quantified using ultra-performance convergence chromatography tandem mass spectrometry (UPC^2-MS/MS) according to Quanson et al. (2016). The Acquity UPC^2 system was fitted with an Acquity UPC^2 BEH 2-EP column (3 mm x 100 mm; 1.7 µm particle size; Waters Corporation, USA) with a mobile phase comprising of carbon dioxide modified with methanol. A four-minute linear gradient from 2 to 9.5 % methanol with a constant flow rate of 2.0 mL/minute was used to separate the C19 steroids within the prepared samples at an injection volume of 2 µL. Column temperature was 60°C and automated back pressure regulator was 2000 psi. Quantification of testosterone was performed using a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, USA) attached to a make-up pump supplying 1% formic acid in methanol at a flow rate of 0.2 mL/minute. Sample were analysed using Multiple Reaction Monitoring mode with an electrospray probe in positive ionization mode under 3.8 kV capillary voltage, 120 °C source temperature, 500 °C desolvation temperature, 1000 L/h desolvation gas and 150 L/h cone gas as specified by Quanson et al (2016). The limit of detection was 0.01 ng/mL for testosterone. Analysis of raw data was performed using MassLynx^{TM} software (Waters Corporation, USA).

3.2.3 Transport, slaughter & offal yields

At 18 months of age (76.8 ± 0.93 kg), all sheep were weighed at 7 am and transported to a commercial abattoir approximately 73 km away, on tarred roads. Upon arrival, the sheep entered lairage until 2 pm. Lairage was shaded and rams were given *ad libitum* access to water and hay. The rams were electrically stunned for four seconds (200 V; 250 AMP), exsanguinated and electrically stimulated for 60 seconds (11 V; 250 AMP). The carcass was dressed, and offal items were weighed. The forelegs were removed at the ulna and the hindlegs at the stifle joint and weighed together as the feet. The head was removed at the base of the skull. The whole scrotum was removed, containing the testes. The testes, still within the scrotum, were

transported to the laboratory directly after the slaughter process was completed to be processed within four hours *post-mortem*. The carcasses were skinned and the esophagus, rumen, reticulum, omasum, abomasum, small intestine, large intestine, caecum, colon and rectum (gastrointestinal tract; GIT) were removed together. The red offal was also removed and weighed, and included the heart, lungs, liver and trachea. Carcasses were weighed before entering the chiller to establish hot carcass weights (HCW).

3.2.4 Testes histology & sperm morphology evaluation

Testes were trimmed free from the scrotum, along with excess tissue and epididymides before weighing (RADWAG PS750/C/2 scale, Wagi Elektroniczne, Poland; accurate to 0.001g). Sperm were harvested from the epididymis (Boshoff, 2014) as soon as possible after slaughter and sperm concentration determined using the hemocytometric method (Rouge, 2004a). Basic morphology and viability were assessed at 10 X magnification on approximately 100 sperm per ram, using sperm smear slides stained using nigrosine-eosin (Rouge, 2014b).

Cut surface CIE Lab colour was measured (Color-guide 45°/0° colourimeter, BYK-Gardner GmbH, Gerestried, Germany) on the testes by dissecting them along the widest section, perpendicular to the longitudinal axis (Lealiifano et al., 2011). The CIE colour space describes colour visible to the human eye using a mathematical three-dimensional model, namely “L” for “lightness” (0 indicates black, whereas 100 indicates diffuse white), “a” and “b” for the colour co-ordinates green (negative values) to red (positive values) and blue (negative values) to yellow (positive values), respectively. No “blooming” time was allocated for the testes samples before CIE Lab colour measurement. Tissue samples (approximately 1 cm x 1 cm) were collected from the mid-section of the testis and placed in buffered formalin to be preserved for preparation of histology slides (Bai et al., 2017). Testes tissue samples were washed with water and dehydrated in a graded alcohol series before embedding in paraffin.

Tissue samples were then sliced into 5 µm thick sections, placed onto microscope slides and stained with haematoxylin and eosin. Seminiferous tubule circumference and epithelium thickness were evaluated using an Olympus IX70 microscope (Olympus Corporation Tokyo, Japan) at 40X magnification and Olympus Image Analysis Software (Olympus Corporation Tokyo, Japan), measuring 100 seminiferous tubules per ram.

3.2.5 Statistical analysis

STATISTICA Version 13.2 (StatSoft Inc.) was used for statistical analysis of the live measurements over time. After normality and homoscedasticity was tested, the Variance Estimation, Precision and Comparison (VEPAC) procedure was followed to compare weekly data. The restricted maximum likelihood (REML) method was used to determine weekly differences with "animal" and "week" used as grouping variables, "week" as the fixed effect and "animal" as the random effect. One-way analysis of variance (ANOVA) was used to compare the data collected at slaughter. Fishers LSD was used to compare weekly means at a significance level of 5 %.

3.3 Results & Discussion

The study quantified the growth and sexual development of Dohne Merino rams after puberty and approaching sexual maturity. The recorded parameters serve to provide baseline values for the growth and certain reproduction-related parameters of Dohne Merino rams that can be used in the comparison in future studies. Based on management of production systems, Dohne Merino ram lambs are culled from a commercial stud flock either after weaning or at one year old, when lambs are too old to be castrated using physical castration methods. Producers then feed/finish the lambs for slaughter as intact rams, with a corresponding decrease in income

generated. This study thus further aims to provide information on Dohne Merino rams which may be slaughtered at a later age due to being kept longer for wool production or stud selection.

Dohne Merino rams are considered to attain puberty at approximately 40 to 50 kg live weight. Rams in this study were thus considered post-pubertal at the start of the trial, with rams having an average (±SE) live weight of 65.1 ± 0.93 kg (Figure 3.1). Live weight increased ($P < 0.001$) over the trial period, with rams achieving what can be considered an adult live weight (76.7 ± 0.93 kg). The Dohne Merino rams were thus approaching full sexual maturity towards the end of the period of study.

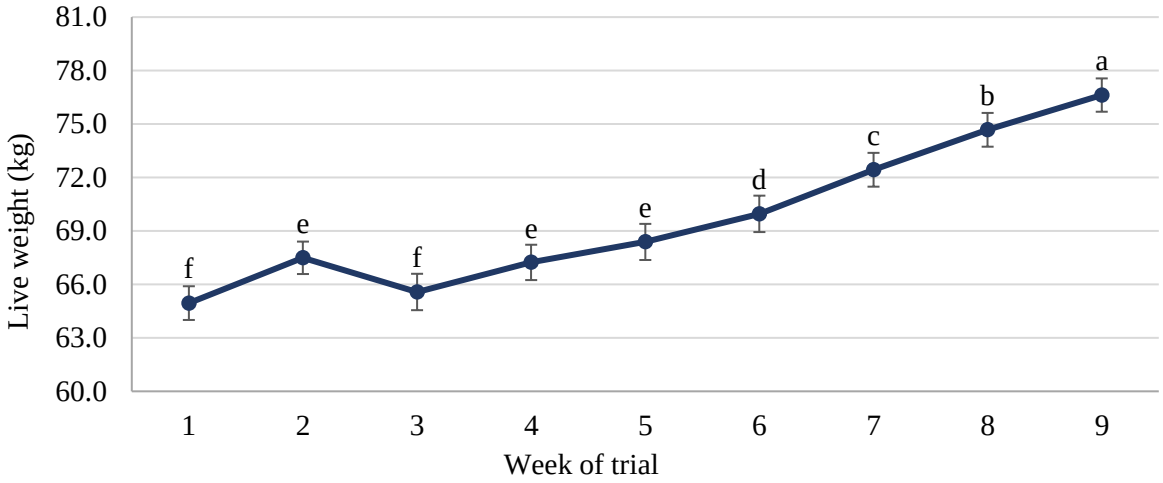

Figure 3.1. Average live weight of Dohne Merino ram lambs from 16 to 18 months of age, maintained on kikuyu pasture for a period of nine weeks. Error bars indicate SEM and letters indicate significant differences between means at a significance level of 5 %.

Serum testosterone concentration increased ($P < 0.001$) progressively from Week 1 to 6, with peak concentrations occurring at Week 6 and 7, before a slight decrease from Week 7 to 9 (Figure 3.2). Serum testosterone concentration ranged from 0.84 ± 0.29 ng/mL to 2.6 ± 0.29 ng/mL. The range measured on this study would allow for normal mounting and mating activities to take place. According to D'Occhio and Brooks (1982a), a threshold testosterone concentration of 0.32 to 0.65 ± 0.01 ng/mL is required for mounting, and 1.26 ± 0.13 ng/mL is required for mating (intromission with ejaculation). Thus, the Dohne Merino rams could be

considered sexually mature from Week 6 of the trial, after which all serum testosterone concentrations were high enough to support sexual reproductive behaviour. A variation in serum testosterone concentrations was expected because of the pulsatile nature of Luteinizing hormone (LH), and consequently testosterone secretion (D'Occhio et al., 1982b). Circulating testosterone concentrations are also influenced by season, as the HPG axis is under the influence of photoperiod (Schanbacher & Ford, 1979). Photoperiod influences LH and follicle stimulating hormone (FSH) release, such that decreased day length stimulates testosterone secretion and enhanced spermatogenesis (Schanbacher & Ford, 1979). Thus, it would be beneficial to not only standardise the time of day for blood collection in future research but also the season.

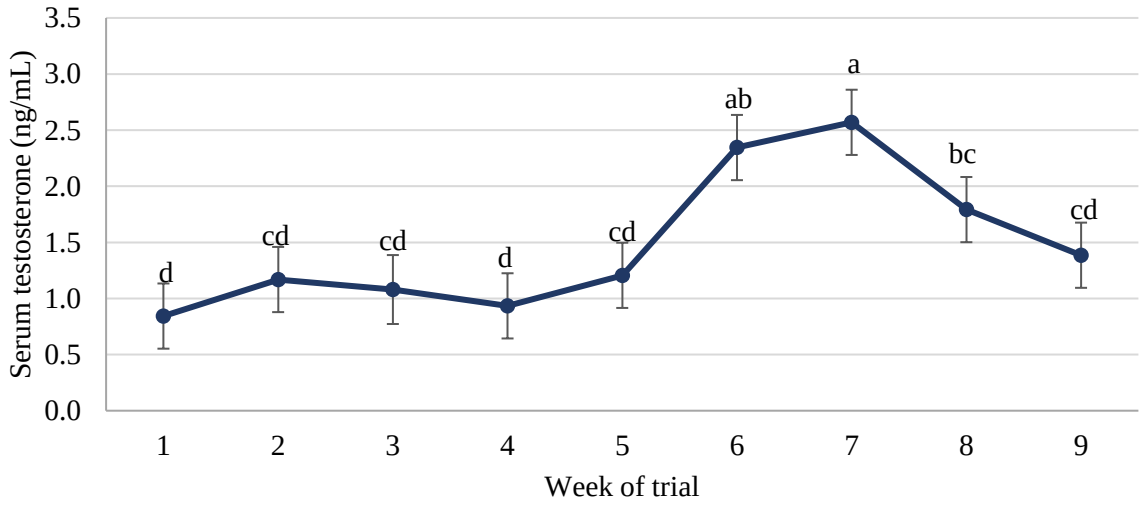

Figure 3.2. The average serum testosterone concentration measured in Dohne Merino rams from 65.1 ± 0.93 to 76.7 ± 0.93 kg live weight. Error bars indicate SEM and letters indicate significant differences between means at a significance level of 5 %.

Scrotal circumference also varied ($P < 0.001$) between 30.7 ± 0.52 and 34.0 ± 0.36 cm over the trial period (Figure 3.3), which is not abnormal as testes size is reported to fluctuate in mature rams due to the seasonal nature of breeding. Typically, increased reproductive activity is observed during periods of a shorter daylight hours, under the influence of LH, thus, testosterone secretion varies throughout the year in rams consequently influencing scrotal

circumference (Schanbacher & Lunstra, 1976). The variation in scrotal circumference followed a similar pattern to that of the serum testosterone concentrations towards the end of the trial period, which may be due to the influence of photoperiod, as discussed. At the start of the trial, scrotal circumferences were on average 33.1 ± 0.42 cm which is considered normal (> 32 cm) for rams older than 14 months (Bedford-Guaus, 2017). The ratio of scrotal size to live weight remained constant throughout the trial period at 0.5.

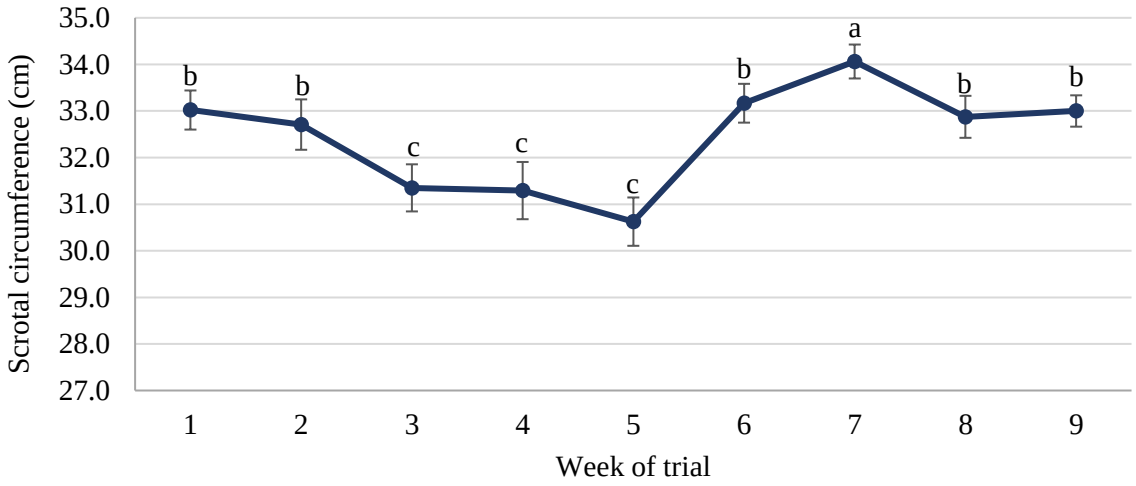

Figure 3.3. The average scrotal circumference of Dohne Merino rams recorded during a nine-week period. Error bars indicate SEM and letters indicate significant differences between means at a significance level of 5 %.

The maintenance of a constant body temperature of rams is not only essential for normal functioning and integrity of cells but is also important for spermatogenesis and integrity of hormone production. The rectal temperature for the first four weeks varied between 38.6 ± 0.05 and 38.7 ± 0.03 °C (Figure 3.4); however, the mean skin surface temperature showed greater fluctuation ($P < 0.001$; Figure 3.4) between 22.2 ± 0.30 and 27.2 ± 0.21 °C. The mean scrotal skin surface temperature recorded in this study varied ($P < 0.001$) between 24.0 ± 2.23 and 29.7± 1.96 °C (Figure 3.4) and followed a similar, but not identical, pattern to the skin surface temperature on the shoulder (Figure 3.4). Changes in scrotal temperatures contribute to seminal degradation and changes in the proteome in rams (Moule & Waites, 1963; Rocha et al., 2015)

and is thus important for sperm quality. The scrotal surface temperature is highly correlated to deep tissue temperature of the testes (Waites & Moule, 1961) and can be considered to provide a reasonable indication of testes temperature. The scrotal surface temperatures are lower than reported by Rocha et al (2015) measured using an infrared thermometer on Morada Nova rams, which averaged 31.2 ± 0.2 °C before testes insulation. However, the average air temperature was 29.5 °C with a relative humidity of 57 % in Brazil where Rocha et al (2015) performed their trial, which was considerably higher than the average of 13.5 °C and 75.5 % relative humidity experienced in Stellenbosch, South Africa (late winter to early spring) during the trial period using Dohne Merinos. The skin surface temperature and scrotal surface temperature was on average 14 and 12 °C and cooler than the mean rectal temperature, respectively, for the trial duration. Due to the deviation in skin surface and scrotal surface temperatures but not rectal temperatures, the fluctuations are likely the result of varying environmental temperatures.

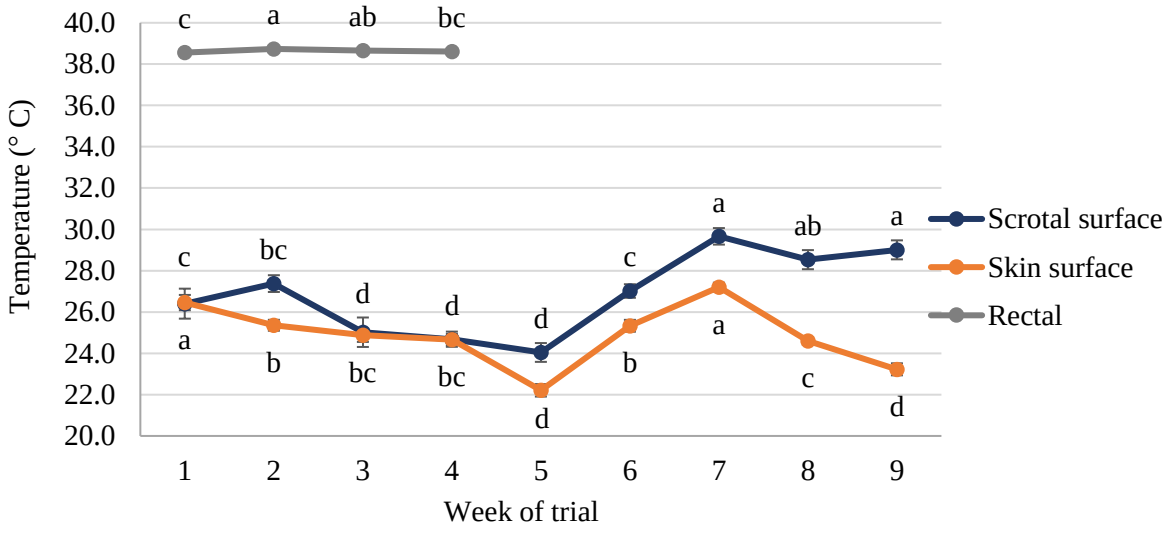

Figure 3.4. Scrotal skin surface, shoulder skin surface and rectal temperature of Dohne Merino rams from 16 to 18 months of age during the South African late winter to early spring months. Vertical bars denote SEM and letters indicate significant differences between means within different temperature measurement sites at $P \leq 0.05$. Rectal temperature was only recorded for the first four weeks of the trial.

Although testes size is typically quantified using scrotal circumference, as the measurement is performed on live breeding rams, trimmed testes weights were evaluated to provide an idea of the typical weight for a post-pubertal ram entering maturity (Table 3.1). Immunocastration in swine causes the testes tissue cut surface colour to be lighter, less red and more yellow (Lealiifano et al., 2011). Testes cut surface colour may provide an indication of the level of tissue activity within the testes and is important for later comparison of reproductive functioning of immunocastrated lambs. Information on testes cut surface colour in rams is scarce and to the best of our knowledge has not been quantified in Dohne Merino rams, thus values within Table 3.1 serves the purpose of establishing a standard for rams reaching sexual maturity. Relative to the values for cut surface colour in swine of similar testes weights, Dohne Merino rams have lighter (L*), less red (a*) and more yellow (b*) testes (Table 3.1). Likewise, seminiferous tubule measurements for Dohne Merino rams are scarce in literature, but mature Dorper rams with an average testis weight of 303.8 g had seminiferous tubule diameters of 209.4 µm, which equates to a circumference of 657.8 µm (Cloete et al., 2000). The Dohne Merino rams thus showed a high degree of seminiferous tubule development within the testes tissue in comparison, with a mean circumference of 980.3 ± 21.57 µm (Table 3.1).

Table 3.1. The mean (± SE) for testes and sperm parameters of Dohne Merino rams (76.7 ± 0.93 kg).

Parameter	**Mean ± SE**
Trimmed testes weight, g	400.1 ± 15.59
CIE colour values	
*L**	64.1 ± 0.22
*a**	1.4 ± 0.13
*b**	12.4 ± 0.16
Seminiferous tubule:	
Circumference, µm	980.3 ± 21.57
Epithelium thickness, µm	64.4 ± 1.81
Sperm concentration, cells/mL	$1.9 \times 10^9 \pm 0.11 \times 10^9$
Total live sperm, %	80.8 ± 4.47
Normal, %	97.7 ± 1.37
Deformed, %	2.3 ± 1.37
Total dead sperm, %	19.1 ± 4.47

Normal, %	81.6 ± 5.12
Deformed, %	14.2 ± 3.74

SE = standard error

Although scrotal circumference is used as an indication of breeding soundness in rams, semen evaluation is important to determine the fertilizing ability of the sperm present. Standard sperm concentration for breeding rams is between 2.5 to 6 x 10^9 cells per mL (Bedford-Guaus, 2017) and although the mean concentration for the 24 Dohne Merino rams is lower than this, the variation is large (Table 3.1). Normal sperm morphology for rams is considered excellent at > 80 %, acceptable at + 50 %, questionable at 30 to 50 % and unsatisfactory at < 30 % (Bedford-Guaus, 2017). The epididymal sperm had a total live sperm percentage over 80 % and under 20 % dead with more than 80 % of all sperm cells with normal morphology (Table 3.1). Even though breeding soundness evaluation of rams is performed using semen samples collected from the live ram and not the epididymis, the Dohne Merino rams produced satisfactory sperm quality. However, if possible, semen should be collected using alternative methods such as electro-ejaculation in future studies.

The average carcass weight for the Dohne Merino rams was 31.3 ± 0.57 kg (Table 3.2) which provides a dressing percentage of ~ 40.8 %. Dohne Merinos (rams and ewes) slaughtered at 20 months of age at a live weight of 56.0 ± 1.8 kg by Cloete et al. (2012) had carcass weights of 22.4 ± 1.0 kg, which is lighter than the current study. However, dressing percentages were similar at 40.5 % (Cloete et al., 2012). When the offal weights are converted to a percentage of the live weight, the head, feet and skins from the Dohne Merinos slaughtered by Cloete et al. (2012) had similar yield percentages to those reported in this study. However, GIT, red offal and reproductive organ weights were not measured by Cloete et al. (2012). In future research, the GIT fat should be separated and weighed as a measure of offal fat yield as well.

Table 3.2. The average live weight, carcass weight and offal yields (offal weights expressed as a percentage of live weight) of post-pubertal Dohne Merino rams.

Parameter	Mean ± SE
Live slaughter weight, kg	76.7 ± 0.93
Hot carcass weight, kg	31.3 ± 0.57
Offal Items	
Head, %	5.8 ± 0.28
Feet, %	2.2 ± 0.02
Skin, %	6.4 ± 0.10
GIT, %	25.5 ± 0.38
Red offal, %	4.2 ± 0.04
Scrotum & testicles, %	1.3 ± 0.05

SE = standard error
GIT = gastrointestinal tract

3.4 Conclusion

Dohne Merino rams at 16 months of age may be considered post-pubertal and entering sexual maturity at 76.7 ± 0.93 kg (~ 18 months old), as supported by testosterone concentrations and scrotal circumferences. The testosterone secretory pattern confirms the need for the time of day and even season, to be standardised regarding sample collection for future research. Testes size and development is an important factor in selecting breeding rams for fertility and is an easy and painless means to quantify development. Scrotal circumferences were normal for the Dohne Merino rams but also fluctuated over the study. Sperm quality at slaughter indicated concentrations just below normal commercial standards for breeding rams but acceptable viability and morphology. Scrotal temperatures are more affected by environmental temperature than rectal temperature and thus scrotal and rectal temperatures should be monitored together. Considering the extent of development in terms of serum testosterone concentrations, scrotal circumferences and sperm quality of post-pubertal young Dohne Merino rams selected out of a commercial stud flock, castration would be beneficial before feeding for slaughter. Thus, immunocastration could potentially suppress these high

testosterone concentrations, thereby controlling reproduction and behaviour in mixed-sex flocks.

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CHAPTER 4

The influence of vaccination administration interval on growth, scrotal circumference and carcass traits of immunocastrated ram lambs[2]

Abstract

This study investigated the effect of different immunocastration vaccine administration intervals;' namely two (IC2), three (IC3) or four (IC4) weeks between primary and secondary vaccination on growth, carcass traits and testis development of ram lambs. The nine-week trial consisted of four treatments with ten, 5.5 months old, Dohne Merino rams per group; the control treatment being intact (R) ram lambs. Slaughter age was fixed at four weeks post-second vaccination for all treatment groups. Live body weight, scrotal circumference and temperature, as well as injection reaction score and temperature, were recorded weekly. At slaughter, the carcass and individual offal items were weighed. At 24 hours *post-mortem*, shoulder circumference, neck circumference and subcutaneous backfat thickness were measured. Vaccination interval did not influence average daily gain, slaughter weight, carcass weight, subcutaneous backfat thickness, and linear carcass measurements. Vaccination schedule influenced the percentage contribution of the skin and red offal to the total live weight. Differences in scrotal circumference between the immunocastrated groups and R were evident from Week 4. The immunocastration protocols followed in the study thus were successful in inhibiting testis growth without negatively affecting the overall growth performance and slaughter performance of the ram lambs.

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[2]*Needham, T., Lambrechts, H., Hoffman, L. 2016. The influence of vaccination interval on growth, carcass traits and testicle parameters of immunocastrated ram lambs. Small Rumin. Res. 145,53-57.*

4.1 Introduction

In livestock production systems, male animals are castrated to control aggression and sexual behaviour, as well as to improve meat quality and fattening of the carcass. Castration methods such as sloughing using an elastrator band, crushing of the spermatic cords using a Burdizzo, or surgical removal results in pain and thus decreased growth performance (Melches et al., 2007). Although the production of intact males is considered beneficial in terms of growth and feed efficiency, relevant management and meat quality issues need to be addressed to ensure the welfare of ram lambs during production. Such management issues include controlling aggressive and reproductive behaviour, which can comprise the welfare of other animals in the flock. Intact males intended for slaughter are typically maintained in single sex groups, away from female slaughter animals or breeding ewes, to prevent unwanted pregnancies. Stocking density of intact males also must be considered to minimize interactions between males to prevent injuries and carcass damage due to fighting and mounting activities that form part of the reproductive behavioural repertoire. Downgrading of carcasses due to bruising contribute to economic losses, which in turn affects the profitability of production systems. There is thus a need to develop welfare-friendly alternatives to physical castration that will ensure efficient management, optimal carcass quality and maintenance of animal welfare.

Immunocastration has proved to be an effective method of suppressing the functioning of the reproductive system in a range of species including swine, cattle, sheep, goats, horses, dogs, elephants and red deer (Lincoln et al., 1982; Schanbacher and Pratt, 1985; Godfrey et al., 1996; Ladd et al., 1994; Dunshea et al., 2001; Ülker et al., 2002; De Nys et al., 2010; Amatayakul-Chantler et al., 2013; Needham & Hoffman, 2015). Immunocastration has also been widely researched and implemented in the swine industry to control aggression (Cronin et al., 2003) and prevent boar taint (Dunshea et al., 2001).

Although rams are physically castrated to aid in fattening of the carcass, and to control behaviour, little research has been done on the use of immunocastration in rams maintained for lamb meat production. The aim of the study is therefore to determine the influence of immunocastration vaccination interval on the growth, testes development and carcass traits in ram lambs raised for lamb meat purposes.

4.2 Materials and Methods

Ethical clearance was obtained from the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00073) and animal husbandry was in accordance specifications of the South African National Standards 10386: 2008.

4.2.1 Growth trial & vaccination

At 5.5 months of age (35.0 ± 2.18 kg live weight), 40 Dohne Merino dual-purpose ram lambs were stratified according to initial body weight, and then randomly allocated to treatments at 10 rams per treatment group. The treatment groups consisted of the control (intact ram lambs, R), and vaccination intervals of two (IC2), three (IC3), and four (IC4) weeks between first (primary) and second (booster) vaccination administration. All immunocastrated groups received the booster vaccination at the same time, four weeks prior to slaughter. The experimental design is presented in Table 4.1.

Table 4.1. Weekly activities indicating vaccination schedules used for immunocastrated ram lambs and slaughtering.

Activity	Week								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
IC4 inject	X				X				
IC3 inject		X			X				
IC2 inject			X		X				
Slaughter									X

IC4 = immunocastrates with a 4-week vaccination interval and vaccinated at 1 & 5 weeks
IC3 = immunocastrates with a 3-week vaccination interval and vaccinated at 2 & 5 weeks
IC2 = immunocastrates with a 2-week vaccination interval and vaccinated at 3 & 5 weeks

The first immunocastration injection (2 mL Improvac®; Reg. no. G3643, Act 36/1947, Zoetis Animal Health) was given just behind the left foreleg and the second (2 mL Improvac®) behind the right foreleg in the area free from wool. For each vaccine administration session, the injection site was disinfected by spraying with a 70 % ethanol solution, and the vaccine was then administered using a self-tenting 2 mL Sekurus™ injector and AC10 needle guard (Simcro™, New Zealand) fitted with an 18-gauge x 1.9 cm needle.

All trial animals were maintained on kikuyu pasture for the duration of the trial, under uniform management conditions. Parameters recorded weekly included live weight, scrotum circumference, scrotal surface temperature, injection site reaction score, and injection site/ skin surface temperature. Scrotal circumference was measured whilst the sheep was in a recumbent/ sitting position and ensuring both testes were descended into the scrotum before using a flexible plastic tape measure around the widest axis of both testes. Scrotal and injection site/ skin surface temperatures were measured by means of an infrared thermometer (Alla France, accuracy: ± 2 °C) by placing the device flush with the skin, thus standardizing distance of measurement. Scrotal surface temperature was measured on the left testis, after parting the wool to expose the skin surface. The injection site score was assigned as described by Pauly et al. (2009) and represented in Table 4.2. The average daily gain (ADG) was calculated weekly, and then further for the periods: pre- and post-booster vaccine as well as the overall nine-week trial period.

Table 4.2. Description of scoring system used to describe the injection site reaction to Improvac® as defined by Pauly et al. (2009).

Score	Degree of reaction	Description
0	Normal	Slight swelling < 0.5 cm diameter
1	Mild	Slight redness & swelling > 0.5 cm
2	Moderate	Considerable redness & scabbing
3	Infection	Discharge
4	Severe	Abscess & open wound

4.2.2 Slaughter

All sheep were slaughtered four weeks after the second vaccination was administered (48.8 ± 4.56 kg live weight) at a commercial abattoir according to practises described in Chapter 3 (3.2.3). On the day of slaughter, all sheep were weighed before and after transport to establish potential effect of transport on weight loss. All sheep were transported together to the abattoir in the morning and remained in shaded lairage for approximately five hours with *ad libitum* access to water and hay before they were slaughtered that same afternoon. Each sheep was electrically stunned (four seconds; 200 V; 250 AMP) by placing electrodes on each side of the head and exsanguinated.

The forelegs were removed between the radius and humerus and the hindlegs between the tibia and femur. These four limbs were weighed together and referred to as the feet. The scrotum containing the testes was then dissected off the carcass, after which the head was removed between the skull and the atlas. The skin was removed thereafter, thus excluding that found on the feet and head. The anus and esophagus were excised free and the gastrointestinal tract (GIT) was removed; the GIT included the esophagus, rumen, reticulum, omasum, abomasum, small intestine, large intestine, caecum, colon and rectum. From this, the fat around the rumen was removed (GIT fat) and weighed separately. The heart, lungs, liver and trachea were removed from the carcass together as the red offal portion.

Hot dressed carcass weight (HCW, ~45 mins *post-mortem*) was recorded, as well as the weights of the head, feet, skin, GIT, GIT fat, red offal and scrotum containing skin and testicles. All weights were expressed as a percentage of the live weight. Dressing percentage was calculated using the HCW and live weight on arrival at the abattoir. The carcasses were maintained at 4 °C for 24 hours, after which the neck circumference, shoulder circumference and subcutaneous backfat thickness were measured (Figure 4.1).

Backfat thickness was measured with an electronic engineering calliper (150mm Electronic Digital Vernier Caliper CE ROHS) at the last rib, 45 mm from the midline, on the right side of each carcass. Shoulder circumference was measured using a flexible plastic tape measure directly behind the shoulder blades, perpendicular to the spine (Figure 4.1). The neck circumference was also measured using a flexible plastic tape, at the base of the neck where the neck meets the shoulder blades (Figure 4.1).

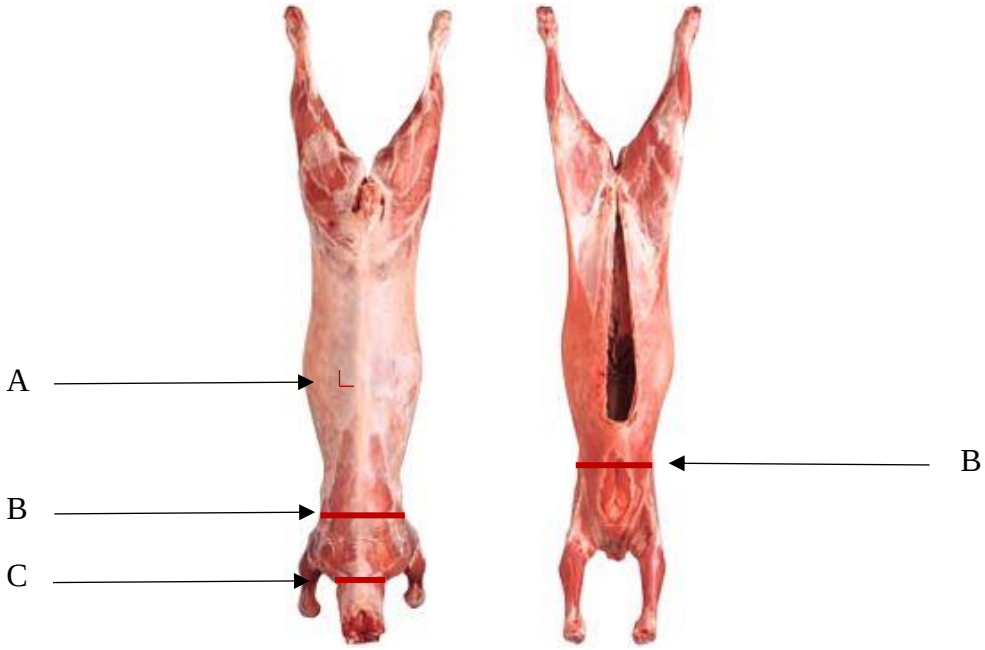

Figure 4.1. The relative positions of the various carcass measurements performed 24 hours *post-mortem*. The backfat thickness (A) was measured 45 mm from the midline with a calliper after creating an L-shaped incision over the last rib, while the shoulder circumference (B) was measured behind the shoulder blades. Lastly, the neck circumference (C) was taken at the base of the neck.

4.2.3 Statistical analysis

Statistical analysis was carried out using STATISTICA 64 (StatSoft Inc., Version 13). Residuals were tested for normality and homogeneity was ensured using Levene's test. For the growth and scrotal circumference data over time, the Variance Estimation, Precision and Comparison (VEPAC) procedure was followed. For the slaughter data, one-way ANOVAs

were used. Comparison of treatment means was done using Fishers Least Significant Difference. In the case where the assumption of homogeneity was not met, Games-Howel post hoc tests were performed. Results are reported at least square means (LSMeans) ± SE, and significant differences at a significance level of 5 %.

4.3 Results

4.3.1 Growth trial, scrotal circumference and reaction to vaccination

The weekly live weight and the corresponding average daily gains of the ram lambs are presented in Figure 4.2 and Figure 4.3, respectively. The calculated ADGs for the time periods between the start of the trial and second vaccination, the ADGs between second vaccination and slaughter, as well as the overall ADGs for the trial period are represented in Table 4.3.

Those lambs vaccinated in the first week of the trial (IC4) showed a decline ($P = 0.02$) in growth during the first week following the primary vaccination (Figure 4.2) and thus a negative ADG (Figure 4.3) which was lower than IC3 ($P = 0.020$) and R ($P = 0.020$) during the same period. Subsequently, their live weight increased ($P < 0.001$) from Week 2 to Week 3 (Figure 4.2), as reflected by the positive ADG during Week 2 (Figure 4.3). After the booster vaccination in Week 5, ADG decreases for IC4 ($P = 0.002$). Again, this is followed by a spike in growth ($P = 0.03$) from Week 6 to 7 to meet that of the previous ADGs seen in the week prior to secondary vaccination (Week 4). The IC3 lambs did not experience a decline in growth rate after the primary vaccination as seen in the IC4 lambs. Thus, the ADG of IC3 instead remains stable until a decrease ($P < 0.001$) in growth rate during Week 5 (Figure 4.3). Again, the ADG increases ($P < 0.001$) subsequently during Week 6 and again in Week 8 ($P = 0.003$) to a similar ADG seen prior to the administration of the booster. The IC2 lambs had improved ADG ($P = 0.014$) during Week 2, most likely indicating compensatory growth as no treatment

was applied. After booster vaccination, the ADG of IC2 also decreased (P =0.011) during Week 5, followed by a spike up to the previous ADG seen in Week 4, and then remained stable.

The only significant differences seen between treatments for ADG were the week following booster vaccination, where only IC3 lambs had lower (P = 0.007) ADGs compared to R; as well as during Week 8, where IC2 had the greatest ADG during Week 8 compared to IC4 (P = 0.006), IC3 (P = 0.0004) and R (P = 0.011).

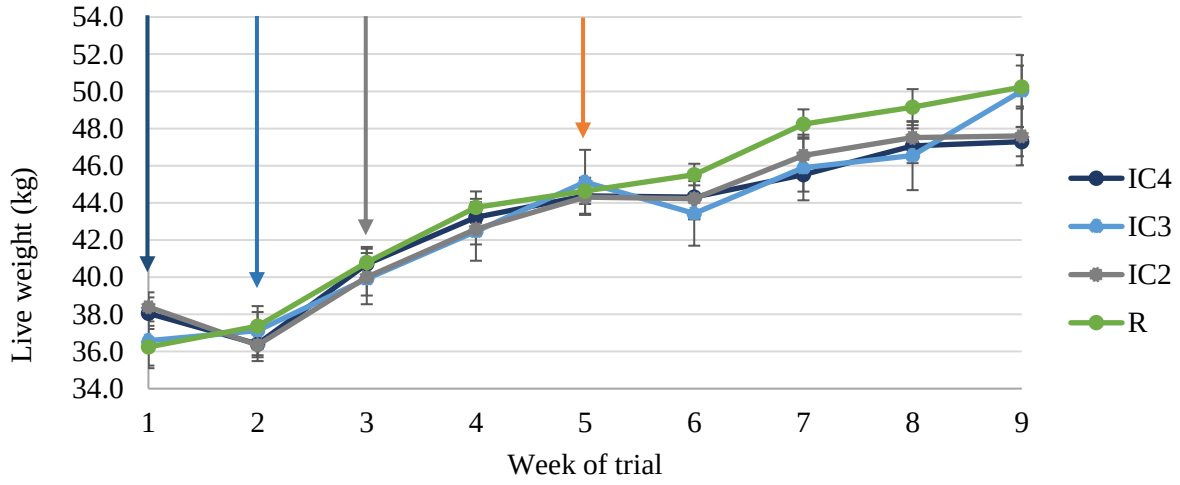

Figure 4.2. Weekly live weights (kg) of immunocastrated and intact (R) Dohne Merino ram lambs. The vaccinations for immunocastrates were given at four (IC4), three (IC3) or two (IC2) week intervals, with the second vaccination at four weeks before slaughter (orange arrow). Primary vaccinations are indicated by the arrows corresponding to the colour legend per treatment. Error bars indicate SE.

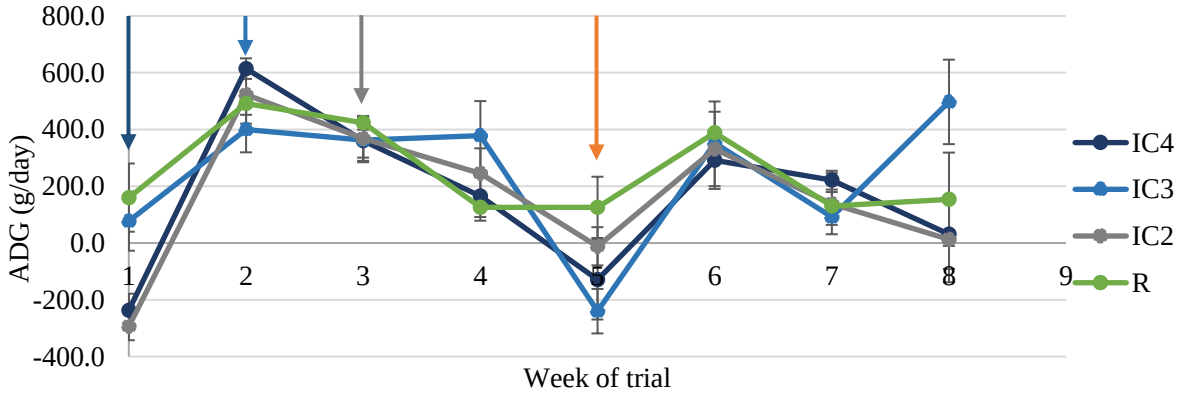

Figure 4.3. The influence of vaccine administration protocol on the average daily gain (ADG; g/day) of Dohne Merino ram lambs. Each data point is represented as the ADG for the duration of that week per treatment. Arrows with colour corresponding to treatment indicate primary vaccination and orange arrow indicates secondary vaccinations. Error bars indicate SE.

When the ADG were calculated and compared between treatments for the four weeks before booster vaccination, the four weeks after booster vaccination and the overall trial, no differences were seen between the treatment groups (Table 4.3).

Table 4.3. The influence of vaccine administration protocol on the average daily gain (g/day) (LSMean ± SE) of Dohne Merino ram lambs over a 9-week treatment period.

Interval	Treatment group			
	Rams	**IC4**	**IC3**	**IC2**
Before second vaccination	342 ± 13.1	325 ± 25.7	358 ± 41.5	336 ± 18.8
After second vaccination	197 ± 34.3	104 ± 37.0	175 ± 33.0	118 ± 45.2
Over total trial period	270 ± 19.4	220 ± 13.5	266 ± 24.7	227 ± 25.0

IC4 = immunocastrates with a 4-week vaccination interval and vaccinated at 1 & 5 weeks
IC3 = immunocastrates with a 3-week vaccination interval and vaccinated at 2 & 5 weeks
IC2 = immunocastrates with a 2-week vaccination interval and vaccinated at 3 & 5 weeks

Scrotal circumferences did not differ between treatments until Week 4 of the trial, when smaller scrotal circumference was reported for IC4 when compared to R ($P = 0.04$; Figure 4.4). From Week 5 until the end of the trial period, smaller scrotal circumferences were reported for IC3 and IC2, when compared to the R ($P = 0.03$; $P = 0.04$, respectively) and remained as such until the end of the trial. Although scrotal surface temperature increased weekly ($P < 0.001$) over the duration of the trial from 25.3 ± 0.35 °C to 29.7 ± 0.35 °C, no differences were observed between treatments (Figure 4.5).

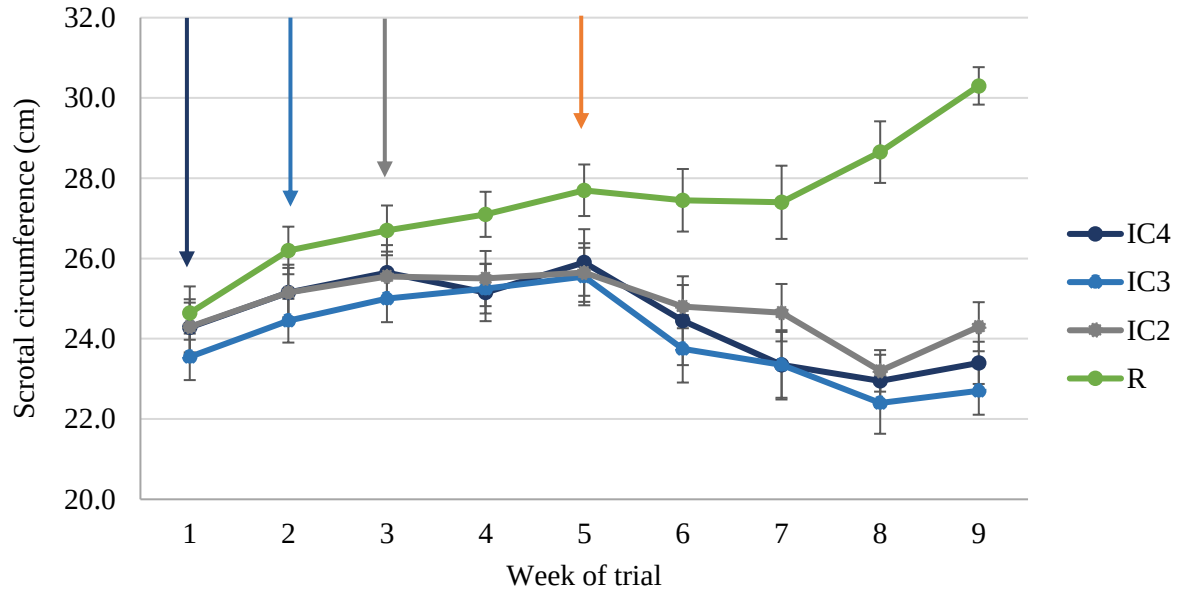

Figure 4.4. The scrotal circumference of immunocastrated and intact rams (R) recorded during a 9-week growth period. Those immunocastrated were done so with either 2 (IC2), 3 (IC3) or 4 (IC4) week vaccination intervals. The colour-coordinated arrows indicate when the respective treatments groups received the primary and booster vaccine injections (orange). Vertical bars denote SE.

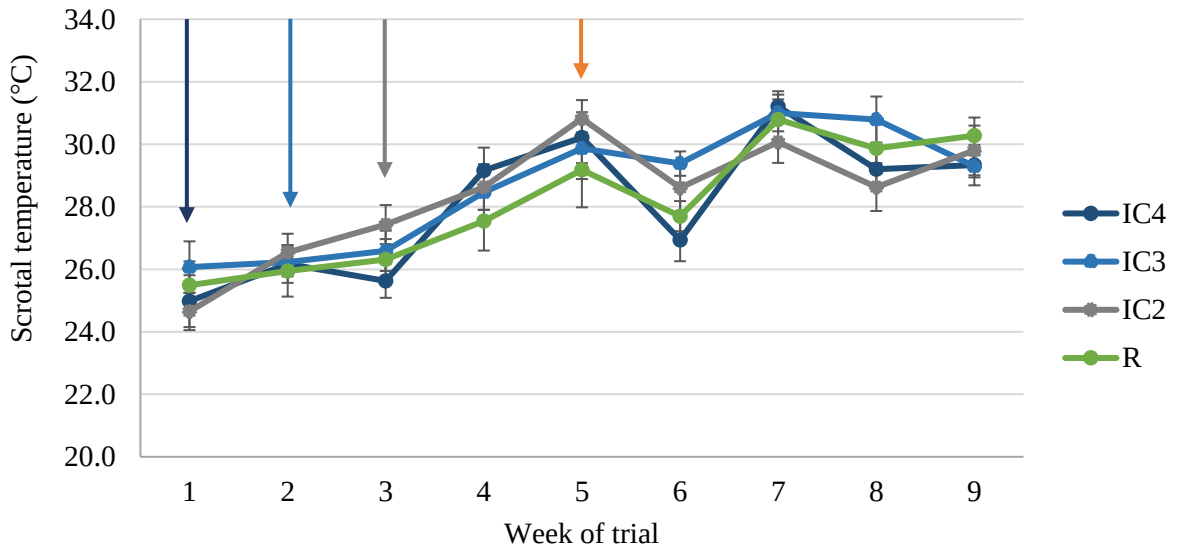

Figure 4.5. The scrotal temperature of immunocastrated and intact rams (R) recorded during a 9-week growth period. Vaccination intervals include 2 (IC2), 3 (IC3) and 4 (IC4) weeks between primary (colour-coordinated) and secondary vaccination (orange). Vertical bars denote SE.

In the week following the first vaccination, one animal in the IC4 group experienced a mild reaction at the injection site (score of 1; Table 4.2), which healed within the following week.

However, three animals in the IC3 group experienced mild to moderate reactions following the first vaccination which only started to improve two weeks later. One animal in the IC2 group experienced a mild reaction for the first two weeks after their first vaccination, until their second vaccination where this number increased to two animals and remained as such until slaughter. The observed reactions did not result in any secondary infections, with the side effects disappearing after one week, and no veterinary intervention was required. In the week after the second vaccination, three animals in each of the IC4 and IC3 groups experienced mild reactions, while two animals in IC2 experienced moderate to severe reactions. After the IC4 lambs healed, their number of animals with a reaction increased after the booster vaccine to three. The IC3 lambs, initially had an increase from 1 to 3 animals after second vaccination, with some reactions only presenting later at Week 8 when the initial three had healed.

The reactions observed after the second vaccine administration were more varied (Figure 4.6) and took longer to subside, with one animal from each treatment group experiencing an infection and consequent abscesses forming during Week 7. The abscesses were cleaned of any debris, sprayed with Necrospray (Bayer© Animal Health) and then Supona™ (Zoetis™ Animal Health) until healed. Monitoring of the animals in which the abscesses developed indicated that the growth of the animals was not affected, and thus they were not removed from the study. Although reactions were observed at the administration sites, there were no differences in the surface temperature at the site of injection over the trial period. The mean skin surface temperature for the IC4, IC3, IC2 and R groups were 34.1 ± 0.28 °C, 33.7 ± 0.28 °C, 33.8 ± 0.28 °C and 33.8 °C ± 0.28 °C, respectively.

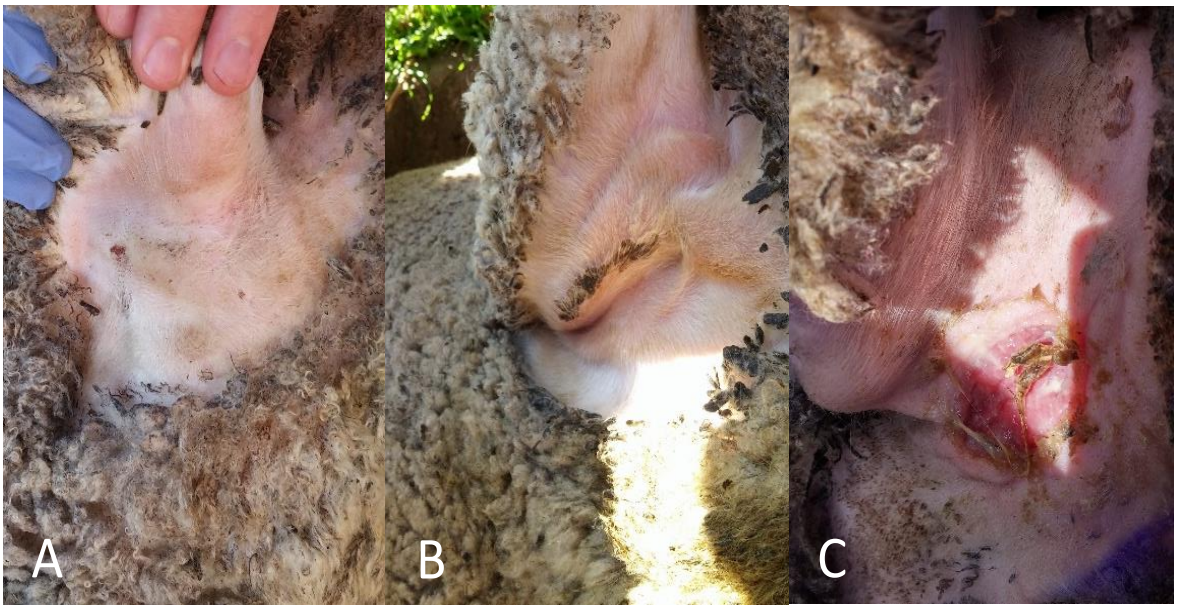

Figure 4.6. The variation in injection site reaction from normal (A), to moderate (B) and severe (C). The accumulation of fluid and subsequent reaction can be seen in B. In the case of C, debris were cleaned from the wound, followed by Necrospray (Bayer© Animal Health) and Supona™ (Zoetis™ Animal Health) application.

4.3.2 Transport loss, slaughter performance and carcass traits

The immunocastration vaccination schedule had no significant effect on the transport losses and slaughter parameters measured, other than some of the offal percentages (Table 4.4). The percentage contribution of the skin to the live weight was greater in IC4 and IC3 than in IC2 and intact R rams ($P = 0.01$). The red offal percentage was lower ($P = 0.04$) for R than the immunocastrated lambs. The scrotum and testicle percentage contribution to HCW for the R rams were higher ($P < 0.001$) than all immunocastrated groups, indicating that immunocastration inhibited testicular growth over all vaccination schedules.

Table 4.4. The effect of vaccination interval on the slaughter and carcass traits of Dohne Merino ram lambs that were vaccinated over a 9-week period.

	Treatment group					
	Rams	**IC4**	**IC3**	**IC2**	**SEM**	**P-value**
Live slaughter weight, kg	52.4	48.6	49.0	51.0	1.41	0.21
Transport loss, kg	2.1	2.1	1.6	2.6	0.31	0.18
Carcass traits						
Hot carcass weight, kg	21.8	20.3	20.5	20.9	0.65	0.38
Cold carcass weight, kg	21.1	19.7	19.8	20.2	0.63	0.38
Dressing percentage, %	43.1	43.6	42.8	43.2	0.51	0.70
Subcutaneous fat depth, mm	1.3	1.5	1.3	1.4	0.20	0.93
Shoulder circumference, cm	75.5	76.8	76.7	76.0	1.00	0.72
Neck circumference, cm	33.2	32.7	32.8	33.8	0.60	0.54
Offal Items						
Head, %	5.5	5.6	5.5	5.5	0.10	0.70
Feet, %	2.3	2.5	2.4	2.4	0.05	0.24
Skin, %	8.0^{b}	9.0^{a}	8.3^{a}	8.0^{b}	0.20	0.01
GIT, %	30.7	29.9	30.7	31.7	0.64	0.56
GIT fat, %	0.4	0.4	0.4	0.3	0.06	0.72
Red offal, %	3.9^{b}	4.1^{a}	4.2^{a}	4.3^{a}	0.08	0.04
Scrotum & testicles, %	1.0^{a}	0.5^{b}	0.5^{b}	0.5^{b}	0.03	< 0.01

$^{a, b}$ LSMeans within rows with different superscripts are significantly different ($P \leq 0.05$)
IC4 = immunocastrates with a 4-week vaccination interval and vaccinated at 1 & 5 weeks
IC3 = immunocastrates with a 3-week vaccination interval and vaccinated at 2 & 5 weeks
IC2 = immunocastrates with a 2-week vaccination interval and vaccinated at 3 & 5 weeks
SEM = standard error of the mean

4.4 Discussion

When considering incorporating immunocastration as part of the management program of commercial production systems, it is important to formulate the most appropriate vaccination schedule. In swine, the recommended vaccination schedule for Improvac® was established to allow sufficient time for the clearance of boar taint compounds. Due to the fast growth of swine and ability to deposit fat, vaccination intervals are generally shorter when compared to that suggested when Bopriva® is administered in cattle. When the formulation of Bopriva® and Improvac® is considered, the same concentration of GnRH-conjugate is administered per recommended dosage/injection; however, the adjuvants used differ.

The recommended vaccination schedule for swine is a minimum of four weeks between vaccinations, with the booster given four to five weeks before slaughter. However, the

vaccination schedule used on cattle is far more flexible than that of swine, allowing both the interval between the two vaccinations as well as the interval between booster vaccination and slaughter to be manipulated. Thus, depending on the production system used, the vaccination schedule can be determined depending on the desired onset and duration of the decreased testosterone effect. This phase in which testosterone is decreased is otherwise termed the "Agreeabull" phase and is defined by testosterone concentrations under 5 ng/mL. The recommended vaccination schedule for cattle is a minimum of three weeks and a maximum of eight weeks between vaccinations, with a duration of "Agreeabull" effect of approximately three months after the booster. The primary focus of the vaccination schedule in cattle is based on the suppression of sexual, aggressive and destructive behaviour. Cattle are slower growing and do not deposit fat as rapidly as swine, which further aids in the ability to manipulate the vaccination schedule.

Although sheep also have a slower rate of fat deposition, their production cycle is much shorter than that of cattle. Typically, in South African systems, sheep are weaned at 100 to 120 days and fattened (or finished) to a live weight of approximately 40 kg. However, a range of both extensive and intensive production systems are used with both early and medium maturing breeds being used in commercial meat producing systems. Thus, a flexible vaccination system would also be preferred, where not only the behaviour of rams can be controlled, but performance will not be compromised. Furthermore, manipulation of carcass fatness using a flexible vaccination schedule would further motivate the use of immunocastration. Thus, the vaccination schedules investigated in this study were chosen in such a way as to fit into a short production time frame and potentially coincide with typical time frames which farmers may handle their sheep for tasks such as vaccination or preparation for feedlotting.

Although ADG fluctuated over the trial period within the treatments, the control ram treatment group also fluctuated in a similar fashion. Thus, environment is likely to be a

contributing factor, which is motivated by the result that no overall differences were seen for the overall ADG for the trial period between treatments and thus no differences were observed for the live weight at slaughter. This is consistent with the findings of Kiyma et al. (2000) using a GnRH-keyhole limpet hemocyanin conjugate vaccine, Janett et al. (2009) using Improvac®, and Ülker et al. (2002) and Gökdal et al. (2010), using recombinant LHRH fusion protein vaccines in their studies on ram lambs. This lack of overall effect on ADG could possibly be attributed to the fact that the duration of the study was too short for the effect of decreased testosterone production to influence overall body growth or possibly the upregulation of the conversion of adrenal steroids such as androstenedione into potent androgenic compounds in the absence of testicular steroids. The period of data collection from the sheep in this study was from ~ 35 kg to ~ 48 kg, which could be considered peri-pubertal as rams tend to reach puberty at 50 to 60 % of their mature body weight (~ 80 kg). Thus, the androgen production concentrations in these animals needs to be established to determine whether, firstly, anabolic steroid hormone concentrations were substantial enough to influence growth and secondly, if conversion of steroids occurred.

Immunocastration successfully suppressed testicular growth in all immunocastrated animals. The IC4 treatment experienced the decrease in scrotal circumference a week earlier than IC3 and IC2 groups, indicating a potential suppressing effect of the primary vaccination on testosterone concentrations. Testosterone concentrations in swine decrease slightly within six days after the first vaccination; however, they increase shortly thereafter until the booster is given (Claus et al., 2007). Ülker et al. (2009) reported a decrease in scrotal circumference in immunized rams within two weeks after the second vaccination; however, their measurements were taken fortnightly.

Although all vaccination intervals had an influence on testicle development, the time at which the effect took place was determined by the vaccination schedule. Thus, vaccination

interval may also have an influence on the point at which testicular steroidogenesis is disrupted, which requires further investigation. This could be done through the repeated collection of semen over time to determine semen quality, sperm concentration and the number of dead versus alive sperm. Improvac® has been shown to cause structural damage to the testes in swine (Kubale et al., 2013). Thus, histological evaluation of the ram testes will indicate whether the various vaccination intervals had different degrees of influence on the testes structure.

Scrotal temperatures were recorded to determine if immunocastration treatment disrupted testicular thermoregulation, resulting in thermal injury. Subcutaneous scrotal temperatures correspond closely to that within the testis itself (Waites & Moules, 1961), thus changes in surface temperatures could indicate altered testis activity, disruption of blood flow (Marti et al., 2010) and possibly changes in overall body temperature. Increased body temperature due to stimulation of the immune system could also influence the ability to thermoregulate the testes and thus influence spermatogenesis. However, no differences were observed in scrotal temperatures between treatments, thus any potential histological changes are unlikely to be caused by thermal injury. The decrease in scrotal temperature across all treatments seen for Week 6 was likely due to environmental influence. From Week 3 to Week 4, both the average maximum and average minimum temperatures increased, while the wind speed decreased, over the time of day when the measurements were consistently performed on the animals. However, the average maximum and average minimum temperatures decreased on the Week 6 measurement, thus likely influencing the scrotal temperature across the treatments (Figure 4.5). It is thus recommended to include the measurement of rectal temperature over time after immunocastration vaccination when conducting further research. The aim of this being to establish whether a rise in body temperature is experienced after vaccination and the relationship between body temperature and scrotal temperature.

The incidences of moderate and severe reactions were less than that reported with the extra-label use of Improvac® in mares, where 89 % of the horses experienced injection site reactions, with an increase in severity after the booster vaccine (Imboden et al., 2006). However, the reaction response was highly variable over the immunocastrated lamb groups and thus warrants further examination. The four-week vaccination interval allowed for a longer recovery time between vaccinations; however, this seemed to have little influence on the number of animals which had reactions after the booster vaccination. Those lambs vaccinated with a two-week interval had a lower number of animals with reactions over their vaccination schedule; however, the reaction was more severe than the other treatment groups. Over all treatment groups, more animals had reactions and more aggressive reactions were seen after the second vaccination, along with notable stiffness in the limb on the side that was injected, which needs to be quantified. Serum cortisol concentrations should be investigated to establish whether this causes stress to the animal after injection and whether the reaction score is adequate in describing this. Although only two animals experienced severe reactions at the injection site, this could result in issues when applied to a large scale commercial unit and thus an alternative injection site should be considered. The 2mL of fluid tended to accumulate subcutaneously, which may be expected with an oil-based adjuvant such as the diethylaminoethyl-dextran used in Improvac®. However, the use of multiple injection sites per dose should be considered to spread the vaccine to improve the incidences of reaction to the vaccine.

The lack of an immunocastration effect on HCW, cold carcass weight and dressing percentage is similar to that reported by Ülker et al. (2002) and Gökdal et al. (2010). Ülker et al. (2002) reported no differences in the subcutaneous fat weight determined by physical dissection of the section between the 6th and 12th rib, while Gökdal et al. (2010) reported no differences in fat depth over the *M. Longissimus thoracis et lumborum*. These results agree

with the finding of the current study; that subcutaneous backfat depth was not influenced by immunocastration. However, the animals in this study were maintained extensively, and results may differ should a feedlotting system be used where animals receive a high energy concentrate diet.

No differences were seen in linear carcass measurements taken in this study or those by Ülker et al. (2002) and Gökdal et al. (2010) thus supporting the findings that immunocastration does not influence the development of the forequarter when applied to lambs later than typical physical castration (shortly after birth). The physical development of the forequarter and neck is often used as an indication of masculinity in abattoirs and has an influence on carcass grading, with males showing heavy masculine features, such as neck and shoulder development, receiving a poorer carcass grading in South African abattoirs. However, should rams be immunocastrated at an early age, one could potentially expect their development to be similar to that of a physically castrated ram.

Testosterone has been shown to increase the clean wool weight in sheep when administered to wethers at high doses (175 mg) (Slen & Connell, 1958) However, in this study, lambs who received their primary vaccination earlier had a greater skin yield than those who received their primary vaccination only two weeks before the secondary vaccination, as well as the intact rams. Thus, the differences between treatments for skin yield is unlikely to be as a result differences in wool growth due to testosterone concentrations.

The differences seen in red offal yield could be due the enlargement of the spleen. The hypothalamic-pituitary-gonadal (HPG) axis appears to interact with the immune system, with sex steroids likely playing a role in immune function. This is based on the premise that sex steroid receptors are expressed in various tissues involved in the immune system, including the spleen (Samy et al., 2000). Han et al. 2016 suggested that GnRH production by the spleen increased in immunocastrated rams because the inhibitory effect of testosterone was removed.

Their results showed that immunocastration increased immune cytokine expressions in spleen and serum concentrations of interleukin-2 (IL-2), IL-4, IL-6, tumour necrosis factor-α (TNF-α) thus having a positive effect on the immune system. Due to the increased activity of the spleen in response to immunocastration, it is likely that enlargement could occur. It is unfortunate that the individual organ weights were not recorded, and this aspect warrants further research.

4.5 Conclusion

Immunocastration vaccine administration interval had no effect on the overall average daily gain and the respective carcass weights and traits measured. However, further investigation into the injection site location and vaccination protocol is required to establish if the incidences of reactions can be improved to that acceptable on a commercial scale. Alternatively, the choice of adjuvant needs to be considered. Serum cortisol concentrations can assist in quantifying the level of potential pain and stress these reactions may cause to the animals.

If the production aims are to increase the fatness of carcasses, a longer period between second vaccination and slaughter should be investigated. Furthermore, should the purpose of immunocastration be to control sexual and aggressive behaviour as well as mating through compromised testicular functioning, further investigation into the effects of vaccination interval on testicular androgen production and testicular morphology is recommended to provide insight into the influence of immunocastration on testicle physiology and functioning.

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CHAPTER 5

Influence of immunocastration vaccine administration interval on serum androgen concentrations and spermatogenic activity in ram lambs[3]

Abstract

The objective of this study was to establish whether different intervals between the primary and secondary administration of Improvac® influenced androgen production and spermatogenic activity in Dohne Merino rams. Forty Dohne Merino rams (5.5 months old) were allocated to the respective treatment groups: no vaccination (R), four (IC4), three (IC3) or two (IC2) weeks between the primary and secondary vaccination. Administration of the secondary vaccination to all IC4, IC3 and IC2 lambs occurred four weeks prior to slaughter. Blood samples were collected weekly during a nine-week growth period, and serum androgen metabolites were determined using ultra-performance convergence chromatography tandem mass spectrometry. Testes were collected at slaughter, weighed and cut surface CIE Lab colour determined, before tissue samples were collected and processed for histological evaluation. Serum testosterone concentrations decreased in IC4, IC3 and IC2 lambs within the first week after the primary vaccination, with testosterone concentrations lower than 0.5 ng/mL were recorded a week after the secondary vaccination. Androstenedione concentrations decreased to boardering non-detectable concentrations in IC4, IC3 and IC2 lambs within the week after the second vaccination. Serum cortisol concentrations were higher than all other treatments in the IC4 rams in the eighth week of the trial. Improvac® administration resulted in a decrease in testes weight, seminiferous tubule circumference and tubule epithelium thickness, which all are indicative of impaired spermatogenesis. The most pronounced influence of Improvac® on spermatogenic activity, based on seminiferous tubule epithelium thickness, was observed in the IC4 lambs. The a* values from the cut surface colour of the testes differed between vaccination intervals for all treatments, potentially indicating differences in the degree of spermatogenic activity. Sperm concentration and viability decreased for all immunocastrated lambs. In conclusion, the IC2, IC3 and IC4 vaccination protocols were successful in suppressing testosterone secretion and interrupting reproductive functioning of the testes in Dohne Merino rams within this study.

[3]*Needham, T., Lambrechts, H., Hoffman, L. 2017. Influence of immunocastration vaccine administration interval on serum androgen levels and spermatogenic activity in ram lambs. Small Rum. Res. Under revision.*

5.1 Introduction

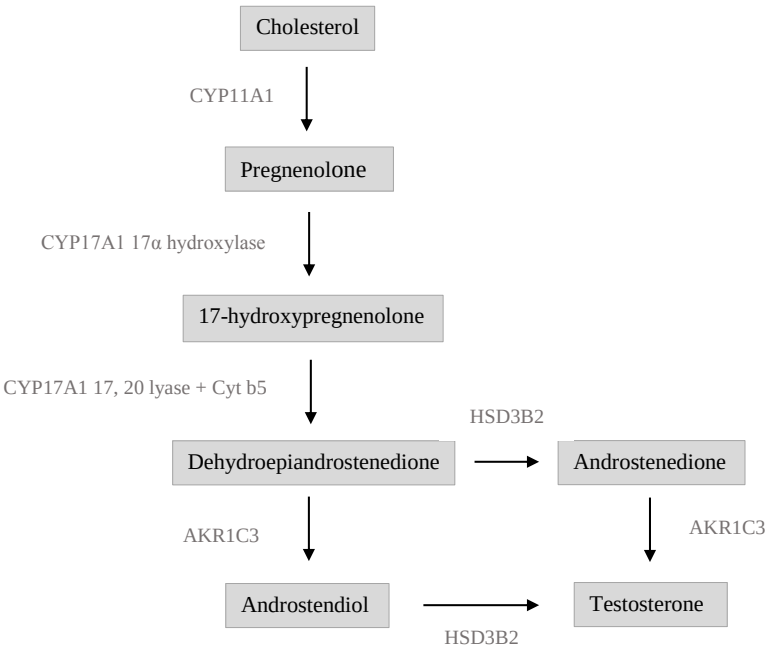
Physical castration of male livestock is considered as a management tool to control aggression and reproduction, with the aim of improving carcass and meat quality. However, increased consumer awareness about animal welfare has placed pressure on the meat industry to reconsider the use of physical castration. The primary concern associated with the various physical castration methods is the pain and stress inflicted on the animals (Melches et al., 2007). Feed efficiency and growth rate are also compromised due to a decrease in circulating concentrations of anabolic steroid hormones, of which testosterone is the most well-documented in this regard (Sales, 2014).

Testosterone production in the testes is governed by luteinizing hormone (LH), which is under endocrine control of gonadotropin-releasing hormone (GnRH) released from the hypothalamus. Together these hormones form part of the hypothalamic-pituitary-gonadal-axis. In the Leydig cells of the testes, cholesterol is continuously converted and metabolised until it forms dehydroepiandrosterone (DHEA), which is subsequently converted to androstenedione (A4), and finally testosterone (T) (Miller & Auchus, 2011).

Testosterone, through a negative feedback mechanism, inhibits the secretion of LH and follicle stimulating hormone (FSH) from the anterior pituitary. The elimination of the negative feedback mechanism of the testosterone on LH and FSH concentrations consequently result in the concentrations of these two hormones being elevated after physical castration in lambs (Schanbacher, 1980). Immunocastration, in contrast to physical castration, results in the inhibition of GnRH-action, which in turn prevents the production of LH and FSH, and ultimately the production of testosterone in the testes (Claus et al., 2007). Although physical castration halts testosterone production by the testes, various androgen precursors such as DHEA, A4 and 11β-hydroxyandrostenedione (11OHA4) can be produced by other tissues such

as the adrenal glands (Figure 5.1; Xing et al., 2011; Schloms et al., 2012; Morimoto et al., 2013).

Leydig cell:



Zona reticularis:

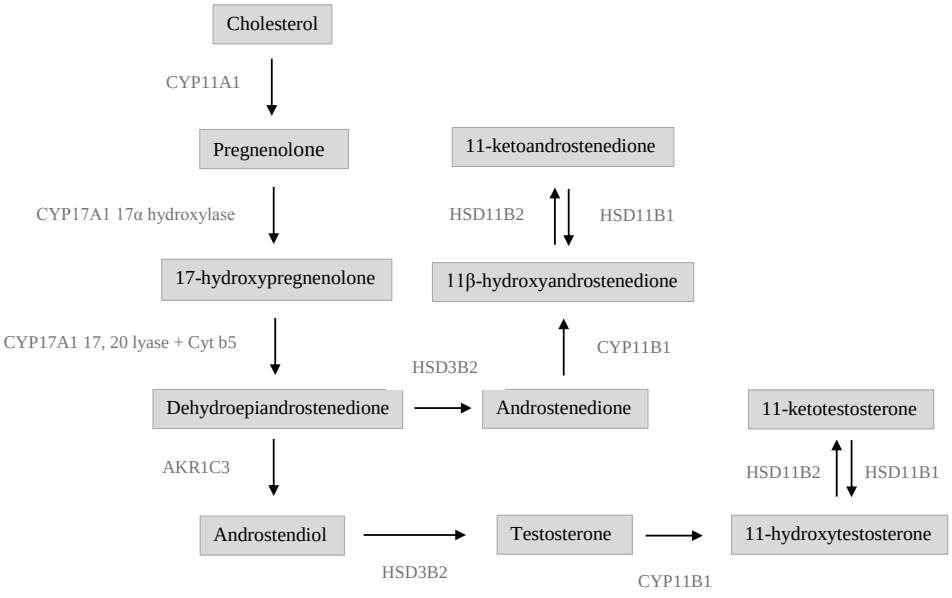


Figure 5.1. Androgen biosynthesis in the Leydig cells of the testes (top) and the *zona reticularis* of the adrenal glands (bottom) (reproduced from Quanson, 2015; Miller & Auchus, 2011; Xing et al., 2011).

The role of the above-mentioned androgen metabolites and their precursors in the various steroid pathways have been studied in humans with prostate cancer. Prostate cancer patients are essentially castrated using androgen deprivation therapy, thus decreasing testosterone concentrations. In response, the prostate tumour makes use of alternate pathways where adrenal A4 is 5α-reduced to 5α-dione and subsequently to DHT (Chang et al., 2011). Adrenal A4 may also be converted to 11OHA4 (Figure 5.1), which forms part of the 11OHA4 pathway implicated in the development of castration-resistant prostate cancer (Storbeck et al., 2013). In the absence of testosterone in castrated animals, androgen precursors may also be converted into active androgens.

Although the influence of immunocastration on production and reproduction parameters in swine and cattle is relatively well-documented, information regarding the effect of immunocastration on production and reproduction in sheep is poorly documented. Immunocastration of rams has been successful in decreasing testosterone secretion in rams using recombinant ovalbumin-GnRH vaccines (Ülker et al., 2003), GnRH-keyhole limpet hemocyanin vaccines (Kiyma et al., 2000) and Improvac® (Janett et al., 2003). However, the efficacy of vaccination intervals as well as the timing of testosterone decrease has not been thoroughly investigated. Furthermore, no commercial vaccination protocol currently exists for rams and before immunocastration can be recommended for use in the sheep industry aspects such as vaccination interval and age at which to administer vaccine needs to be determined.

The aim of this study was to determine whether the interval between first and second vaccination influences the pattern of testosterone suppression in rams and production of androgen metabolites involved in alternative steroid metabolism pathways which may be upregulated. The influence of varying vaccination schedules on testis functioning and

spermatogenesis was determined, as well as the effect of injection site reactions (Chapter 4; 4.3.1), on cortisol secretion.

5.2 Materials and methods

Ethical clearance was obtained from the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00073) and animal husbandry was in accordance specifications of the South African National Standards 10386: 2008.

5.2.1 Animals, vaccination and blood collection

The animals used in this trial formed part of a growth study, of which details are provided in Chapter 4. Briefly, forty Dohne Merino ram lambs (35.0 ± 2.18 kg; 5.5 months of age) were allocated to one of four treatment groups. Three injection intervals were investigated, namely two (IC2), three (IC3) or four (IC4) weeks between primary and secondary vaccinations (Figure 5.2) with the fourth treatment group being the control/intact rams (R). All secondary vaccinations were given four weeks prior to slaughter for all immunocastrated treatments. Immunocastration was performed using 2 mL Improvac® (Reg. no. G3643, Act 36/1947, Zoetis Animal Health) per dose, injected subcutaneously using a Sekurus™ injector (Simcro™, New Zealand), into the bare area behind the foreleg, alternating first left for the primary injection and then right side for the secondary injection. Each injection site was disinfected using a 70 % ethanol spray prior to vaccine administration.

Blood samples were collected weekly by means of venepuncture from the jugular vein using 6 mL Z Serum Clot Activator Vacuettes® for each immunocastrated treatment group, starting from their respective first vaccination as indicated within the sampling schedule (Figure 5.2). Intact controls were sampled every week for the duration of the entire nine-week trial. During the sixth week of the trial, all animals were sampled three times, at intervals of

two days (D1, D3 and D5) to ascertain how rapidly the effects on androgen production take place. Thereafter, blood was sampled again in the eighth week of the trial. Blood samples were taken prior to any other measurement on the lambs, attempting to minimise the effect of handling stress on the serum cortisol results as much as possible. However, it is inevitable that any human interaction will influence cortisol secretion and thus animals were not sampled according to their treatment group or allocated number, but rather at random from the group. Blood collection was performed from 9 am on the respective sampling days indicated in Figure 5.2 from late winter (daylength ~ 10 to 11 hours) to early spring (daylength ~ 11 to 13 hours). The total period taken to withdraw blood from all the trial animals was approximately one hour. Blood samples were centrifuged as soon as possible after collection for 15 minutes at 1500 RCF and 4 °C, and the serum samples stored at – 20 ° C until later analysis.

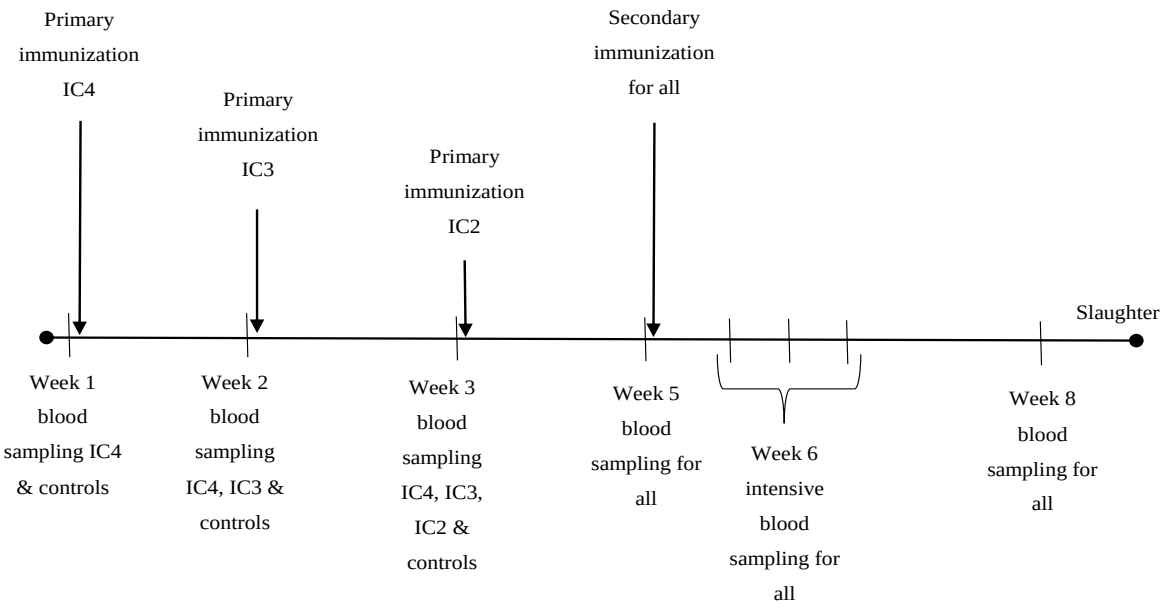


Figure 5.2. The vaccination protocol of immunocastrated (IC) and intact rams (control/R) during a nine-week growth period. Vaccination administration intervals were either two (IC2), three (IC3) or four (IC4) weeks, with the second vaccination given to all immunocastrates four weeks prior to slaughter, as indicated by the arrows. Weekly blood collection is indicated per treatment group, with an intensive blood sampling performed in all groups during Week 6.

5.2.2 Androgenic steroid analysis

Steroid extraction and analysis was based on the ultra-performance convergence chromatography tandem mass spectrometry (UPC^2-MS/MS) methodology developed by Quanson et al. (2016) and discussed within Chapter 3. Steroids were extracted from 500 µL serum samples, as discussed for testosterone extraction within Chapter 3 (3.2.2); however, the internal standards were expanded to include cortisol-9, 11, 12, 12-d4 (15 ng per sample), testosterone-1, 2-d2 (1.5 ng per sample) and progesterone-2, 2, 4, 6, 5, 17α, 21, 21, 21-d9 (15 ng per sample; Cambridge Isotope Laboratories, Andover, USA) within deionized water, added to the serum at 50 µL per sample. These internal standards were also used to generate a standard curve, as discussed within Chapter 3. The liquid-liquid extraction was then performed as detailed by Quanson et al. (2016) using a ratio of 3:1 volume of MTBE to serum. Samples were vortexed (1000 RPM; 10 minutes), then frozen (– 80 °C; 60 minutes) followed by transferring the non-polar phase and evaporating at 55°C under a constant nitrogen gas stream. The dried steroids were reconstituted in 150 µL 50 % methanol and stored at – 20 °C prior to analysis by UPC^2MS/MS under conditions discussed in Chapter 3. The following steroid metabolites were assayed: testosterone (T), cortisol (C), progesterone (PROG), androstenedione (A4), 5α-androstanedione (5α-dione), 5α-dihydrotestosterone (DHT) and 11-ketodihydrostestosterone (11KDHT).

5.2.3 Testes collection and measurements

All sheep were slaughtered according to standard commercial practices, as detailed within Chapter 3 (3.2.3) and Chapter 4 (4.2.2). The testes were collected on the slaughter line and transported on ice to the laboratory for further processing as described within Chapter 3 (3.2.4).

Briefly, trimmed testes weights were measured as a pair before cut surface CIE Lab colour measurement (Color-guide 45°/0° colourimeter, BYK-Gardner GmbH, Gerestried, Germany) without blooming (Lealiifano et al., 2011). Tissue samples were collected and processed for histological analysis, of which included staining with haematoxylin and eosin (Bai et al., 2017) before seminiferous tubule circumference and epithelium thickness determination on 100 seminiferous tubules per lamb (40X magnification; Olympus IX70 microscope and Olympus Image Analysis Software, Olympus Corporation Tokyo, Japan).

After the epididymides were trimmed from the testes, they were placed into petri dishes containing Ham's F10 Nutrient Mixture (Merck, Germany) at room temperature to maintain cell integrity. Nigrosine-eosin stained smears (Rouge, 2004b) of sperm harvested from the cauda epididymis were used to evaluate basic morphology and viability within four hours *post-mortem* (Boshoff, 2014). Sperm concentration was determined within 24 hours *post-mortem* by means of the hemacytometer method (Rouge, 2004a). For viability, the number of alive sperm was expressed as a percentage of the total number of sperm counted per slide. For morphology, the total number of abnormalities (cytoplasmic droplets, detached heads, and coiled/bent tails) was expressed as a percentage of the total number of sperm counted. An average of 100 sperm were counted in three fields of each slide for each parameter per animal at 10 X magnification.

5.2.4 Statistical analysis

Statistical analysis was performed using STATISTICA 13 (StatSoft Inc.). Normality of residuals was ensured, and homogeneity was tested using Levene's test. The Variance Estimation, Precision and Comparison (VEPAC) procedure was used to assess the androgenic steroid concentrations over the trial period using a mixed model repeated measure analysis of

variance (ANOVA). Fixed effects included treatment and week, with animals being the random variable. One-way analysis of variance (ANOVA) was used to evaluate the data for the testes and sperm parameters. Fishers LSD was the chosen post-hoc test to compare treatment means. In the case where the assumption of homogeneity was not met (testes weights), Games-Howel post hoc tests were performed. Significant differences are reported at a significance level of 5 %.

5.3 Results

5.3.1 Androgenic steroid concentrations

Results for serum testosterone (T) concentrations are presented in Figures 5.3 and 5.4, and results for cortisol and androstenedione (A4) concentrations are presented in Figures 5.5 to 5.6, and Figure 5.7, respectively. Concentrations for 5α-dihydrotestosterone, progesterone, 5α-androstanedione and 11-ketodihydrostestosterone were non-detectable over the trial period, and thus are negligible.

Serum testosterone concentration decreased ($P = 0.03$) for IC4 within the first week after the primary vaccination; however, no significant change in testosterone concentrations was detected again until Week 6 (Figure 5.3). Testosterone concentrations for IC4 were significantly lower than R rams only during Week 5 ($P < 0.001$) and Week 8 ($P = 0.02$) of the trial period. Both IC3 and IC2 lambs experienced a decrease in T concentrations ($P = 0.04$; $P < 0.001$) within the first week after the primary vaccination but did not experience a significant change in concentration for the rest of the trial. The T concentrations remained significantly lower than pre-vaccination concentrations for all immunocastration treatments until slaughter. However, the decrease in T concentration for three and two-week vaccination intervals was more rapid and sustained than of the four-week interval (Figure 5.3).

Although no significant change in T concentrations occurred within the immunocastrated lambs during Week 6 (Figure 5.4), T concentrations were significantly lower for all immunocastrated lambs than R rams. The intact rams experienced a significant increase in T concentrations from Week 4 to 5 ($P < 0.001$), followed by a decline in T ($P = 0.004$; Figure 5.3), indicative of variable secretion.

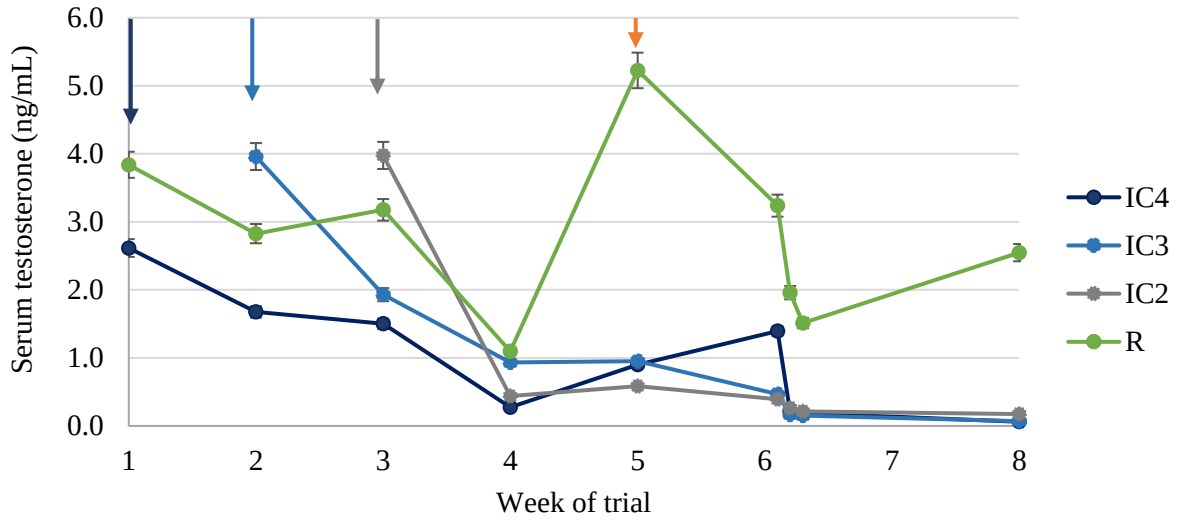


Figure 5.3. Average serum testosterone concentration measured in immunocastrated (IC) and intact rams (R) during a nine-week growth period. Primary vaccinations (colour-coordinated arrows) were administered to the immunocastrated groups at Week 1 (IC4), week 2 (IC3) or week 3 (IC2). The second vaccination was administered to all immunocastrates at Week 5 (orange arrow). Vertical bars denote 95 % confidence intervals.

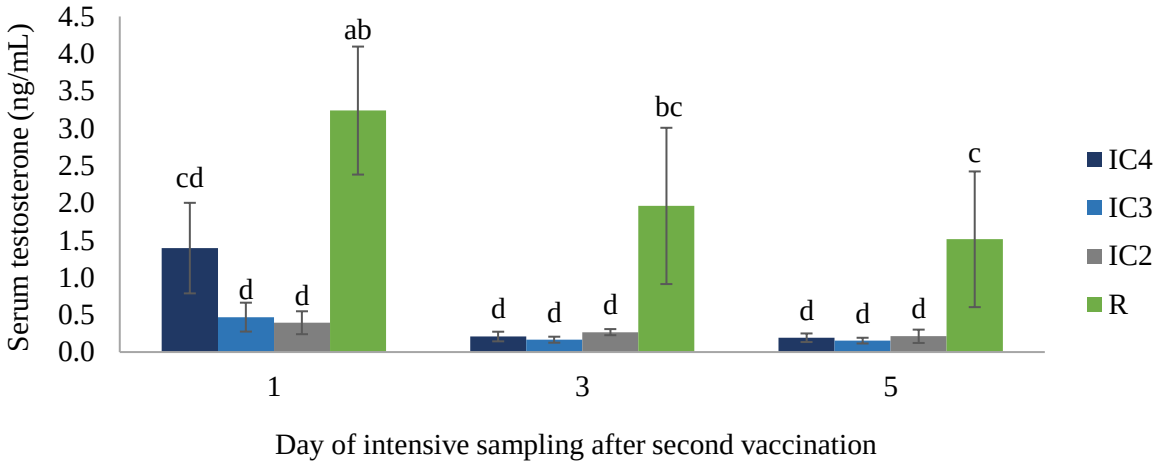


Figure 5.4. The average serum testosterone concentration measured in immunocastrated (IC) and intact rams (R) during the intensive blood sampling period during Week 6, following the secondary vaccination. Vaccinations were given at an interval of four (IC4), three (IC3) or two (IC2) weeks. Letters indicate significant differences between means at a significance level of 5 %.

When the serum cortisol concentrations are compared between treatments, IC4 lambs had higher C concentrations from Week 5 than both IC3 ($P = 0.02$) and IC2 ($P = 0.04$; Figure 5.5) and remained as such until the end of the trial (IC3, $P = 0.009$; IC2, $P = 0.04$; R, $P = 0.05$). No differences were seen for cortisol concentrations within the intensive blood sampling period of Week 6 (Figure 5.6).

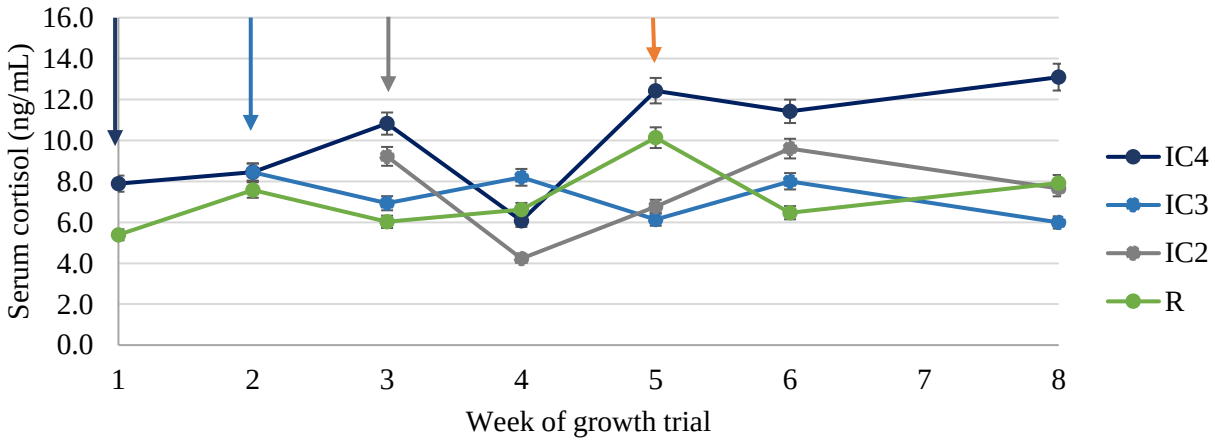


Figure 5.5. Average serum cortisol concentrations (ng/mL) measured in immunocastrated (IC) and intact rams (R) during a nine-week growth period. Primary and secondary vaccinations are indicated by the colour-coordinated arrows. The secondary vaccination was administered at Week 5 (orange arrow). Vaccination intervals include four (IC4), three (IC3) and two (IC2) weeks. Vertical bars denote 95 % confidence intervals.

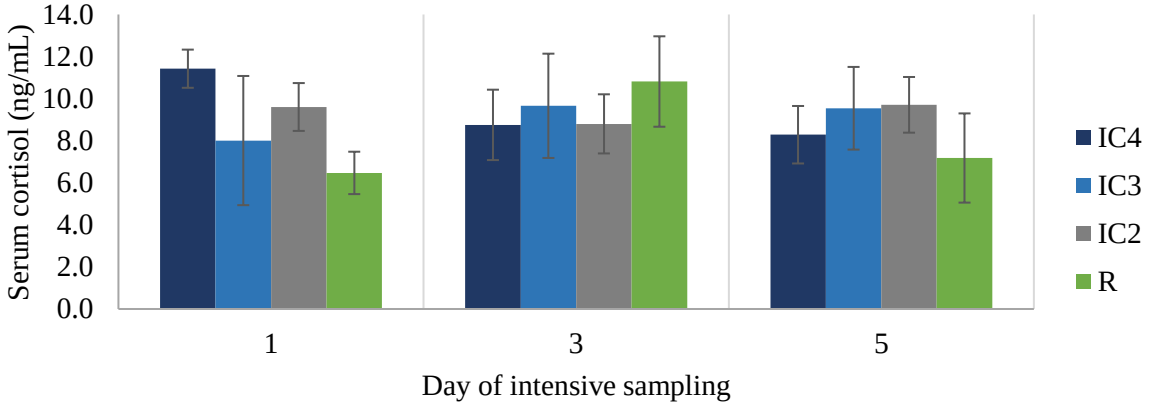


Figure 5.6. Average serum cortisol concentration measured in immunocastrated (IC) and intact rams (R) during Week 6, following the second vaccination. Immunocastrates were vaccinated at intervals of either four (IC4), three (IC3) or two (IC2) weeks. Letters indicate significant differences between means at a significance level of 5 %.

Serum androstenedione concentration decreased ($P \leq 0.05$) after the respective primary vaccination for all immunocastrated lambs but show variable responses at Week 5 (Figure 5.7). During the intensive blood sampling, all three immunocastrated treatment groups reached non-

detectible A4 serum concentrations on D3, while R lambs had A4 concentrations of 0.10 ± 0.039, 0.07 ± 0.044 and 0.03 ± 0.033 ng/mL on D1, D3 and D5 (Figure 5.7).

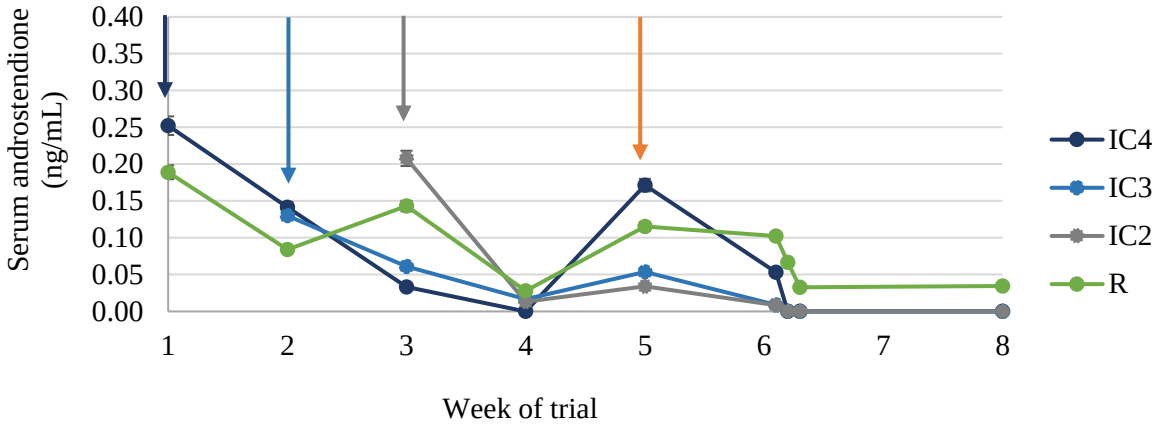


Figure 5.7. The average serum androstenedione concentrations (ng/mL) measured in immunocastrated (IC) and intact rams (R). Colour-coordinated arrows indicate the primary vaccinations for respective treatments and the orange arrow indicates the secondary vaccination administration for all immunocastrated groups. Vaccination intervals were either four (IC4), three (IC3) or two (IC2) weeks. Vertical bars denote 95 % confidence intervals.

5.3.2 Testes and sperm parameters

Results for testes weight, testis tissue colour, seminiferous tubule circumference and seminiferous epithelium thickness are presented in Table 5.1.

Immunocastration resulted in a decrease in trimmed testis weight, regardless of vaccination treatment ($P < 0.001$; Table 5.1 & Figure 5.8). Treatment influenced the cut surface CIE colour values, with the IC3 testes having higher a* colour values than IC2 testes ($P = 0.02$; Table 5.1). All immunocastration treatments resulted in an increase in b* values ($P < 0.001$). Immunocastration resulted in adhesions being formed between the epididymis and the tunica albuginea, which complicated dissecting the epididymis free from each testis (Figure 5.9). Darkening of epididymal tissue was observed in one if the IC2 lambs, indicating a possible thrombosis (Figure 5.9).

Table 5.1. The effect of vaccination interval on the testis weight, CIE colour values and seminiferous tubule parameters (LSMean ± SE) of Dohne Merino ram lambs.

	Treatment group			
	IC4	**IC3**	**IC2**	**R**
Trimmed testes weight, g	$80.0^{b} \pm 8.15$	$76.7^{b} \pm 4.71$	$90.1^{b} \pm 9.44$	$258.5^{a} \pm 19.58$
Seminiferous tubule				
Circumference, µm	$534.7^{b} \pm 17.23$	$527.3^{b} \pm 18.89$	$547.2^{b} \pm 17.59$	$791.5^{a} \pm 34.84$
Epithelium thickness, µm	$20.9^{b} \pm 1.08$	$17.5^{c} \pm 1.00$	$17.2^{c} \pm 1.07$	$52.5^{a} \pm 1.04$
CIE colour values				
*L**	67.3 ± 0,74	67.2 ± 0,64	68.7 ± 0,52	66.2 ± 0,88
*a**	$3.8^{ab} \pm 0.25$	$4.1^{a} \pm 0.35$	$3.0^{b} \pm 0.44$	$0.7^{c} \pm 0.16$
*b**	$13.3^{a} \pm 0.46$	$14.1^{a} \pm 0.46$	$13.8^{a} \pm 0.46$	$9.7^{b} \pm 0.49$

[a, b] LSMeans within rows with different superscripts are significantly different ($P \leq 0.05$)
R = intact control rams
IC4 = immunocastrates with a 4-week vaccination interval and vaccinated at 1 & 5 weeks
IC3 = immunocastrates with a 3-week vaccination interval and vaccinated at 2 & 5 weeks
IC2 = immunocastrates with a 2-week vaccination interval and vaccinated at 3 & 5 weeks
SE = standard error

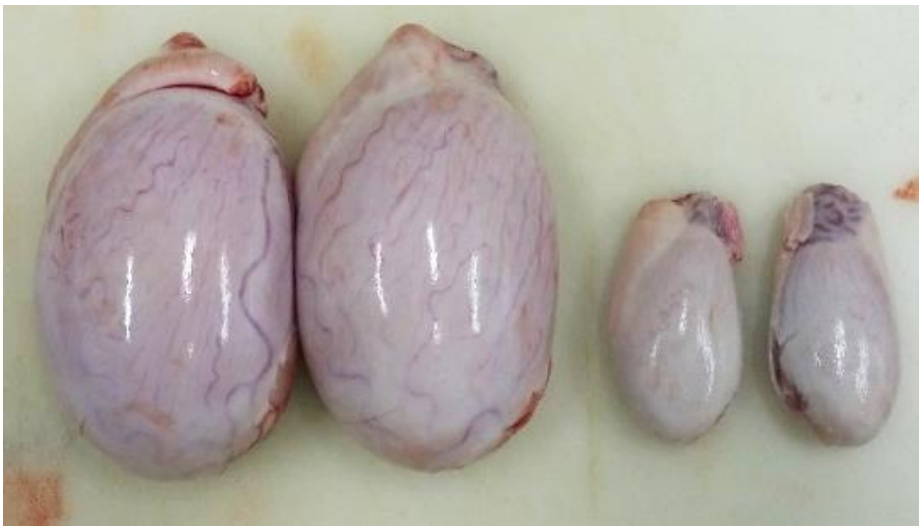


Figure 5.8. The testes (with epididymides still attached) of an intact control ram (left) compared to a ram immunocastrated using Improvac® (right).

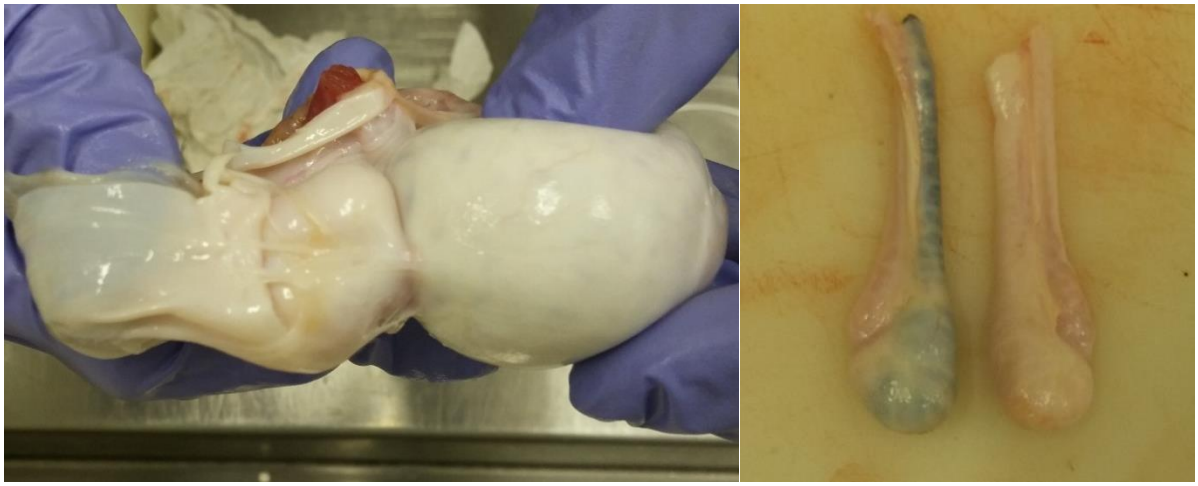


Figure 5.9. Thickening and adhesion of the tunica albuginea observed in immunocastrated lambs (left). Possible thrombosis of the epididymis was observed in one immunocastrated lamb (right) following a two-week interval between vaccinations.

Histological evaluation of the testes indicated that regardless of vaccination interval, immunocastration interrupted spermatogenesis, as evident in the thinner seminiferous epithelium, and smaller seminiferous tubule circumferences ($P < 0.001$) compared to non-vaccinated controls (Table 5.1; Figure 5.10). The non-vaccinated rams had thicker seminiferous tubule epithelium compared to immunocastrated lambs ($P < 0.001$); however, differences also existed between immunocastrated treatments (Table 5.1). The lambs immunocastrated with a four-week interval had thicker seminiferous tubule epithelium than both those vaccinated with a three and two-week interval ($P = 0.03$ and $P = 0.02$ respectively).

Epididymal sperm could only be obtained from five immunocastrated rams. Of these, three animals were part of the IC2 treatment group, and one animal from each of the IC4 and IC3 treatment groups, respectively. When these five immunocastrated rams were compared to intact rams, sperm concentration was lower ($P < 0.001$) in treated animals ($3.3 \times 10^7 \pm 11.0 \times 10^7$ vs. $130 \times 10^7 \pm 8.1 \times 10^7$ for treated and untreated respectively). Viability of sperm obtained from immunocastrated rams were similarly affected, with lower viability recorded for the

immunocastrated animals, when compared to the intact animals (55.6 ± 8.87 % vs. 78.0 ± 6.27 %; Figure 5.11). No differences were observed in terms of sperm abnormalities recorded.

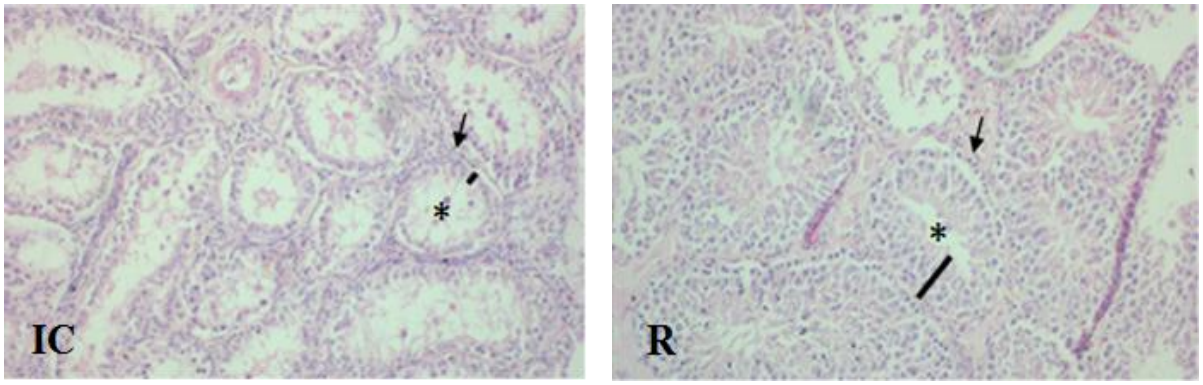


Figure 5.10. Micrographs of cross-sections of testicular tissue from immunocastrated (IC) and intact (R) rams. Slides were stained with haematoxylin and eosin and analysed at a magnification of 40X. Immunocastration resulted in atrophy of the seminiferous tubules (arrow), decreased epithelium thickness (line) and resultant increased lumen space (asterisk).

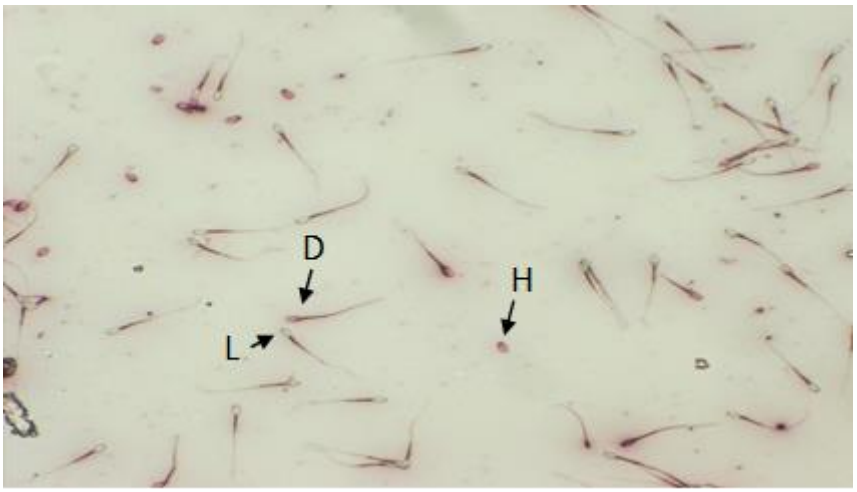


Figure 5.11. Nigrosine-eosin stain of sperm cells indicating normal dead (D) stained cells, live (L) cells and detached heads (H) of sperm cells collected from the cauda epididymis of an intact ram (10 X magnification).

5.5 Discussion

In this study, the effects of varying intervals between primary and secondary (booster) vaccinations for immunocastrating rams using Improvac® on serum cortisol and androgen concentrations, testes size, histology parameters, and sperm morphology and viability were evaluated.

Extra-label use of Improvac® in mares reported reactions at the injection site in 89 % of the horses, which could result in pain and stress (Imboden et al., 2006). Thus, serum cortisol concentrations were determined throughout this study to ascertain whether reactions at the injection site, or a particular vaccination interval, elicits a stress response within immunocastrates due to pain or inflammation. The differences in cortisol concentrations do not show a definite trend following the presence of reaction sites for the respective treatments described in Chapter 4. Although, the high cortisol concentration (Figure 5.5) at the end of the trial experienced in the lambs immunocastrated with a four-week interval may indicate a preference for a shorter vaccination interval. However, due to the small sample size used in this study, further investigation into injection protocol and related influence on the stress levels of immunocastrated animals is required. Additionally, development of a more descriptive injection site scoring system and more frequent monitoring would be beneficial for further research.

All vaccination intervals used for immunocastration in this study were successful in suppressing serum testosterone concentrations to negligible concentrations after the second vaccination (D3 of Week 6; Figure 5.4). All immunocastration treatments also experienced a decrease in testosterone within the first week after the primary vaccination. Although two doses of Improvac® is required to significantly influence the testosterone secretion of swine (Claus et al., 2007), one dose seems to already have a significant effect on GnRH antibody production

in rams, thus decreasing testosterone secretion. While the lambs immunocastrated with a four-week interval between vaccinations showed a slight increase in testosterone concentrations from Week 4 to Week 6 (Figure 5.3), the lambs vaccinated with a three or two-week interval showed no increase in testosterone concentrations between the primary and secondary vaccinations. Although the efficacy of immunocastration was the same for all vaccination intervals, the three and two-week vaccination intervals resulted in a rapid and sustained decrease in testosterone concentrations throughout the trial. Thus, the shorter inter-vaccination periods may be preferred in terms of testosterone reduction efficacy as well as cortisol stress-response levels. Even though the blood samples were taken at the same time of day for the sampling periods, the results for the intact rams indicate the pulsatile secretion of testosterone over time but also between individuals (D'Occhio et al., 1982). Both testosterone and cortisol secretion are under the influence of circadian rhythms as well as various environmental factors. Despite controlling the time of day for blood withdrawal, interpretation of the results of one blood withdrawal per day is thus limited. Although studies (Kiyma et al., 2000; Ülker et al., 2005; Ülker et al., 2009; Gökdal et al., 2010) have made use of a single blood sampling per day, multiple blood withdrawals should be performed per day to improve the accuracy of serum testosterone and cortisol concentration determination and limit the variability. However, the multiple baseline values after the second immunocastration vaccination indicate that testosterone secretion has indeed been compromised.

Declining A4 concentrations after the second vaccination (Figure 5.7) may indicate the conversion of this androgen metabolite to further androgen precursors (Figure 5.1). However, no differences were seen for the androgen precursors 5α-dione, PROG as well as DHT and 11KDHT. Dihydrotestosterone is a potent androgen involved in androgen receptor activation and plays an integral role in both the central and alternative steroid synthesis pathways.

Furthermore, 11KDHT is also produced from the adrenal androgen precursor 11OHA4 through a series of conversions involved in the novel 11OHA4 pathway (Storbeck et al., 2013). However, numerous factors may have influenced the extremely low concentrations of the analysed androgen metabolites in this study. Firstly, conversion of these precursors take place in peripheral tissues and thus perhaps extraction from androgenic tissue itself may provide a better representation of the influence of immunocastration treatment of these pathways. Increasing the number of metabolites analysed may also give a better indication of whether these pathways are activated or not. Additionally, upregulation of androgens depends on both the availability of androgen precursors as well as the various enzymes involved in various tissues. The upregulation of androgen receptors first needs to take place; whilst the period of castration in the immunocastrates was relatively short in this investigation and this was most probably the reason for no effect being recorded.

In response to decreased testosterone secretion, immunocastration reduced testes activity and size, interrupting spermatogenesis. Immunocastration with a range of experimental vaccines have been shown to successfully suppress testes development in rams over a wide range of ages (Kiyma et al., 2000; D'Occhio et al., 2001; Janett et al., 2003). Evaluation of the testis tissue from immunocastrated lambs indicated atrophy of the seminiferous tubules and interrupted spermatogenesis. The presence of sperm in the epididymides of the five immunocastrated rams may therefore be attributed to incomplete clearance as a minimum amount of time is required for the epididymides to be free of sperm. Sperm take approximately 40 to 60 days (average 56 days) to form and be transported through the epididymis for ejaculation in rams (Foote, 1978; Gimenez & Rodning, 2007), undergoing maturation within the epididymis between 7 to 14 days (Dacheux & Dacheux, 2014). The difference in the respective testis cut surface colour values may also be an indication of different levels of

spermatogenetic activity occurring in the testes between treatment groups (Lealiifano et al., 2011). Immunocastrated rams had “redder” and “yellower” testis surface colour which is possibly an indication of decreased sperm and fluid within the testis, the presence of which could contribute to the less red appearance of the testes in intact males when cut open.

Sperm concentrations were reduced within four weeks after second vaccination in all immunocastrated lamb samples and thus it is possible that the initial decline in testosterone concentration had an influence on spermatogenesis. Lambs receiving their primary vaccination earliest may thus experience inhibited testes functioning sooner compared to lambs vaccinated at a later stage. Semen quality, sperm concentration and sperm viability therefore need to be investigated over time *ante-mortem*. Establishing when exactly the decrease in testosterone secretion begins to interrupt spermatogenesis may indicate at what time point immunocastrated lambs are considered infertile and can safely be placed in mixed sex flocks.

Although an attempt was made to minimize the time between slaughter and sperm viability measurement, the time between animal death and sperm sampling contributed to sperm cell death, which potentially is responsible for the lack of differences in sperm viability between treatments. Thus, semen should be collected *ante-mortem* to improve the accuracy of sperm viability determination after immunocastration.

5.6 Conclusion

Improvac® can successfully be used in ram lambs to decrease testosterone production and testicular functioning over a flexible vaccination interval ranging from four, three or two weeks between the primary and secondary vaccination. Although the intervals between the primary and secondary vaccination were successful in suppressing reproductive functioning, the period between second vaccination and slaughter may have a more pronounced influence on the

overall impact of the technique. Further research is required into the effects of the interval between second vaccination and slaughter regarding the duration of testosterone suppression, reproductive capacity, fat deposition and carcass traits. The need to investigate intervals between secondary vaccination and slaughter is further motivated by the desire for flexible vaccination schedules that can be used in various production systems used in lamb meat farming, while ensuring efficient suppression of reproductive functioning. The influence of the lower testosterone concentration on sexual and aggressive behaviour in immunocastrated ram lambs also needs to be quantified.

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CHAPTER 6

Growth, scrotal circumference and behaviour of immunocastrated lambs, as influenced by administration interval between second vaccination and slaughter

Abstract

The study compared the growth rate, testes size, and behaviour of immunocastrated lambs, rams physically castrated with a Burdizzo clamp, and intact ram lambs Dohne Merino ram lambs (n = 40; average live weight = 45.4 ± 3.68 kg) were randomly allocated to the four treatment groups, control (intact; R), Burdizzo-castrated (B; on Day 2), immunocastrated with a four-week (ICS4), or a six-week (ICS6) interval between the second Improvac® vaccination and slaughter. Within the immunocastration treatments, the reaction to vaccination on the shoulder was assessed through injection site scoring, recording the local injection site temperature, and assigning a forequarter walking score. The influence of the Burdizzo castration was assessed by assigning a procedure reaction score, a testes palpation score, a hindquarter walking score, and measuring testis temperature. Additional parameters recorded include growth, serum cortisol concentration, scrotal circumference and rectal temperature. Frequency of normal and abnormal behaviours were determined to assess the short-, medium- and long-term post-castration effects. Reactions to immunocastration vaccination included tissue hardening and bruising, with little effect on walking comfort and no effect on site temperature or rectal temperatures. Burdizzo-castration resulted in both palpation and walking discomfort from Day 3 to 8 of the trial, although discomfort lessened by Day 15. Serum cortisol concentrations were elevated in Burdizzo-castrated lambs on Day 3, Day 15 and 29 compared to baseline values on D1 (prior to Burdizzo-castration) indicating physiological stress. Shortly after Burdizzo-castration, there were higher incidences of standing abnormally compared to other periods. Immunocastration improved the welfare of castrated lambs as assessed by cortisol secretion, scrotal swelling and short-term behaviour, without influencing growth.

6.1 Introduction

In lamb meat producing enterprises, the castration of rams aids in fattening during the finishing phase, and minimises the incidence of aggressive behaviour that impact negatively on production. In extensive production systems, castration is common practice where rams may reach puberty prior to slaughter. Physical castration in lambs can be carried out using bloodless castration methods that involves the placement of an elastrator ring at the top of the scrotum preferably shortly after birth (Baird & Wolfe, 1998), or closed-crushing with an emasculator (Burdizzo) when late castration is required. Negative side effects of these two methods include infection and chronic pain when rubber rings are used, while the less successful Burdizzo technique results in high levels of acute stress (Hosie et al., 1992; Melches et al., 2007)). Thus, methods for improving the welfare of rams castrated for meat production purposes are required. According to Melches et al. (2007), anaesthesia and analgesics may be used, but this approach does not eliminate the pain response to castration. Immunocastration of lambs potentially presents an opportunity to prevent castration-related pain and the accompanying loss in production.

Intervals of two, three and four weeks between first and second vaccination using Improvac® in Dohne Merino ram lambs were equally effective in suppressing reproductive functioning (Chapter 5) without negatively influencing growth and slaughter performance (Chapter 4). However, the interval between second vaccination and slaughter may have a greater effect on the performance of immunocastrated lambs. In Chapter 5, a shorter vaccination interval was preferred in terms of fast androgen suppression and thus it was chosen as the inter-vaccination period for this trial which investigates both a six and four-week interval between second vaccination and slaughter. The commercially recommended pre-slaughter booster vaccination interval for Improvac® in swine is between four and six weeks and although

it factors in boar taint clearance time, it is effective in suppressing testosterone production (Lealiifano et al., 2011).

Although the influence of immunocastration on lamb production and reproduction has been compared to intact male rams, to the best of our knowledge, no study has compared the use of immunocastration in rams to closed-crushing or Burdizzo castration. Elastrator bands cannot be stretched over the larger testes in older animals and thus closed-crushing castration is generally preferred for older or larger animals. However, lambs over 10 weeks of age showed an immediate pain response in terms of behaviour and cortisol secretion after Burdizzo castration (Melches et al., 2007). Behavioural studies involving immunocastrated ram lambs focused predominantly on sexual behaviours (Kiyma et al., 2000) and not the frequency of normal behaviours or stress behaviours. Quantifying the stress responses and behavioural reactions to the two castration techniques may motivate the use of immunocastration for potential to improve animal welfare. Thus, the reaction to both immunocastration and Burdizzo-castration in lambs was investigated in this study to elucidate their respective and relative effects on cortisol secretion, behavioural responses and growth.

6.2 Materials and Methods

Ethical clearance was obtained from the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00073) and animal husbandry was in accordance specifications of the South African National Standards 10386: 2008.

6.2.1 Animals, feeding & experimental design

The study was conducted at the Welgevallen Sheep Research Section of Stellenbosch University (Western Cape Province, South Africa). Forty Dohne Merino (dual-purpose;

medium maturity type) ram lambs were stratified according to initial weight (~ 6.5 months of age; 45.4 ± 3.68 kg live weight), and randomly allocated to one of four treatment groups, with 10 animals per treatment group. Treatments included a group of immunocastrated lambs, receiving their second vaccination four weeks prior to slaughter (ICS4); lambs receiving their second immunocastration vaccination six weeks prior to slaughter (ICS6); a Burdizzo-castrated group (B) and an intact male group (R, control).

The duration of the growth trail was 57 days. The sheep grazed on kikuyu pasture during the day with *ab libitum* access to water. At 4 pm daily, the sheep were housed indoors on wooden slatted floors to avoid stock theft, with lucerne (*ad libitum*) and a commercial grower feed provided (approximately 16.4 MJ/kg gross energy; 120 g/kg crude protein; 23.9 % derived from urea) at 500g per sheep per day.

6.2.2 Immunocastration vaccination & physical castration protocols

The interval between first and second vaccination is flexible in terms of production and slaughter performance when Improvac® is used on ram lambs at 5.5 months of age (Chapter 4). However, a shorter interval tended to be preferred in terms of decreased testosterone secretion and cortisol concentrations (Chapter 5) and for this trial, a minimum period between vaccinations of two weeks was used. Immunocastrated rams received a total of two doses of 2mL Improvac® (Reg. no. G3643, Act 36/1947, Zoetis Animal Health) per ram, each administered two weeks apart, with the second vaccination being either six (ICS6) or four (ICS4) weeks prior to slaughter (Table 6.1). Thus, ICS6 were vaccinated on Day 1 (D1) and D15 of the trial, and ICS4 were injected on D15 and D29.

The vaccine was injected subcutaneously on the shoulder blade area, the first 2 mL dose given on the left side, and the second dose given on the right side of the animal two weeks

later. Prior to vaccine administration, a 10 x 10 cm area on each shoulder blade was trimmed free of wool (Figure 6.1) to allow for the scoring of any injection site reactions, and then disinfected with a 70 % ethanol solution. Each 2 mL vaccine dose was administered in 0.5 mL "sub-doses" and administered at each corner of the wool-free square to ensure as best possible a uniform dispersal of the vaccine. Vaccine was administered using a sterile disposable 1.27 cm 20-gauge needle per animal to prevent contamination. Intact controls received no placebo vaccination.

Table 6.1. Timeline of activities indicating vaccination schedules used for immunocastrated lambs, timing of Burdizzo castration and the subsequent measurements.

Action	**Day**														
	1	**2**	**3**	**5**	**8**	**15**	**17**	**19**	**22**	**29**	**31**	**33**	**36**	**43**	**50**
Injection ICS6:	X					X									
Injection site scoring	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Forequarter walk score	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Injection ICS4:						X				X					
Injection site score						X	X	X	X	X	X	X	X	X	X
Forequarter walk score						X	X	X	X	X	X	X	X	X	X
Burdizzo castration:		X													
Reaction score		X													
Testes palpation score	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Hindquarter walk score	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Temperatures:															
Skin	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Rectal	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Scrotal	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Scrotal circumference	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Weighing	X				X	X			X	X	X	X	X	X	X
Blood collection	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Observations		X	X	X	X	X	X	X	X	X	X	X	X	X	X

ICS6 = lambs receiving second vaccination 6 weeks before slaughter
ICS4 = lambs receiving second vaccination 4 weeks before slaughter

Figure 6.1. The square area on the shoulder blade which was trimmed free of wool for injection of immunocastration vaccine. Injections were done on each of the four corners (marked X) to disperse the vaccine over a larger surface area and prevent subcutaneous accumulation.

The physical castration treatment (B) involved the closed-crushing castration of 10 lambs on D2 of the trial, using a Burdizzo clamp. The oral use of the non-steroidal anti-inflammatory drug (NSAID) Meloxicam® has been successful in reducing the frequency of abnormal behaviours in knife-castrated lambs at 7 to 10 weeks of age (Small et al., 2014). Thus, prior to castration, each lamb was injected with Metacam® (0.25 mL of 20 mg Meloxicam/mL per 10 kg body weight) according to veterinary recommendations. Lambs were placed in a recumbent position and the Burdizzo clamp was applied to the scrotal neck of each testis for 30 seconds (Figure 6.2). The application of the Burdizzo clamp to the second scrotal neck was done distally (~ 0.5 cm) to the first, with both the first and second applications being distal to the nipples. The scrotal area was then sprayed with Necrospray (Oxytetracycline hydrochloride 40 mg; gentian violet 4 mg; Bayer© Animal Health) and Supona™ (Chlorfenvinphos 0,48 % m/m; dichlorphos 0,74 % m/m; gentian violet 0,145 % m/m; Zoetis™ Animal Health). Subsequently, three doses of Metacam® were administered to the Burdizzo-castrated group at three-day intervals.

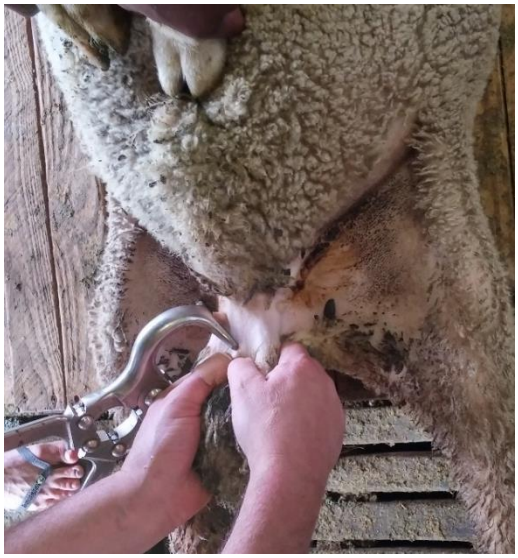

Figure 6.2. The physical castration procedure, using the Burdizzo clamp, applied to the ram while ensuring both testes are within the scrotum with the ram in the vertically recumbent position.

6.2.3 Assessment of bodyweight, temperature, injection site reaction & scrotal circumference

All sheep were weighed weekly using a livestock scale (Model SI2963, Scales Incorporated, South Africa accurate to 200 g). Maximum scrotal circumference, scrotal surface temperature, skin surface temperature on the clean sheared shoulder blade (Alla France infrared thermometer, accuracy: ± 2 °C) and rectal temperature (Vet Thermometer, Kruuse, Denmark, range: 32 – 44 °C, accuracy: ± 0.10 °C) were measured following the outlay specified in Table 6.1. The injection site reaction of immunocastrates was scored (Table 6.2) on a system adapted from Pauly et al. (2009) and Chapter 4 (4.2.1) following the programme in Table 6.1. The maximum testes circumference was measured as performed in Chapter 3 (3.2.1) with the sheep in the standing position, and placing a flexible plastic tape measure around the widest axis of both testes, ensuring both testes were descended into the scrotum. The surface temperature was measured on the left testis, by parting the wool to expose the skin surface and placing the infrared thermometer flush with the skin.

Table 6.2. Immunocastration injection site scoring system used to describe the vaccination after primary and secondary administration of Improvac®.

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; > 1cm diameter	Moderate	Moderate; > 1cm 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

6.2.4 Recording of behavioural responses

During the Burdizzo castration procedure, the immediate lamb reaction was scored according to Melches et al. (2007) for the duration of the procedure. Reactions while being castrated were categorised as no response (score = 0), moderate response with wriggling (score = 1) or severe response with kicking, struggling and vocalisation (score = 2). After the routine scrotal measurements, the Burdizzo-castrated lambs were assessed for pain responses to testes palpation of the scrotum, avoiding the tissue area to which the closed-crushing clamp was directly applied. Lamb responses to palpation were either no response (score = 0), moderate response of wincing and wriggling (score = 1) or severe response of struggling and attempting to escape (score = 2) as defined by Melches et al., (2007). Palpation measurements were done for the first week of the trial (D1, D3 & D5) and then weekly (Table 6.1). A forequarter walking score (FQWS) was given to each immunocastrate after temperature and scrotal measurements. In contrast, the Burdizzo castrated lambs were given a hindquarter walking score (HQWS).

Both immunocastrated and Burdizzo-castrated lambs were categorised into normal walking gait (score = 0), slight stiffness in gait (score = 1), clear limp or compensation (score = 2) or reluctant to place any weight on limb(s) (score = 3).

The influence of immunocastration and Burdizzo castration on behaviour and posture was assessed according to Molony et al., (2002) as defined in Table 6.3. Immediately after Burdizzo castration, lambs were separated and placed into one of four pens according into their respective treatment groups. Lucerne and water were available *ad libitum*. The first observation period started one hour after the Burdizzo castration procedure on D2 of the trial and continued for five hours from 11am to 4pm (short-term behavioural response). Recordings lasted a total of 10 minutes each for each hour that the animals remained in their respective groups. Observations were recorded from outside of the pens, without disturbing the activities of the lambs.

Table 6.3. Description of the various behavioural and postural observations recorded during the 57-day growth trial of immunocastrated, Burdizzo-castrated and intact ram lambs.

Parameter	Description
Eating	Normal ingestion of lucerne.
Standing	Standing with weight on all limbs without showing signs of discomfort or abnormalities.
Lying	Lying on sternum and abdomen with legs tucked in.
Walking	Normal gait.
Drinking	Normal ingestion of water.
Standing abnormally	Easing quarters, foot stamping, statue-standing > 10 seconds and standing with head in a corner of the pen.
Lying abnormally (ventral)	Lying on sternum with hindlegs extended or dog sitting (keeping scrotal region off the ground).
Lying abnormally (lateral)	Lying on side with one or both forelegs extended and both hindlegs extended.
Walking abnormally	Walking unsteady, hunched or swaying; limping; walking with hindlegs apart.
Lying and kicking	Abnormal lying posture and kicking with hindlegs.
Headbutting	Initiates headbutt with another animal.
Mounting	Mounting the hindquarters of another animal.

Within each 10-minute recording period, the number of animals per treatment exhibiting one of the behaviours or postures described in Table 6.3 was recorded every two minutes. The total number of occurrences of a certain behaviour or posture within the 10-minute period was expressed as a percentage of the total of all behavioural parameters recorded within that group for the session to establish its frequency. Animals were also separated into groups, as described, for a 10-minute observation period on D3, D5 and D8 at 11am for the first week of the trial (medium-term behavioural response), followed by weekly measurement (long-term behavioural responses; Table 6.1).

6.2.5 Blood collection & determination of cortisol concentrations

Blood samples were collected from the jugular vein into 6 mL Z Serum Clot Activator Vacuettes® according to the schedule indicated in Table 6.1. Serum cortisol concentrations were determined to establish the effect of castration treatment on the stress hormone using the ultra-performance convergence chromatography tandem mass spectrometry methodology described in Chapter 3 and under conditions indicated by Quanson et al. (2016).

6.2.3 Statistical analysis

Abnormal postures were analysed separately for the short-term period (standing, lying and walking abnormally) but also pooled and analysed over the short-term, medium-term and long-term. Normality of residuals and homogeneity was tested using STATISTICA 13 (StatSoft Inc.), before the Variance Estimation, Precision and Comparison (VEPAC) procedure was followed to determine treatment effects using mixed model repeated measure analysis of variance (ANOVAs) for weight gain, scrotal circumference, temperature measurements, behaviour, reaction scores and cortisol concentrations over the study period. Initial weight was

considered by stratifying body weights initially before the random allocation of lambs to one of four treatments. Fishers LSD was used to compare treatment means and significant differences are reported at 5 %.

6.3 Results

6.3.1 Reaction to immunocastration vaccination

The primary vaccinations for both ICS4 and ICS6 elicited normal injection site reactions with no influence on forequarter walking score (FQWS) for the first week after injection. However, two weeks after primary vaccination, one ICS4 and two ICS6 lambs had hardening of the tissue (induration) at the injection site (score = 2; Table 6.2).

Two days after the secondary vaccination of ICS6 lambs, two lambs (20 %) had mild reactions to the vaccine with slight bruising or contusion (score = 1) and three ICS6 animals (30 %) showed stiffness in their limb on which they were injected (FQWS = 1). Four days after secondary vaccination, the mild reaction recorded on D3 showed tissue hardening. One animal from the ICS6 group had tissue hardening at the injection site for the duration of the growth period, with some tissue hardening in other animals occurring later in the trial (Table 6.4).

Two days after the second/booster vaccination for ICS4, one lamb had bruising (score = 1) and one had tissue hardening (score = 2). At four days after the booster, the number of animals with hardened tissue at the injection site increased to three and then to four animals in the following week. These four animals had tissue hardening for the duration of the study, as also noted for the ICS6 treatment (Table 6.4). However, the hardening tissue did not crack or pull away from the underlying skin for the duration of the trial and no infections occurred. None of the ICS4 lambs showed any stiffness or soreness in their forequarter walking gaits.

Table 6.4. The number of animals per immunocastration treatment (n = 10) which had normal, mild or moderate reactions to the vaccination. Major and severe scores have been omitted as no animals exhibited either of these degrees of reactions throughout the trial. Vaccination administration occurred on D1 and 15 for ICS6 and D15 and 29 for ICS4.

Degree of reaction	Day of Trial																					
	1		3		5		8		15		17		19		22		29		33		36		43		50	
	ICS6		ICS6		ICS6		ICS6		ICS6	ICS4	ICS6	ICS4	ICS6	ICS4	ICS6	ICS4	ICS6	ICS4	ICS6	ICS4	ICS6	ICS4	ICS6	ICS4
Normal (0)	10		10		10		10		9	10	8	10	8	10	9	10	9	9	9	7	8	6	7	6	9	6
Mild (1)	0		0		0		0		0	0	2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Moderate (2)	0		0		0		0		1	0	0	0	2	0	1	0	0	0	1	3	2	4	3	4	1	4

D = day
ICS6 = lambs receiving their second vaccination six weeks before slaughter
ICS4 = lambs receiving their second vaccination four weeks before slaughter

The skin surface temperature fluctuated considerably over time for all the treatments (Figure 6.3). However, the skin temperature of ICS6 remained stable and lower than that of the other treatments from D1 ($P < 0.05$) and D5 ($P < 0.001$). Following the second vaccination for ICS6 and first vaccination for ICS4, both immunocastrated treatments had elevated skin surface temperatures from D17 ($P < 0.001$) to D22 ($P < 0.05$). No differences were seen for rectal temperatures over the trial period (Figure 6.4).

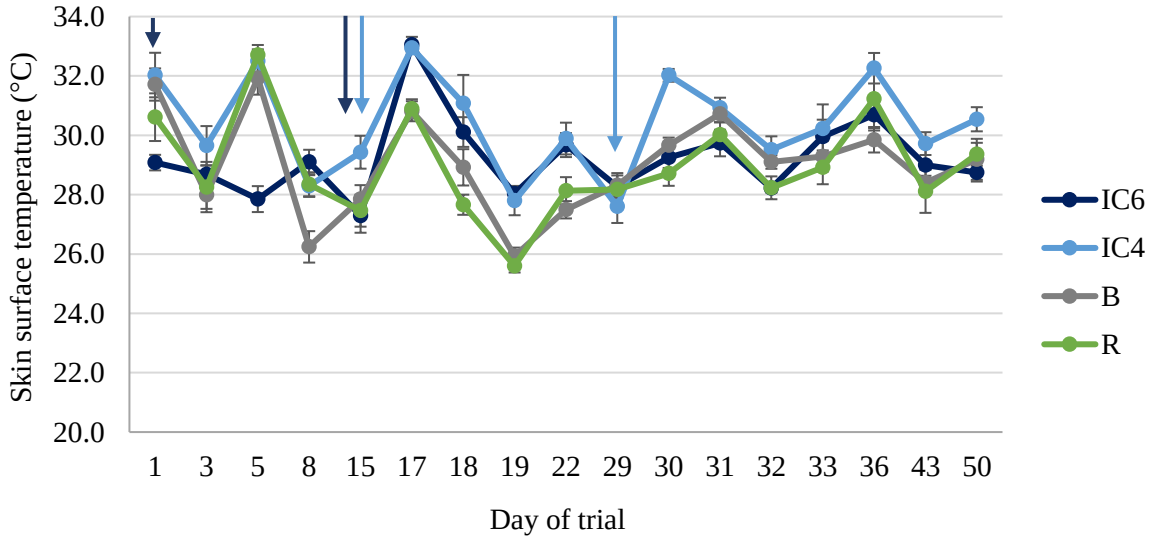

Figure 6.3. Surface skin temperature of lambs immunocastrated at either six (ICS6) or four (ICS4) weeks before slaughter (Day 57), Burdizzo-castrated on D2 (B) and intact rams (R). Vaccinations are indicated by colour-coded arrows. Vertical bars denote SEM.

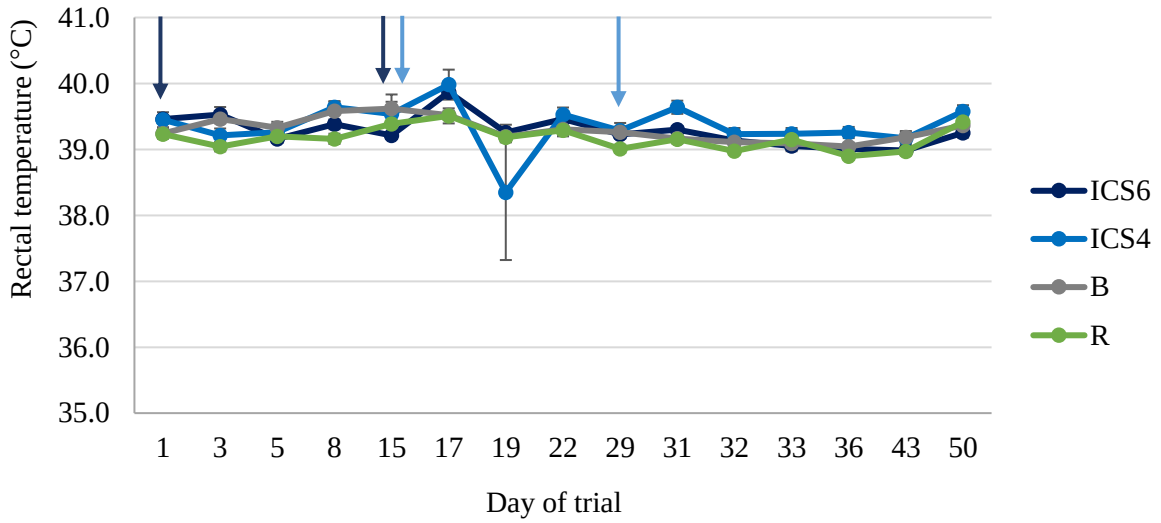

Figure 6.4. The rectal temperature of lambs immunocastrated with varying vaccination intervals of either six (ICS6) or four (ICS4) weeks before slaughter (colour-coded arrows), compared to intact rams (R) and lambs Burdizzo-castrated (B) on Day 2. Vertical bars denote SEM.

6.3.2 Immediate pain response during castration, palpation & hindquarter walking score

Only one lamb which was Burdizzo-castrated had a moderate reaction (score = 1) during the castration, compared to the remaining nine lambs that did not show any reaction. Palpation of the testes and hindquarter walking scores (HQWS) on all lambs on Day 1 showed no response/ abnormalities for baseline values prior to castration (Table 6.5). The day following the castration procedure (D3), 50 % of the castrated lambs showed no reaction to palpation, 40 % showed a moderate response, and 10 % showed a severe response. On D3, 70 % of B lambs walked with stiffness in their hindquarters (HQWS = 1) and 30 % had a normal walking gait. The same frequency of palpation responses was recorded on D5 but 80 % of lambs had a HQWS of 1, thus decreasing the number of B lambs with a normal HQWS score to 20 %. The number of non-reaction to palpation increased to 70 % on D8 of the trial, with 30 % still

showing a moderate response. The frequency of normal HQWS increased to 70 % on D8, with only 30 % still walking stiffly on their hindlegs. Two weeks after castration (D15), all B lambs showed no response to palpation (Table 6.5) and had a normal walking gait that persisted throughout the rest of the trial.

Table 6.5. The number of animals per Burdizzo-castrated treatment (n = 10) which showed varying degrees of reaction to testes palpation (P) and discomfort walking with their hindquarters (HQ) after castration on Day 2 of the trial.

Degree of reaction (score)		**Day of Trial**									
		1		**3**		**5**		**8**		**15**	
Palpation	**HQWS**	**P**	**HQ**	**P**	**HQ**	**P**	**HQ**	**P**	**HQ**	**P**	**HQ**
Normal (0)	**Normal (0)**	10	10	5	3	5	2	7	7	10	10
Moderate (1)	**Stiffness (1)**	0	0	4	7	4	8	3	3	0	0
Severe (2)	**Limping (2)**	0	0	1	0	1	0	0	0	0	0
-	**Reluctance (3)**	-	0	-	0	-	0	-	0	-	0

ICS6 = lambs receiving their second vaccination six weeks before slaughter
ICS4 = lambs receiving their second vaccination four weeks before slaughter
HQWS = hindquarter walking score
P = palpation
HQ = hindquarter

6.3.3 Cortisol secretion, behavioural and postural responses to castration

When weekly serum cortisol data is compared, serum cortisol concentration increased ($P < 0.001$) from D8 to D15, decreased ($P < 0.001$) from D15 to D22, and increased ($P < 0.001$) again from D22 to D29 for B lambs (Figure 6.5). However, the serum cortisol concentrations of B were only significantly different to R on D15, and ICS4 and R on D29 ($P < 0.001$). On D36, ICS6 had higher cortisol concentrations than R and ICS4 ($P < 0.001$). There were no differences between the last two weeks of the growth trial for all treatments.

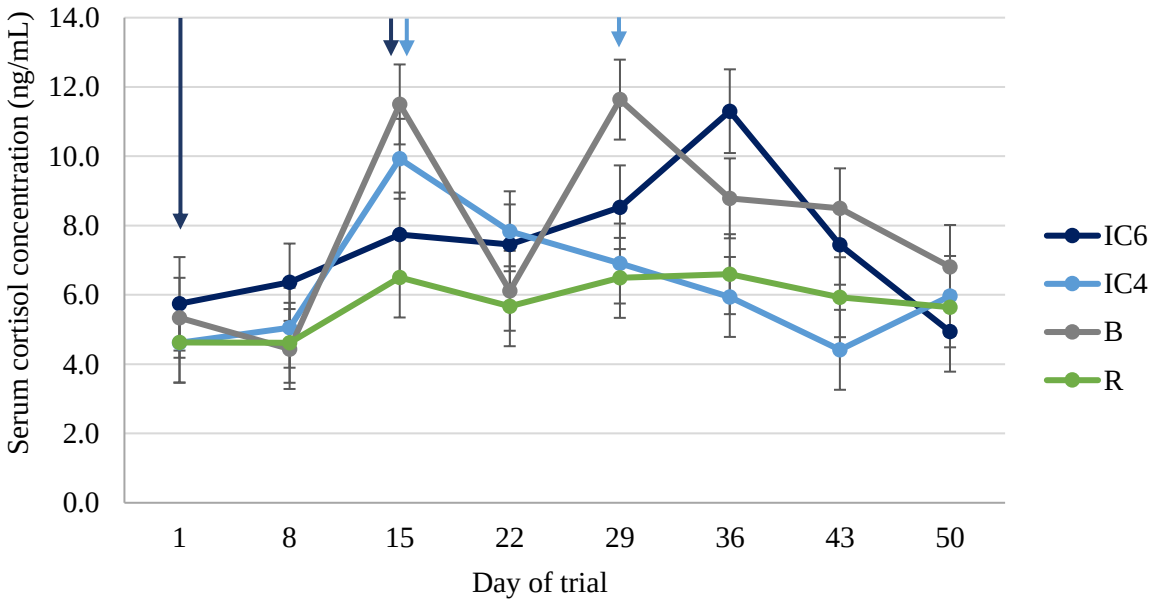

Figure 6.5. Serum cortisol concentrations (ng/mL) for immunocastrated (IC), Burdizzo-castrated (B) and intact rams (R). Arrows indicate the primary and secondary vaccinations for respective treatments. Secondary vaccination intervals include six (ICS6) or four (ICS4) weeks before slaughter. Vertical bars denote SEM.

However, when the intensive blood collection period is evaluated from D1 to D8 (Figure 6.6), Burdizzo-castration and primary vaccination of ICS6 increased serum cortisol concentrations from D1 to D3 ($P < 0.001$; $P= 0.02$). The B lambs had the highest ($P < 0.001$) serum cortisol concentrations on D3, followed by ICS6 which had elevated cortisol concentrations compared to R ($P = 0.03$). However, by D5, cortisol concentrations had decreased, and treatments did not differ from each other. The intact rams showed no changes in serum cortisol concentrations during the first intensive sampling period from D1 to D8.

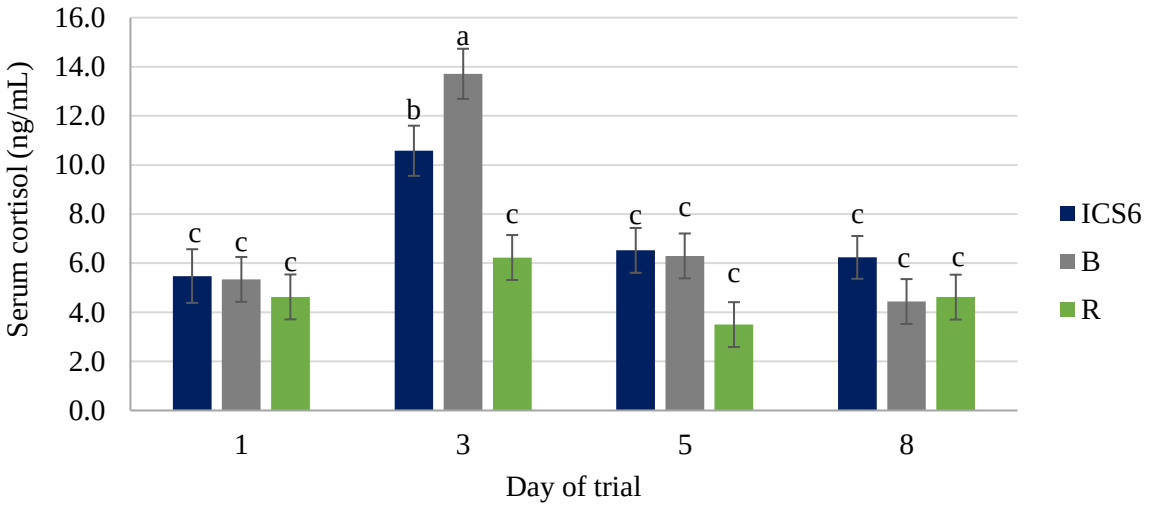

Figure 6.6. The average (± SE) serum cortisol concentrations (ng/mL) of lambs receiving their first immunocastration vaccination at D1 (ICS6), lambs Burdizzo-castrated on D2 and intact rams (R) for the intensive blood sampling period (D1-8). Letters indicate significant differences between means at a significance level of 5 %.

Intensive blood sampling from D15 to D22 indicated no differences in serum cortisol concentrations between treatments on D15, the day of secondary vaccination administration for ICS6 and primary vaccination administration for ICS4 (Figure 6.7). Although, serum cortisol concentrations did not differ between immunocastrated treatments and the intact rams, concentrations were higher in ICS6 than ICS4 ($P = 0.01$) on D17, as cortisol concentrations declined from D15 to D17 for ICS4 ($P = 0.01$). Subsequently, ICS6 cortisol concentrations declined ($P < 0.001$) from D17 to D19 but were not different to those within R lambs. The ICS4 lambs had lower ($P = 0.04$) cortisol concentrations than R on D19 but no different to ICS6. The ICS4 lambs experienced an increase in cortisol concentrations from D19 to D22 ($P =0.02$) and no differences were seen between treatments for D22. The R lambs showed no significant change in serum cortisol concentrations within the second intensive sampling period again. The last intensive sampling period was from D29 to D36 following the second vaccination for ICS4 and showed no differences for the duration of sampling (Figure 6.8).

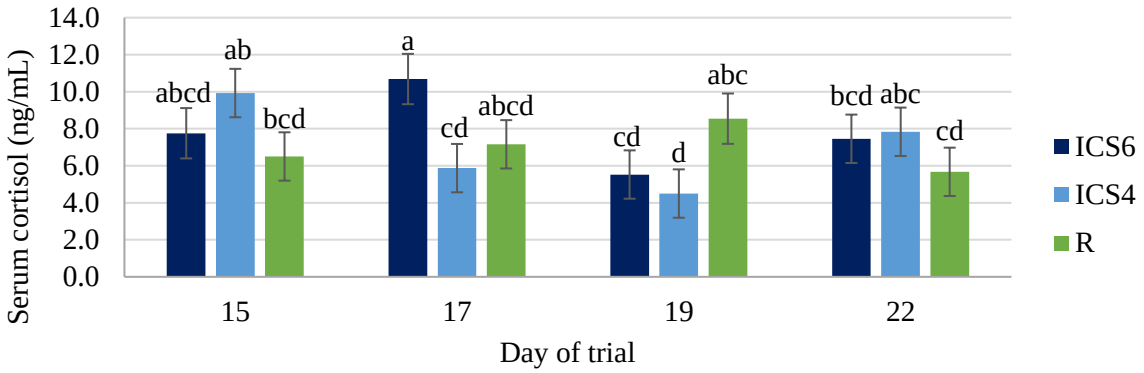

Figure 6.7. The average (± SE) serum cortisol concentrations (ng/mL) of lambs receiving their second immunocastration vaccination at D15 (ICS6), lambs receiving their first immunocastration vaccination at D15 (ICS4) and intact rams (R) for the intensive blood sampling period (D15-22). Letters indicate significant differences between means at a significance level of 5 %.

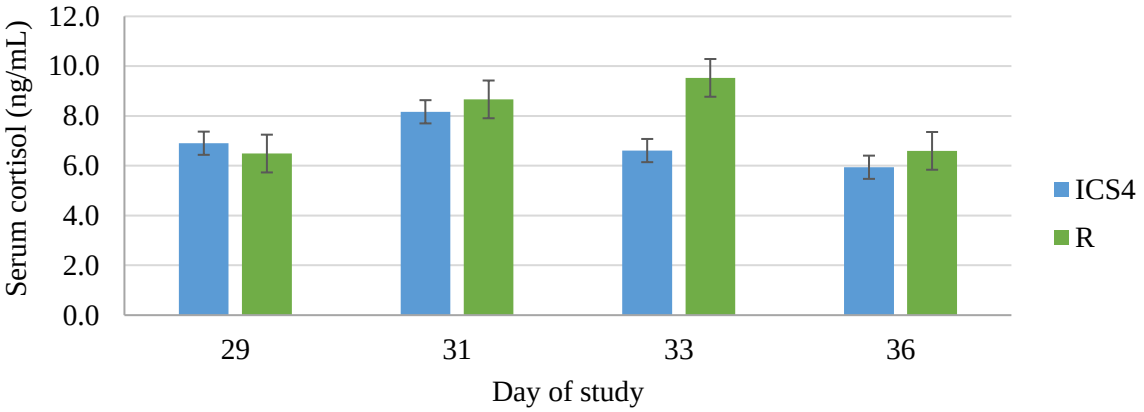

Figure 6.8. The average (± SE) serum cortisol concentrations (ng/mL) of lambs receiving their second immunocastration vaccination at D29 (ICS4), and intact rams (R) for the intensive blood sampling period (D29-36).

The day after first vaccination of ICS6 (D2), vaccinated lambs spent the most time ($P < 0.001$) lying down normally (Figure 6.9). On this day (D2), lambs were Burdizzo-castrated and exhibited a higher frequency of abnormal postures ($P < 0.001$) and the least walking and drinking behaviour (Table 6.5). Most of this abnormal behaviour being described as abnormal standing (Table 6.3).

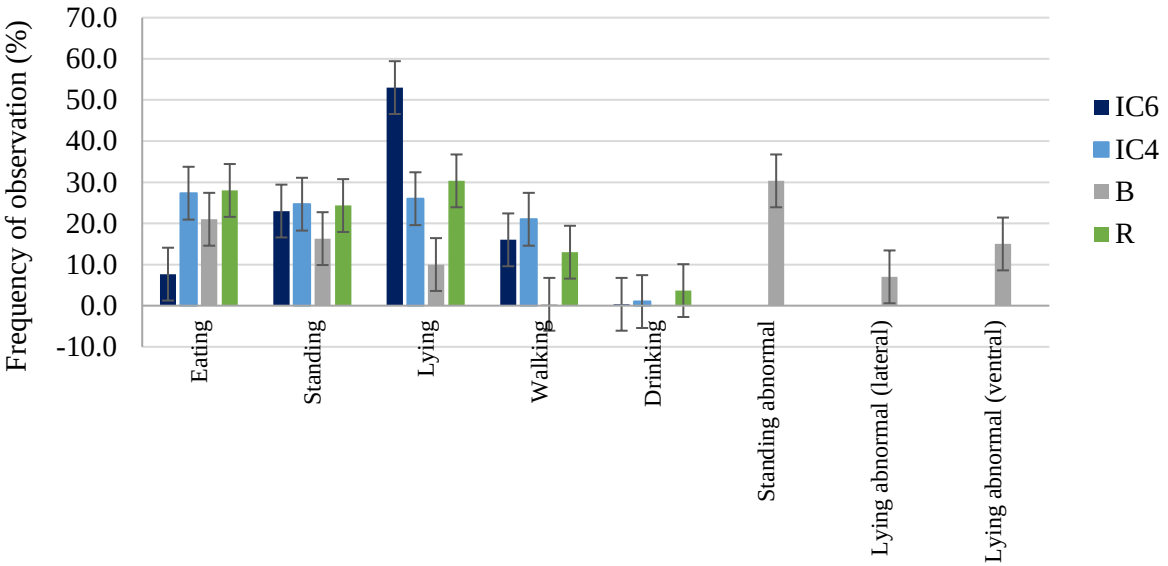

Figure 6.9. Frequency of behaviours and postures observed for lambs immunocastrated at four (ICS4) or six (ICS6) weeks before slaughter, Burdizzo-castrated (B) and intact rams (R) over the short-term period after physical castration (Day 2). Vertical bars indicate SEM.

The incidences of abnormal behaviour in B lambs decreased ($P < 0.001$) over the trial period (Figure 6.10). No differences were observed between treatments for the medium-term behavioural responses ($P = 0.96$); however, differences were recorded within behaviour categories ($P < 0.001$). A higher frequency ($P < 0.001$) of eating behaviour was recorded during the medium-term timeframe compared to standing, lying, walking and drinking (Table 6.6). Animals spent more time standing and lying in the medium-term than drinking ($P = 0.004$), all of which were no different to the amount of time spent walking. The frequency of standing behaviours increased over the long-term period, such that eating, and standing were both the highest recorded behaviours ($P < 0.001$). Again, drinking was the lowest recorded behaviour compared to eating ($P < 0.001$), standing ($P < 0.001$) and lying down ($P = 0.005$) but no different to the time spent walking (Table 6.6).

Table 6.6 Average (±SE) frequency (%) of behaviours exhibited by immunocastrated, Burdizzo-castrated and intact rams after castration on the week post-castration (medium-term) and weekly for the rest of the trial (long-term).

Time after castration	Activity				
	Eating	Standing	Lying	Walking	Drinking
Medium-term	$43.9^{a} \pm 3.95$	$18.9^{b} \pm 3.95$	$18.8^{b} \pm 3.95$	$8.2^{bc} \pm 3.95$	$1.5^{c} \pm 3.95$
Long-term	$39.8^{a} \pm 3.62$	$34.5^{a} \pm 3.62$	$14.9^{b} \pm 3.62$	$8.6^{bc} \pm 3.62$	$0.4^{c} \pm 3.62$

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

Comparison of the total activity over all periods of recording (Figure 6.7) showed a change in the frequencies of certain behaviours over the trial period ($P < 0.001$). Eating behaviours were more frequent over all treatments in the medium and long-term periods than in the immediate period after castration ($P = 0.02$; $P = 0.03$). More lambs spent time lying down during the short-term than the long-term ($P = 0.01$), but an equal frequency was recorded between medium and long-term periods. No changes in walking or drinking behaviour frequencies were recorded for the duration of the trial.

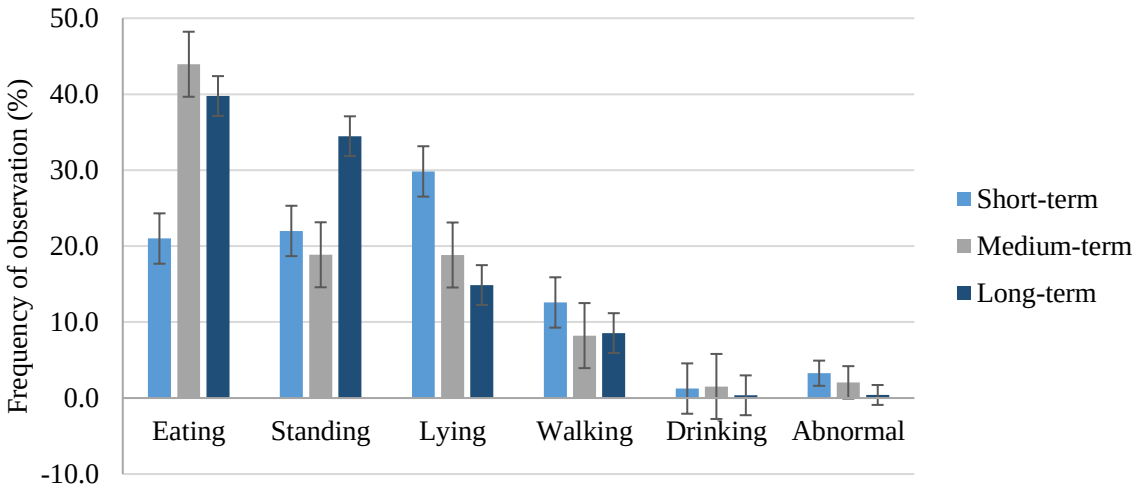

Figure 6.10. Frequency of behaviours and postures observed for immunocastrated, Burdizzo-castrated and intact rams over a 57-day growth period. Observations were performed on the first day of Burdizzo-castration (short-term), the week after castration (medium-term) and once a week for the remainder of the trial (long-term). Vertical bars indicate SEM.

6.3.4 Scrotal circumference & temperature

No differences were seen for scrotal circumference between treatments until D5 (Figure 6.11). The Burdizzo-castrated lambs had larger scrotal circumferences compared to ICS4 (P = 0.018) and R (P = 0.023), due to swelling after the castration procedure which occurred on D2. At D15, the swelling had subsided, and B lambs had smaller scrotal circumferences than all other treatments until D22. However, the scrotal swelling in B lambs did not increase the scrotal surface temperature compared to the other treatments (Figure 6.12).

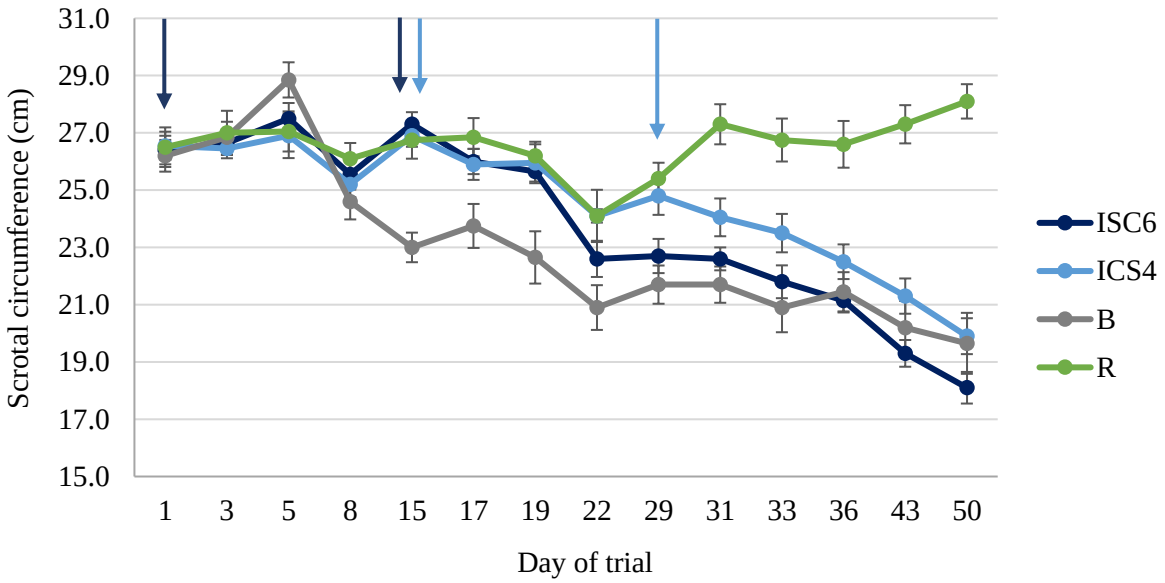

Figure 6.11. Scrotal circumference of immunocastrated (IC), Burdizzo-castrated (on Day 2; B) and intact rams (R). Those immunocastrated were vaccinated with either six (ICS6) or four (ICS4) week intervals between second vaccination and slaughter. Vaccination administration is indicated by arrows. Vertical bars denote SEM.

The scrotal circumference of ICS6 decreased (P = 0.001) from D5 to D8, one week after primary vaccination. However, the scrotal circumference of ICS6 lambs did not decrease further until after the second vaccination. Within a week after the second vaccination, ICS6 had decreased (P < 0.001) scrotal circumferences (D22). From D29, ICS6 lambs had scrotal circumferences no different to B lambs and smaller than both ICS4 (P = 0.017) and R (P =

0.003) lambs. Similarly, from D22 ICS4 lambs experienced a decrease (P = 0.002) in scrotal circumference within a week after primary vaccination. The ICS4 lambs had scrotal circumferences significantly smaller than R from D31 (P < 0.001), within the week after secondary vaccination. The scrotal circumference of ICS4 lambs continued to decrease until all castration treatments no longer differ from one another at D36. Scrotal surface temperatures fluctuated over the trial period, with no treatment differences (Figure 6.12)

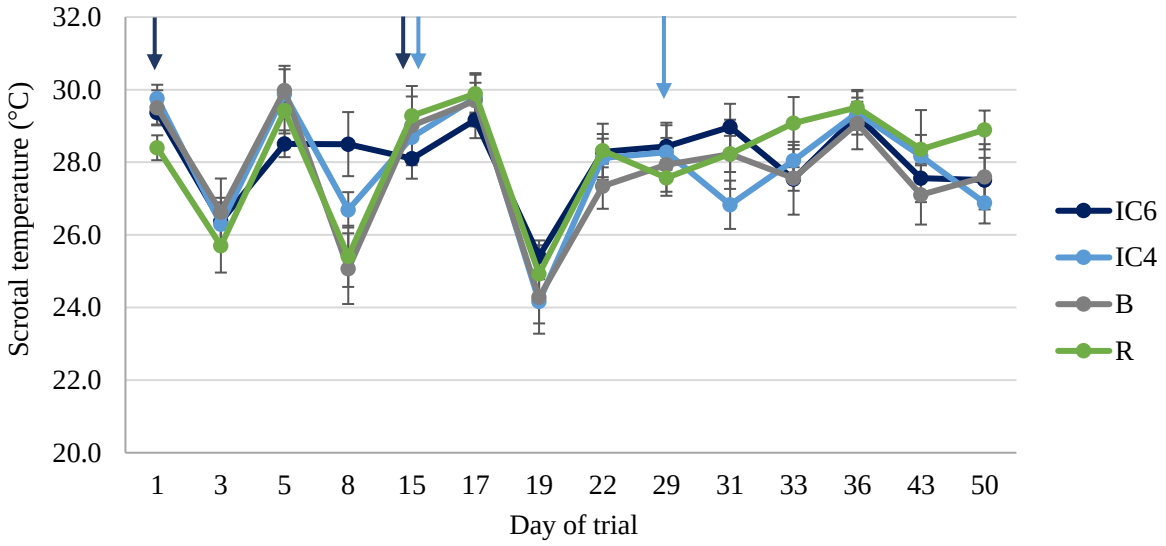

Figure 6.12. Scrotal surface temperature of immunocastrated (IC), Burdizzo-castrated (on Day 2; B) and intact rams (R). Those immunocastrated were vaccinated with either six (ICS6) or four (ICS4) week intervals between second vaccination and slaughter (vaccinations indicated by arrows). Vertical bars denote SEM.

6.3.5 Bodyweight & average daily gain

No treatment differences were observed for live weight over the trial period (Figure 6.13). All treatments followed the same weight gain pattern, resulting in an average live weight of 52.6 ± 4.73 kg at the end of the trial. Although ADG fluctuated within treatment groups, no treatment differences were observed for average daily gain (ADG; g/day) over the duration of the trial (Table 6.7). The ADG decreased during the second week of the trial, followed by a

compensatory spike in ADG, before a steady increase in ADG was realised. The mean ADG for the treatments were 514 ± 61.5 g/day for ICS6, 636 ± 76.1 g/day for ICS4, 495 ± 59.3 g/day for B and 551 ± 65.9 g/day for R.

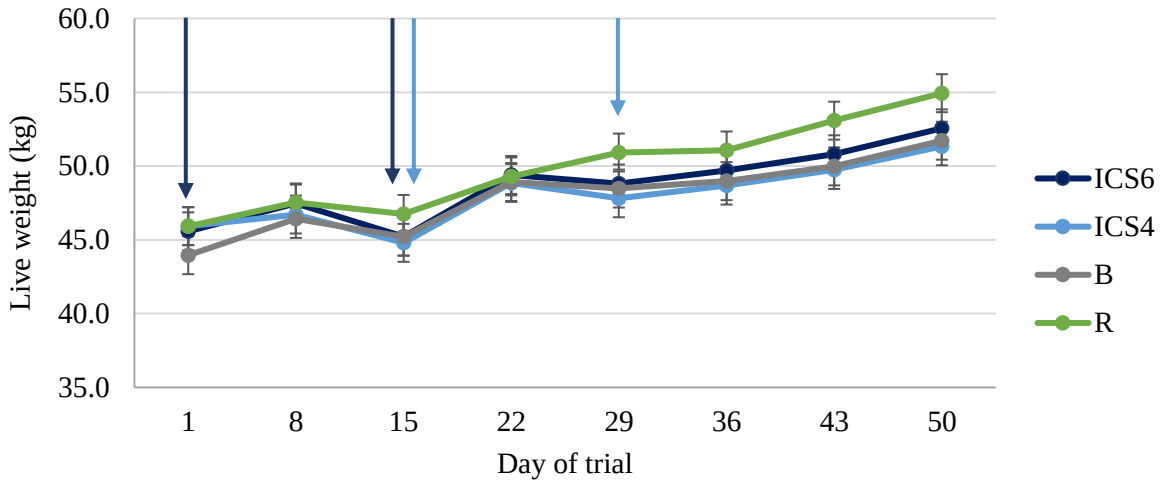

Figure 6.13. Live weights (kg) for Dohne Merino ram lambs immunocastrated with either a six (ICS6) or four-week (ICS4) interval between second vaccination administration and slaughter in comparison to both physically castrated (B) or intact (R) lambs. Primary and secondary vaccinations are indicated by the colour-coordinated dashed. Error bars indicate SEM.

Table 6.7. Average daily gain (ADG, g/day) for the immunocastrated lambs, Burdizzo-castrated (B) lambs and intact ram controls (R) for weekly time periods. Two intervals between second vaccination and slaughter was used for the immunocastrated lambs, namely six (ICS6) or four (ICS4) weeks.

Period	Treatment			
	R	ICS6	ICS4	B
D1-8	$231^{b} \pm 55.9$	$269^{b} \pm 58.6$	$111^{b} \pm 107.6$	$351^{b} \pm 50.4$
D8-15	$-111^{d} \pm 24.6$	$-320^{d} \pm 55.0$	$-274^{d} \pm 31.6$	$-171^{d} \pm 52.9$
D15-22	$363^{a} \pm 220.2$	$597^{a} \pm 265.6$	$580^{a} \pm 313.8$	$529^{a} \pm 248.0$
D22-29	$231^{cd} \pm 253.8$	$-83^{cd} \pm 253.3$	$-149^{cd} \pm 289.9$	$-63^{cd} \pm 262.9$
D29-36	$20^{bc} \pm 71.5$	$126^{bc} \pm 65.6$	$123^{bc} \pm 53.9$	$71^{bc} \pm 44.3$
D26-43	$337^{b} \pm 231.3$	$183^{b} \pm 58.0$	$177^{b} \pm 201.6$	$167^{b} \pm 84.8$
D43-50	$233^{b} \pm 195.9$	$220^{b} \pm 28.8$	$200^{b} \pm 146.9$	$218^{b} \pm 73.0$

[a, b] LSMeans within columns with different superscripts are significantly different ($P \leq 0.05$)
D = day
R = intact ram controls
ICS6 = lambs receiving their second vaccination six weeks before slaughter
ICS4 = lambs receiving their second vaccination six weeks before slaughter
B = lambs physically castrated on day 2 using a Burdizzo clamp

6.4 Discussion

In South Africa, Dohne Merino lambs are typically slaughtered at approximately five months of age (~ 40 kg), dependent on their plane of nutrition. Other than physical castration and tail docking, lambs need only be handled at weaning (three to four months of age), when vaccinating for diseases such as pulpy kidney should be considered before maternal passive immunity completely subsides. An immunocastration vaccination schedule should thus preferably be designed to fit in the time-frame between weaning and slaughter, the latter varying depending on factors such as type of lamb finishing system and quality of nutrition available. Given the relative flexibility regarding lamb finishing, a two-week inter-vaccination interval and a four or six-week interval between second vaccination and slaughter can be considered feasible options for lamb production in South Africa.

The reactions to immunocastration vaccination with Improvac® in lambs in Chapters 4 and 5 motivated the need to reconsider the initial vaccination protocol. The vaccine administration was thus adapted to allow for vaccine administration at four separate injection sites per dose, when administered in the shoulder area. The distribution of the vaccine over four sites resulted in a decreased incidence of adverse reactions; however, changing the injection site to an area free from skin folds where no friction can occur post-administration (as seen in Chapter 4) appeared to have the largest effect. Furthermore, animals could not make contact between the injection site and the ground when lying down, which may have contributed to the incidences of infection in Chapter 4. Most of the side effects observed included tissue hardening (induration) and bruising (contusion). Tissue hardening occurred two weeks after the primary vaccination but within a week after secondary vaccination, likely due to the secondary immune response elicited by the booster vaccination. Hardening of the tissue can also be a consequence of an oil-based adjuvant such as diethylaminoethyl-dextran.

The absence of a change in skin surface temperature at the administration sites can be ascribed to no infection or swelling recorded for the injection sites. Consequently, the fluctuations observed for skin surface temperature can potentially be a result of ambient influences, as discussed in Chapter 4. Rectal temperature remained stable over treatments, with no indication of elevated body temperatures, which may be expected in the case of an immune response in immunocastrates or wound healing complications in Burdizzo-castrates. On average, skin surface temperature on the clean sheared shoulder site (29.4 ± 2.22 °C) was 10 °C lower than rectal temperature (39.3 ± 0.57 °C) throughout the trial. Like scrotal surface temperatures, skin temperature is under greater influence from ambient temperature than rectal temperature, as discussed in Chapter 3.

Although cortisol peaks were observed on D15 for ICS4 and D36 for ICS6, it is difficult to establish a link between the cortisol concentrations and immunocastration reaction site incidences observed, walking discomfort or behaviour as the peaks do not coincide with the reported reactions or discomfort observed. Also, these do not correspond closely to administration of vaccinations as ICS4 received their primary vaccination on D15, but blood was sampled first before any further animal handling. Furthermore, D36 was two weeks after the administration of the second vaccination for the ICS6 lambs. The ICS4 lambs did not follow the same changes in cortisol concentrations as ICS6 during their respective intensive blood sampling after vaccinations. Serum cortisol concentrations increased for ICS6 after primary and secondary vaccination. The initial increase in cortisol secretion early in the trial for ICS6 after primary vaccination but not ICS4 after primary vaccination later in the trial may be attributed to the initial handling stress of the animals at the start of the trial. Vaccinated animals may have been more stressed initially as they were not habituated to the data collection and extra handling for vaccination purpose above that performed the sampling from intact rams.

Although there was an increase in cortisol concentrations again after second vaccination for ICS6, these were not different to those within intact rams, thus further motivating the possible influence of acclimatization to handling on the animals.

Administration of the primary vaccination resulted in a decreased scrotal circumference within a week in both the four and six-week vaccination interval groups. However, a further decrease in scrotal size were only realised after second vaccination again for both immunocastration treatments, as noted within Chapter 4 (4.3.1). Thus, the influence of immunocastration on spermatogenesis needs to be evaluated throughout the growth period to ascertain when the decrease in testicular size begins to influence semen quality and sperm parameters. Sperm parameters that need to be considered include concentration, viability, motility, morphology, and acrosome integrity, with these traits that can potentially provide an indication of the field fertility of the ram. All scrotal circumferences of immunocastrated treatments at the end of the trial where less than 28 cm, which is the acceptable normal scrotal circumference for rams between 8 to 14 months of age (Bedford-Guau, 2016).

Melches et al. (2007) reported a high incidence of moderate responses in Burdizzo-castrated lambs using the same scoring system, despite the use of anaesthesia. The day after castration (D3), the lambs showed discomfort to testes palpation (50 %) and walking (70 %) as well as elevated serum cortisol concentrations (Figure 6.6). Walking discomfort increased within two days post-castration (D5) and so did scrotal circumference (Figure 6.11) indicating scrotal swelling. Serum cortisol concentrations decreased from D3 to D5 but were still higher than that within intact rams, indicating stress. Burdizzo-castrated lambs also showed scrotal swelling for three days after castration using a Burdizzo clamp but applying it for 30 seconds (Melches et al., 2007). A week after castration (D8), pain responses to palpation began to decrease and 70 % of the castrated sheep were walking normally, until two weeks after

castration (D15) when no testes or walking discomfort was recorded. No differences were seen in serum cortisol concentrations between Burdizzo-castrated lambs and intact rams by D8, likely due to the administration of Metcam® not only before, but also after the castration procedure. Metacam® administration was required under the Stellenbosch University ethical guidelines for the handling of the castrated lams and was administered as a pain killer and anti-inflammatory to the Burdizzo-castrated group. It would be expected that without the use of pain mitigation, incidences of discomfort and abnormal behaviour may be increased. Burdizzo-castrated lambs have also been shown to reach baseline cortisol concentrations within six hours after the procedure when anaesthesia was used (Melches et al., 2007). The lambs Burdizzo-castrated by Melches et al. (2007) had painful responses to palpation for a mean of 1.3 ± 1.0 days. Thus, within two weeks after castration, pain score systems indicated that the lambs had healed and recovered from the procedure. Two peaks were seen in serum cortisol concentrations later in the trial for the castrated lambs on D15 and D29, after the scrotal swelling had subsided, possibly indicating physiological stress caused by tissue damage. Scrotal tissue hardening and wool-loss on the testes were also recorded in some of the Burdizzo-castrated animals throughout the study. Thus, tissue histology and serum androgen analyses need to be conducted on Burdizzo-castrated lambs to further investigate the effects on tissue structure and functioning after castration. Scrotal surface temperature (28.0 ± 2.50 °C) did not differ despite swelling and was on average 12 °C lower than rectal temperature (39.3 ± 0.57 °C).

Researchers investigating the effect of immunocastration on sheep behaviour have limited their observations to sexual and mounting behaviours and not normal behaviours or physical activity level (Kiyma et al., 2000; Parthasarathy et al., 2002). The effect of immunocastration using Bopriva® has been investigated in pubertal bulls, showing lower

physical activity in immunized bulls after the second vaccination (Janett et al., 2012). Eating behaviour and physical activity levels are also affected by castration in Holstein bulls, with steers visiting the feeder more often and spending less time lying down within the two weeks after castration (Devant et al., 2012). Although these differences were difficult to interpret over the long-term, castrated cattle showed consistently decreased physical activity (Devant et al., 2012). In swine, physical castration and immunocastration similarly decreased the amount of social behaviour exhibited and increased feeding behaviour in group-housed pigs compared to intact males (Cronin et al., 2003).

An increase in abnormal postures was recorded on the day of Burdizzo-castration. These postures were predominantly abnormal standing, followed by abnormal lying (both ventrally and laterally). Although eating frequency was no different to controls, Burdizzo-castrated lambs spent less time drinking and walking, which could negatively influence their performance in an extensive environment. This contrasts with what Melches et al. (2007) recorded, where Burdizzo-castrated lambs spent less time eating between 2.5 and 9 hours after castration compared to intact controls. However, due to the use of different anaesthesia and pain mitigation products, comparison of the results between studies may be confounded. Furthermore, those Burdizzo-castrated lambs did not show increased incidences of abnormal postures, but did show less active behaviour compared to intact rams within the first day of castration with anaesthesia (Melches et al., 2007). The recovery of Burdizzo lambs in this study improved over the week following castration until no abnormal behaviours or pain responses were recorded. Thus, Burdizzo lambs, immunocastrated lambs and intact rams did not differ for the incidences of observed behaviours and postures during the medium and long-term periods in this study, as defined in Table 6.3. The increase in overall lamb feeding behaviour from the short to medium and long-term periods could be explained by the acclimatization of

the animals to the handling procedures and thus were more relaxed than after the first data collection and handling time point.

Although meta-analysis has indicated that physical castration generally decreases feed efficiency and average daily gain (Sales, 2014), no differences were found in terms of growth between lambs castrated together with local anaesthesia using surgical, Burdizzo and elastrator ring methods and control intact rams 21 days after the procedures (Melches et al., 2007). No differences were reported in Chapter 4, when a two, three or four-week inter-vaccination period was used, with four weeks between second vaccination and slaughter. Similarly, no growth differences were reported between treatments for growth rate in the current study; however, the use of pain mitigation may have influenced the growth of Burdizzo-castrated lambs. The use of recombinant ovalbumin-GnRH vaccines to immunocastrated Karakas ram lambs at 18 and 26 weeks old was successful in interrupting testes growth without influencing growth compared to intact rams (Ülker et al., 2002). However, lambs immunocastrated earlier (10, 14 and 22 weeks old) showed similar rates of gains to physically castrated lambs and decreased feed efficiency and growth compared to intact rams, with a total feeding period to reach slaughter weight being intermediate to the other two sexes (Ülker et al., 2003). Although the timing of immunocastration relative to lamb age influences growth rates, the level of nutrition may also influence the sexual differentiation for growth rates and while this has been investigated in pigs (Boler et al., 2011; Needham et al., 2017), it should be considered in future lamb studies.

6.5 Conclusion

Extending the immunocastration vaccination interval between second vaccination and slaughter does not influence body weight gain and is effective in disrupting testes growth.

Vaccinating immunocastrated lambs subcutaneously in the shoulder area minimized the incidences of reactions at the injection site. However, further investigation into an easy and safe injection system that ensures good hygiene practices when immunocastration is carried out on a commercial scale is warranted. Visual and palpation examination of discomfort in Burdizzo-castrated lambs indicated wound healing and recovery within two weeks after the procedure, without wound healing complications. Serum cortisol concentrations indicated physiological stress experienced by Burdizzo-castrated animals, which potentially indicate necrosis of testes tissue, however the latter can only be confirmed after *post-mortem* evaluation. Shortly after Burdizzo-castration, more incidences of abnormal standing behaviour were exhibited, likely due to pain and discomfort experienced in the Burdizzo-castrated treatment. Although pain mitigation may have improved the discomfort and growth performance of Burdizzo-castrated lambs, it is highly unlikely that farmers would consider handling their lambs as intensively for its administration in the short-term period after castration. Despite minor injection site reactions, immunocastration offers an alternative approach to physical castration, resulting in the improved welfare of castrated lambs, which may be realised in carcass characteristics.

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CHAPTER 7

Changes in the reproductive capacity of immunocastrated ram lambs after extending the slaughter interval between second vaccination and slaughter

Abstract

The effect of extending the interval between second immunocastration vaccination (Improvac®) and slaughter from four to six weeks on the reproductive capacity of Dohne Merino lambs was examined. Forty Dohne Merino lambs were stratified according to initial weight (45.4 ± 3.68 kg; ~ 6.5 months old) and randomly assigned to four treatments that included intact rams (R), Burdizzo-castrated lambs (B), and lambs immunocastrated with four (ICS4) or six (ICS6) weeks between second vaccination and slaughter. Blood and semen samples were collected throughout the trial period to determine testosterone concentrations, evaluate semen quality and assess sperm viability. Semen colour scores varied throughout the trial, but mass motility scores decreased for the immunocastrated lambs. Burdizzo castration resulted in low testosterone concentrations as well as sperm concentration, motility and viability throughout the collection period. However, an increase in percentage live sperm in lambs castrated with the Burdizzo at the end of the trial period indicated that the technique can be ineffective in suppressing spermatogenesis. Semen samples from intact rams showed improvement over the trail, indicating that lambs are pubertal but not sexually mature. Lambs immunocastrated with a six-week interval showed a consistent and continuous decline in testosterone concentrations and sperm viability, with increased dead abnormal sperm in semen samples at the end of the study. The four-week interval was successful in interrupting reproductive functioning; however, not as consistently as the six-week interval. Primary immunocastration vaccination influenced testosterone concentrations and negatively influenced testicular structure. Although all castration treatments influenced testes size and colour, the six-week vaccination to slaughter interval caused a greater decrease in testes cut surface L* colour values and in seminiferous tubule circumference. Burdizzo-castration resulted in tissue necrosis and abscessing, explaining previously reported physiological stress and increased cortisol concentrations. Extending the interval between second immunocastration vaccination and slaughter resulted in a more consistent and reliable influence on reproductive capacity, despite all semen samples collected being sub-standard quality.

7.1 Introduction

Immunocastration is considered as a suitable alternative to physical castration in the livestock industry for the finishing of male animals for meat production (Thompson, 2000). The application and effects of immunocastration have been predominantly investigated in swine and cattle, with commercial vaccination protocols and schedules established for both species. However, little has been researched in terms of developing a commercial vaccination schedule to immunocastrate ram lambs. The effective immunocastration of male livestock relies on a vaccination schedule that not only elicits a strong suppressive response in terms of androgen production, but also a sustained response to ensure sustained growth until slaughter. Chapters 4 and 5 reported on findings that indicated that a two, three or four-week interval between first and second Improvac® vaccination and a four-week interval between second vaccination and slaughter for all treatments, interrupted functioning of the reproductive organs in ram lambs. However, the interval between second vaccination and slaughter needs to be flexible considering the use of various production systems, slaughter ages, breeds and unpredictable levels of nutrition, such as natural grazing, in South Africa.

Thus, prolonging the effect of vaccination on reproductive functioning and androgen suppression needs to be investigated before establishing the possible effect on slaughter performance. Extending the interval between second vaccination and slaughter of immunocastrated lambs from four to six weeks decreased scrotal circumferences compared to intact rams, but scrotal circumference at the end of the trial did not differ between immunocastrated treatments (Chapter 6). All immunocastrated lambs had smaller scrotal circumferences within a week after primary vaccination; however, a more pronounced effect was observed two weeks after the secondary vaccination. Furthermore, immunocastrated lambs in Chapter 5 showed a rapid decrease in serum testosterone concentrations after second

vaccination (5.3.1). Thus, it would be beneficial to establish the point at which semen quality and spermatogenesis is affected. Furthermore, the collection and evaluation of sperm from the epididymides in Chapter 5 (5.3.2) proved suboptimal and thus *in vivo* studies need to be considered to properly evaluate the effect of immunocastration on spermatogenesis.

The physical castration method used in Chapter 6 was Burdizzo castration and was chosen because older rams were used for the study, as well as the fact that Burdizzo castration has shown to be the preferred option for lambs older than 10 weeks old (Melches et al., 2007). The efficacy of Burdizzo castration has been questionable though (Hosie, et al., 1992). Therefore, the efficacy of Burdizzo castration need also to be established, relative to the healing period. Furthermore, the fluctuation in serum cortisol concentrations in Burdizzo-castrated lambs (Chapter 6, Section 6.3.3) motivates the evaluation of the testes *post-mortem* to establish possible causes of physiological stress.

The aim of this study was therefore to evaluate the influence of castration method on the semen quality, sperm viability and morphology, androgen secretion over time as well as *post-mortem* testes histology in immunocastrated and Burdizzo-castrated lambs.

7.2 Materials and Methods

Ethical clearance was obtained from the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00073) and animal husbandry was in accordance specifications of the South African National Standards 10386: 2008.

7.2.1 Animals, castration and husbandry

The animals used to collect data for this study formed part of the growth and behaviour study performed in Chapter 6 and thus further details can be found in Section 6.2. Briefly, Dohne

Merino ram lambs (45.4 ± 3.68 kg; ~ 6.5 months old) were randomly allocated to four treatment groups, namely: intact control rams (R), Burdizzo castrated lambs (B), and lambs immunocastrated with either a four (ICS4) or six-week (ICS6) interval between second vaccination and slaughter (Figure 7.1). A two-week interval was used between primary and secondary vaccination of Improvac® for both immunocastrated treatments. Burdizzo castration was performed on Day 2 (D2) of the trial using a Burdizzo-clamp (30 seconds per testes) after Metacam® (20 mg Meloxicam/mL) administration at 0.25 mL/ 10 kg body weight. Sheep were maintained on kikuyu pasture and fed a supplementary commercial lamb finisher pelleted diet at 500 g per lamb per day with Lucerne and water available *ad libitum*.

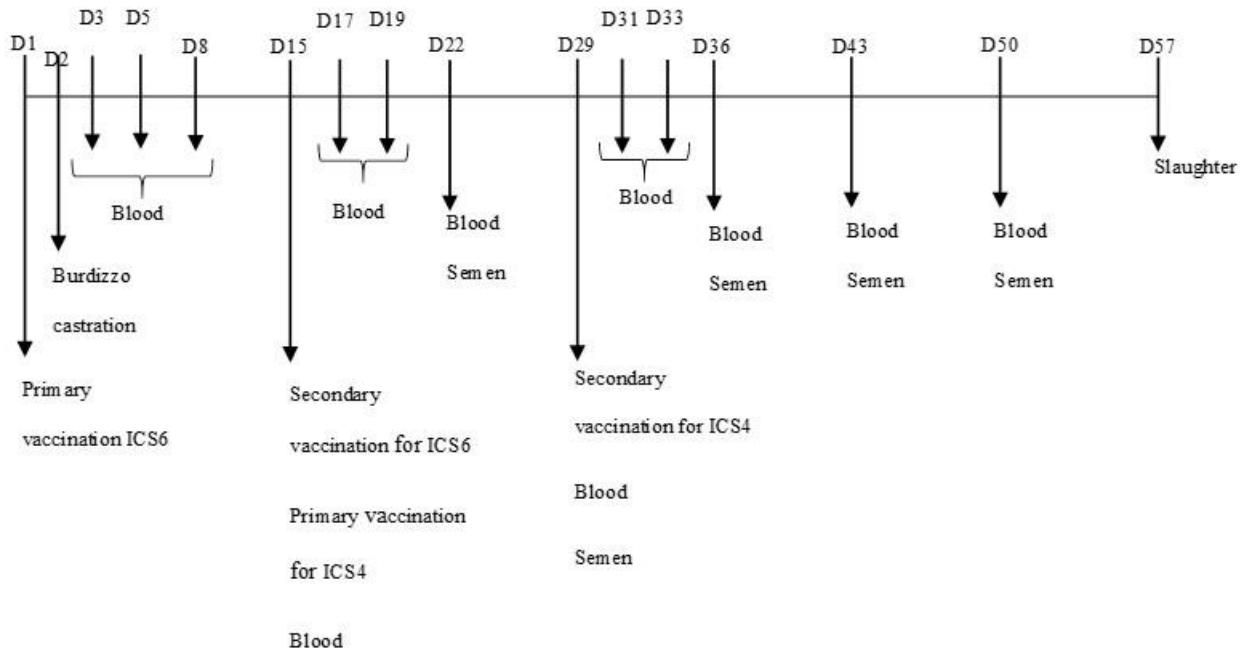


Figure 7.1. Immunocastration vaccination schedule, timing of Burdizzo castration and sample collection timeline for the 57-day growth period involving Dohne Merino lambs (45.4 ± 3.68 kg; ~ 6.5 months old).

7.2.2 Semen collection and quality evaluation

Semen was collected weekly from D15 (Figure 7.1) to allow time for wound-healing of the Burdizzo-castrated animals. Five lambs per treatment were randomly sampled on D15 and semen was collected each week from the same animals using the electroejaculation method. Lambs were placed in lateral recumbency and the prepuce was wiped clean before the penis was exteriorised. The penis was held with a piece of gauze and placed within a sterile plastic collection tube. A manual pulse electro-ejaculator (Bailey, Western Instrument Company, Colorado) probe was inserted into the rectum and the area of the pelvis where the accessory glands and nervous system controlling ejaculation were massaged. During massaging, electrical pulse stimulation was performed for two to three seconds followed by a five second rest, for a maximum of five stimulations per lamb.

Semen samples were analysed immediately after collection between animals. After colour was scored (Table 7.1) according to the system described by Hafez and Hafez (2008), 50 µL of raw semen was pipetted onto a clean microscope slide and viewed using a light microscope (Zeiss, West Germany). Mass motility was accessed and scored according to Table 7.1 (Hafez & Hafez, 2008). Sperm concentration was determined using the haemocytometer method (Rouge, 2004a). Viability was accessed, as described by Rouge (2004b) as well as in Chapter 3 (3.2.4) of at least 100 sperm cells per slide. The number of total alive and total dead sperm were determined, they were then further analysed for normal or abnormal morphology (detached heads, curled tails, cytoplasmic droplets).

Table 7.1. Scoring system used to evaluate the colour and mass motility of semen samples as adapted from Hafez and Hafez (2008) and Ramsem (2017).

Score	Class	Colour	Motility
5	Excellent	Thick, creamy.	Dense, very rapidly moving waves; 90% of sperm are motile.
4	Good	Creamy.	Vigorous wave movement; 70-85% of sperm cells are active.
3	Fair	Thin, creamy.	General but slow-moving waves; 45-65% sperm are motile.
2	Poor	Milky.	Slow movement with no waves; 20-40% poor motility.
1	Very poor	Thin, milky.	Only weak individual movement; only about 10% motile.
0	Unsatisfactory	Watery.	Total immobility.

7.2.3 Blood collection and androgen analysis

Blood samples were collected intensively after vaccination and physical castration, followed thereafter by weekly sampling (Figure 7.1) during the trial period from late winter to early spring (as discussed in Chapter 5). Sampling and serum steroid extraction was performed in accordance to the protocol discussed in Chapter 3 (3.2.2) and Chapter 5 (5.2.2). Androgen analyses of extracted serum samples was performed with ultra-performance convergence chromatography tandem mass spectrometry (UPC^2-MS/MS) using an Acquity UPC^2 system fitted with an Acquity UPC^2 BEH 2-EP (3 mm x 100 mm; 1.7 µm) column (Waters Corporation, USA) as developed by Quanson et al. (2016) and detailed within Chapter 5 (5.2.2). Internal standards used were: cortisol-9, 11, 12, 12-d4 (15 ng per sample), testosterone-1, 2-d2 (1.5 ng per sample) and progesterone-2, 2, 4, 6, 5, 17α, 21, 21, 21-d9 (15 ng per sample; Cambridge Isotope Laboratories, Andover, USA). Steroids evaluated were testosterone (T), androstenedione (A4), 5α-androstanedione (5α-dione), 5α-dihydrotestosterone (DHT) and 11-ketoandrostenedione (11KA4). Androstenone concentration was also evaluated, using 5α-androst-16-en-3-one as a standard for quantification.

7.2.4 Testes histology

At slaughter (52.6 ± 4.73 kg), testes were collected and processed according to Chapter 3 (3.2.4). Measurements included trimmed paired testes weights and cut surface CIE Lab colour (Color-guide 45°/0° colourimeter, BYK-Gardner GmbH, Gerestried, Germany) before testes tissue samples were taken for histology preparation (Bai et al., 2017). The seminiferous tubule circumference and epithelium thickness of 100 seminiferous tubules were analysed per lamb on the haematoxylin and eosin stained slides (40X magnification; Olympus IX70 microscope and Olympus Image Analysis Software, Olympus Corporation Tokyo, Japan). The cauda epididymis was used to collect sperm as soon as possible *post-mortem* and sperm concentration, viability and morphology was assessed as described in Section 7.2.2.

7.2.5 Statistical analysis

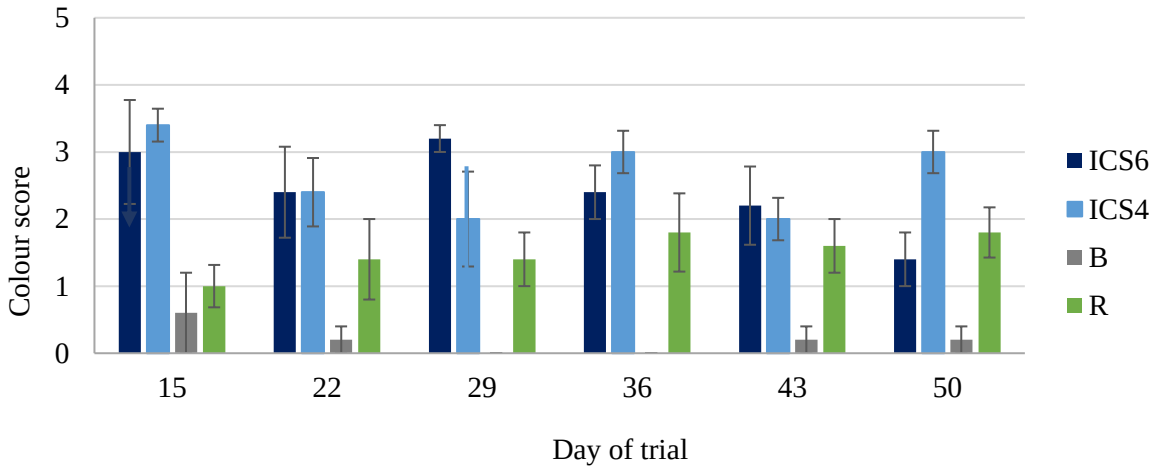
Statistical analysis was performed following the Variance Estimation, Precision and Comparison (VEPAC) procedure in STATISTICA 13 (StatSoft Inc.). The restricted maximum likelihood (REML) method was used to determine treatment differences over the study period (semen quality, sperm quality, serum androgen concentrations). The grouping variables were animal, treatment, day; fixed effects were treatment, day and treatment*day and the random effect was animal. For data collected *post-mortem*, ANOVAs were used to compare treatments (testes cut surface colour, testes histology). Residuals were tested for normality and homoscedasticity using Levene's test. In the case where these assumptions were met, Fishers LSD was the chosen post-hoc test to compare treatment means. When the assumption of homogeneity was not met, Games-Howel post hoc tests were used (testes surface colour). When the assumption of normality was not met, as in the case with sperm concentration, a log

transformation was used. Significant differences were reported as such at a significance level of 5 %.

7.3 Results

7.3.1 Semen quality, sperm concentration and sperm viability

Subjective semen colour scores varied over the trial period between treatments (P = 0.03). The semen samples from ICS6 showed a decline (P = 0.002) in colour score over the study period (Figure 7.2). However, semen colour scores for ICS4 fluctuated over the treatment period, with neither immunocastration treatment scores being different to intact rams at the end of the study. On the final day of semen collection (D50), colour scores for ICS6 were no different to B and



R but on average, lower than ICS4 (P = 0.01). However, semen colour scores for B were lower than both R (P = 0.03) and ICS4 (P < 0.001) and colour scores for ICS4 did not differ from R.

Figure 7.2. Mean (± SE) semen colour scores for intact Dohne Merino rams (R), Burdizzo-castrated lambs (B) and lambs immunocastrated with their second vaccination either four (ICS4) or six (ICS6) weeks before slaughter. Colour-coordinated arrows indicate second vaccination for respective treatments. Sample collection started at 45.5 ± 3.65 kg.

Semen mass motility scores also differed over the study period between treatments (P = 0.001). The mass motility score for R improved over the trial period (Figure 7.3), while scores decreased for both ICS6 and ICS4. However, B scores showed no change over the trial. Motility scores decreased for ICS6 and ICS4 after their respective second vaccinations, with ICS4 reaching lower scores than R on D36 (P = 0.01) and ICS6 reaching lower scores than R on D43 (P < 0.001). Scores for ICS6 and ICS4 semen declined further until at D50, they both no longer differed from the motility scores of B, showing very poor to unsatisfactory semen mass motility.

Sperm concentration was lower (P < 0.001) for B semen than ICS6, ICS4 and R semen samples for the entire growth period. However, ICS6, ICS4 and R semen samples did not differ in sperm concentration. The average sperm concentrations (±SD) were 0.9 x 10^8 ± 17.85 (R), 7.9 x 10^8 ± 5.10 (ICS4), 2.4 x 10^8 ± 80.03 (ICS6) and 146.4 ± 1142.9 (B) sperm cells per mL.

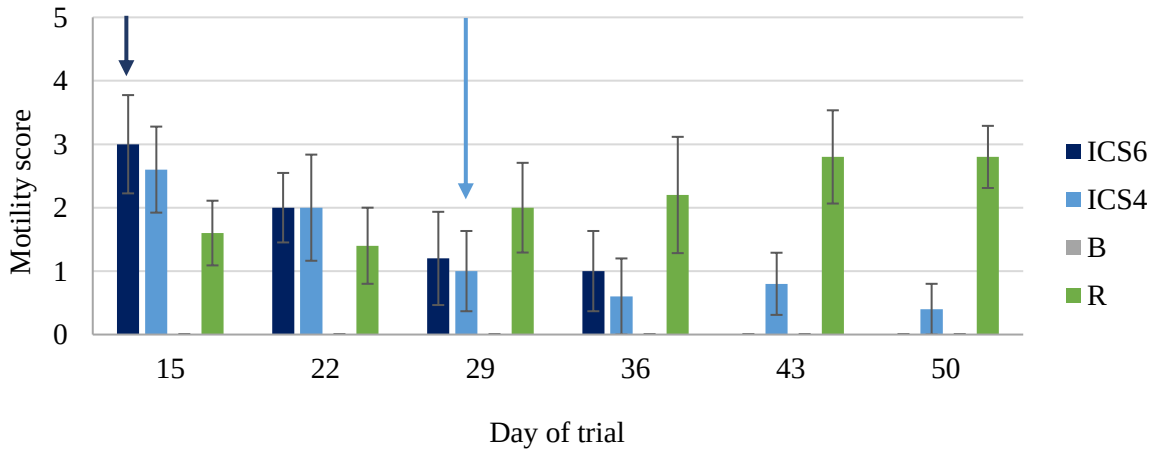
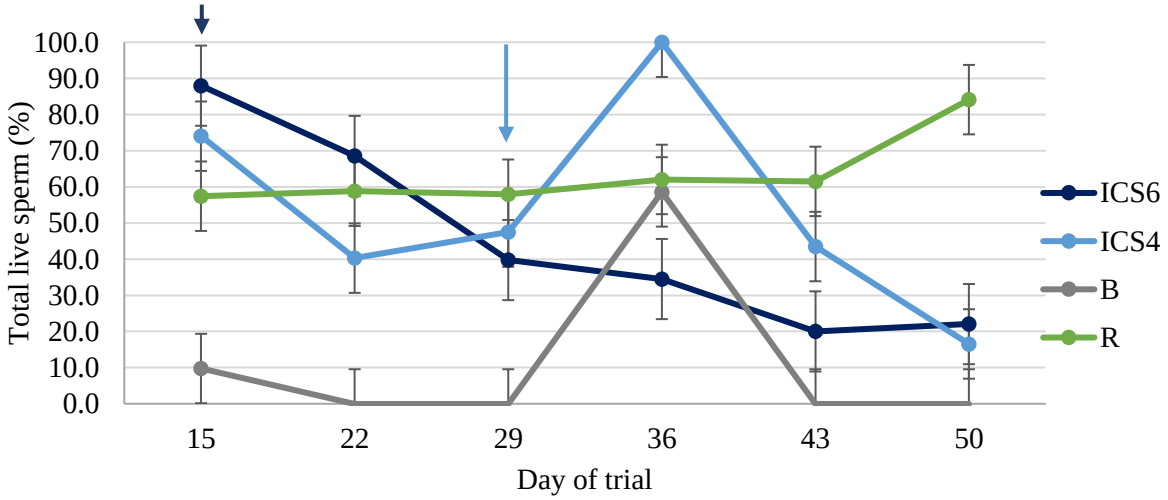


Figure 7.3. Mean (± SE) semen mass motility scores for Dohne Merino lambs immunocastrated at four (ICS4) or six (ICS6) weeks between second injection and slaughter, compared to intact rams (R) and Burdizzo-castrated lambs (B). Arrows indicate timing of second vaccination.

Although sperm concentrations did not differ between immunocastrated and intact rams, the total percentage of alive sperm differed between all treatments over time (Figure 7.4; Table

7.2). From D15 to D36, B lambs had a lower percentage ($P < 0.001$) of live sperm compared to ICS6, ICS4 and R. The ICS6 lambs showed a decrease ($P = 0.03$) in percentage live sperm from D15, until at D43 when they were no different to B lambs. The ICS6 lambs had lower percentages of live sperm than R from D36, three weeks after the second vaccination. The ICS4 semen samples showed an increase ($P < 0.001$) in percentage live sperm on D36, the week after second vaccination, followed by a sharp decrease ($P < 0.001$) until D50 where they no longer differed from B. The ICS4 lambs had lower percentages of live sperm at D43 than R, two



weeks after the second vaccination, while the intact rams showed a constant mean (±SD) percentage of live sperm of 62.9 ± 28.31 % throughout the trial (Figure 7.4). The corresponding means for the total dead sperm percentages per treatment over the trial period can be found within Table 7.3.

Figure 7.4. Percentage of total live sperm within semen collected from Dohne Merino lambs immunocastrated at four (ICS4) or six (ICS6) weeks between second injection and slaughter, intact rams (R) and Burdizzo-castrated lambs (B). Arrows indicate timing of second vaccination.

The distribution of percentages normal and abnormal live sperm ($P < 0.001$; $P = 0.03$) as well as percentages normal and abnormal dead sperm ($P < 0.001$) differed over the study period for the various treatments (Figures 7.5 & 7.6; Table 7.2 & 7.3). The ICS6 lambs showed a decrease ($P < 0.001$) in percentage normal live sperm (Figure 7.5), and an increase in percentage

abnormal live sperm (Table 7.2), from D15 to D29, after which it remained unchanged. At D36, ICS6 semen samples had lower percentages of normal live sperm compared to R (P = 0.01) and ICS4 (P < 0.001), but was only equivalent to that of B on D43. The percentage of dead sperm with normal morphology remained relatively stable over the trial period for ICS6 but the percentage dead sperm with abnormal morphology increased from D22 until the end of the trial (Figure 7.6).

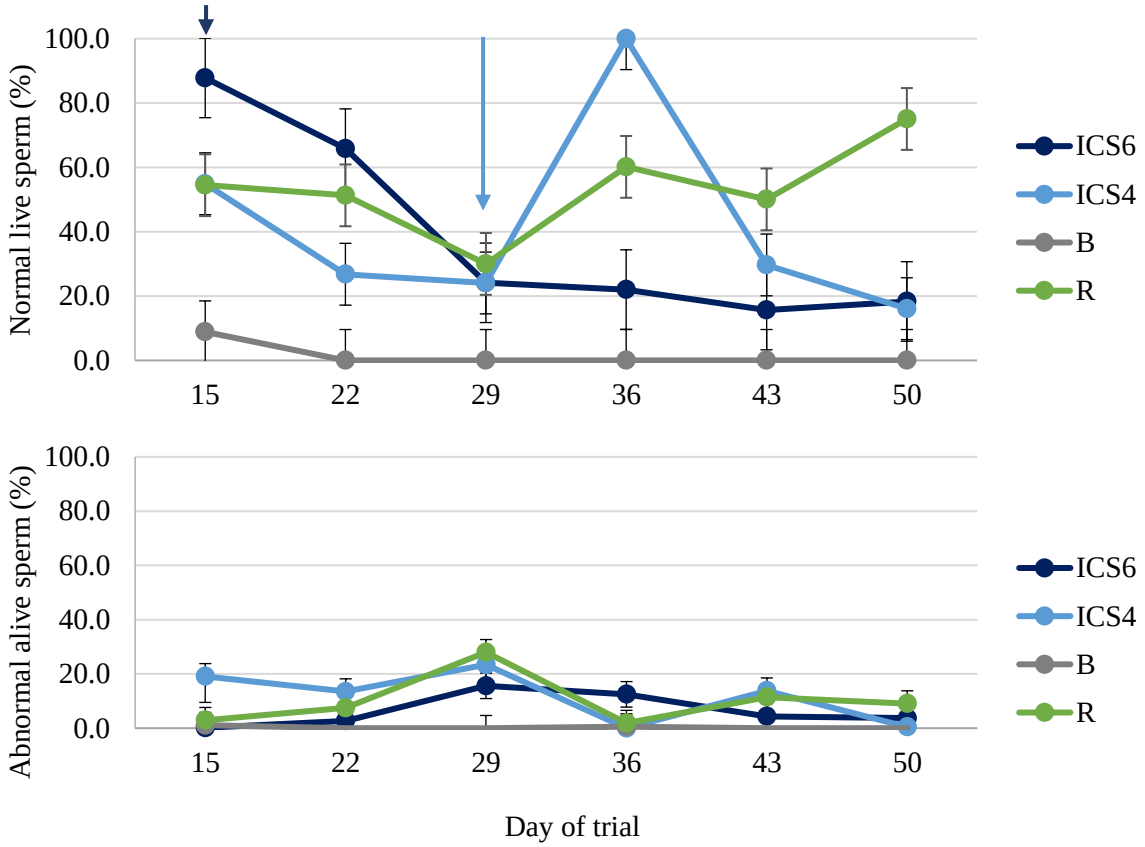
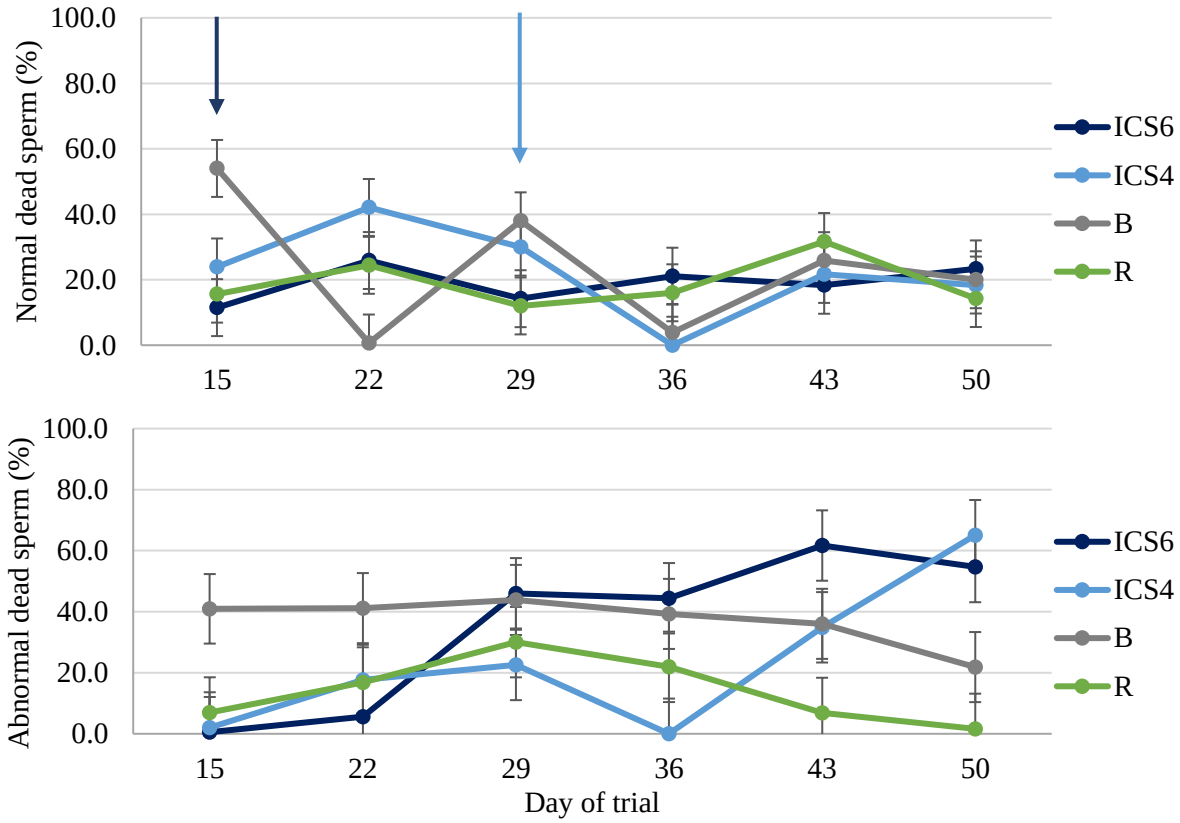


Figure 7.5. Percentage of live sperm with normal morphology (top) and abnormal morphology (bottom) within semen collected from lambs immunocastrated at four (ICS4) or six (ICS6) weeks between second injection and slaughter, intact rams (R) and Burdizzo-castrated lambs (B). Arrows indicate timing of second vaccination.

The viability of sperm cells in semen samples from ICS4 lambs did not follow the same trend as ICS6 after castration, having a peak (P < 0.001) in the percentage of normal live sperm the

week after the second vaccination. The increase in percentage live sperm was followed by a sharp increase ($P < 0.001$) in the percentage of dead sperm with abnormally morphology in



ICS4 semen samples from D36 to D50. At the end of the trial (D50), all castrated treatments had lower ($P < 0.001$) percentages of normal live sperm compared to intact controls (Figure 7.5; Table 7.2).

Figure 7.6. Percentage of dead sperm with normal morphology (top) and abnormal morphology (bottom) within semen collected from lambs immunocastrated at four (ICS4) or six (ICS6) weeks between second injection and slaughter, intact rams (R) and Burdizzo-castrated lambs (B). Arrows indicate timing of second vaccination.

The Burdizzo-castrated lambs had very low sperm concentrations in their semen samples, the majority of which were dead throughout the trial (Table 7.3) and thus poor motility scores (Figure 7.3). The percentage normal/abnormal live/dead sperm cells fluctuated between 30 to 70 %, 0 to 30 %, 10 to 30 % and 5 to 30 %, respectively for intact rams (Table 7.2 & 7.3).

Table 7.2. Percentage of total live sperm, live sperm with normal morphology and live sperm with abnormal for semen samples collected from immunocastrated lambs (IC), intact rams (R) and Burdizzo-castrated lambs (B). Sample collection started at 45.5 ± 3.65 kg.

Day	Total live sperm				Normal live sperm				Abnormal live sperm			
	ICS 6	ICS4	B	R	ICS6	ICS 4	B	R	ICS 6	ICS4	B	R
15	88.0^{ab}	74.0^{abcd}	9.7^{kl}	57.4^{defg}	87.8^{ab}	54.9^{cd}	8.9^{gh}	54.5^{cd}	0.2^{g}	19.1^{abc}	1.1^{efg}	2.9^{defg}
22	68.5^{bcde}	40.3^{fghij}	0.0^{l}	58.8^{cdefg}	65.8^{bc}	26.8^{efgh}	0.0^{h}	51.3^{cde}	2.7^{efg}	13.5^{bcdef}	0.0^{g}	7.5^{cdefg}
29	39.8^{fghij}	47.5^{defgh}	0.0^{l}	58.0^{cdefg}	24.1^{efgh}	24.1^{fgh}	0.0^{h}	30.0^{defg}	15.6^{abcd}	23.4^{ab}	0.0^{g}	28.0^{d}
36	34.5^{ghijk}	100.0^{a}	58.6^{cdefg}	62.1^{bcdef}	22.0^{gh}	100.0^{a}	0.0^{h}	60.2^{c}	12.5^{bcdefg}	0.0^{g}	0.6^{g}	1.9^{efg}
43	20.0^{ijkl}	43.5^{efghi}	0.0^{l}	61.5^{bcdef}	15.7^{gh}	29.7^{defg}	0.0^{h}	50.1^{cdef}	4.3^{defg}	13.8^{bcde}	0.0^{g}	11.5^{cdefg}
50	22.0^{hijkl}	16.5^{jkl}	0.0^{l}	84.1^{abc}	18.3^{gh}	16.1^{gh}	0.0^{h}	75.1^{abc}	3.7^{defg}	0.5^{g}	0.0^{g}	9.1^{cdefg}
SE	9.69	9.69	9.69	9.69	9.69	9.69	9.69	9.69	4.70	4.70	4.70	4.70

[a, b] LSMeans with different superscripts between treatments within main heading columns (Total live sperm, Normal live sperm, Abnormal live sperm) over Day are significantly different (P ≤ 0.05)
R = intact ram controls
ICS6 = lambs receiving their second vaccination six weeks before slaughter
ICS4 = lambs receiving their second vaccination six weeks before slaughter
B = lambs physically castrated on day 2 using a Burdizzo clamp

Table 7.3. Percentage of total dead sperm, dead sperm with normal morphology and dead sperm with abnormal for semen samples collected from immunocastrated lambs (IC) intact rams (R) and Burdizzo-castrated lambs (B). Sample collection started at 45.5 ± 3.65 kg.

Day	Total dead sperm				Normal dead sperm				Abnormal dead sperm			
	ICS6	ICS4	B	R	ICS6	ICS4	B	R	ICS6	ICS4	B	R
15	12.0^{gh}	26.0^{efgh}	93.0^{h}	22.6^{fgh}	11.5^{def}	23.9^{bcdef}	54.0^{a}	15.6^{cdef}	0.5^{gh}	2.1^{gh}	40.9^{abcd}	7.0^{efgh}
22	31.5^{defgh}	59.7^{abcd}	40.1^{a}	41.2^{defg}	25.9^{bcde}	42.1^{ab}	0.7^{f}	24.4^{bcdef}	5.6^{fgh}	17.6^{defgh}	41.2^{abcd}	16.8^{efgh}
29	60.2^{abcde}	52.5^{cdefg}	80.1^{ab}	42.0^{cdefg}	14.2^{cdef}	29.9^{abcd}	38.0^{abc}	12.0^{def}	46.0^{abcd}	22.6^{cdefgh}	43.9^{cdefgh}	30.0^{bcdefgh}
36	65.5^{abc}	0.0^{h}	41.5^{cdefg}	37.9^{cdefg}	21.1^{bcdef}	0.0^{f}	3.9^{ef}	16.0^{cdef}	44.4^{abcd}	0.0^{gh}	39.3^{gh}	21.9^{defgh}
43	80.0^{ab}	56.5^{cdefg}	60.1^{bcdef}	38.5^{cdefg}	18.3^{bcdef}	21.6^{bcdef}	25.8^{bcde}	31.7^{bcde}	61.7^{ab}	34.9^{bcdef}	36.0^{bcdef}	6.8^{fgh}
50	78.0^{ab}	83.5^{ab}	40.1^{cdefg}	15.9^{gh}	23.3^{bcdef}	18.4^{bcdef}	20.0^{bcdef}	14.3^{bcdef}	54.7^{abc}	65.1^{a}	21.9^{a}	1.6^{h}
SE	12.59	12.59	12.59	12.59	8.70	8.70	8.70	8.70	11.54	11.54	11.54	11.54

[a, b] LSMeans with different superscripts between treatments within main heading columns (Total dead sperm, Normal dead sperm, Abnormal dead sperm) over Day are significantly different (P ≤ 0.05)
R = intact ram controls
ICS6 = lambs receiving their second vaccination six weeks before slaughter
ICS4 = lambs receiving their second vaccination six weeks before slaughter
B = lambs physically castrated on day 2 using a Burdizzo clamp

7.3.2 Testes size, colour and histology

Immunocastration and Burdizzo-castration impact negatively on trimmed testis weights ($P < 0.001$) compared to intact controls (Table 7.4). The cut surface colour values differed between treatments, with ICS6 lamb testes having lower L* values than ICS4 ($P = 0.02$) and R ($P < 0.001$). Due to the high variation around the mean L* values for B caused by tissue necrosis and abscessing (Figure 7.7), testes L* values for B did not differ from all treatments. However, Burdizzo-castrated lambs had the greatest a* ($P \leq 0.001$) and b* ($P \leq 0.01$) values and R testes were the lowest, with both immunocastrated treatments being intermediate (Table 7.4). The intact rams had greater seminiferous tubule circumferences ($P < 0.001$) and seminiferous tubule epithelium depths/thickness ($P < 0.001$) compared to all castrated treatments. The ICS6 lambs had the smallest seminiferous tubule circumferences relative to ICS4 ($P = 0.007$) and B ($P = 0.006$) but did not differ from the other castrated lambs for seminiferous tubule epithelium depths.

Table 7.4. The mean effect (± SE) of immunocastration vaccination interval (six or four weeks between second vaccination and slaughter) on the testes weight, CIE colour values and seminiferous tubule parameters of Dohne Merino ram lambs (52.6 ± 4.73 kg).

Parameter	**Treatment group**			
	ICS6	**ICS4**	**B**	**R**
Testes weight, g	$79.2^{b} \pm 3.91$	$92.4^{b} \pm 7.34$	$112.3^{b} \pm 21.34$	$279.3^{a} \pm 20.14$
CIE colour values				
*L**	$61.2^{b} \pm 0.71$	$65.0^{a} \pm 0.96$	$63.0^{ab} \pm 2.07$	$65.4^{a} \pm 0.27$
*a**	$4.9^{b} \pm 0.32$	$3.7^{b} \pm 0.31$	$9.3^{a} \pm 0.78$	$1.3^{c} \pm 0.20$
*b**	$14.0^{b} \pm 0.44$	$14.4^{b} \pm 0.41$	$15.7^{a} \pm 0.31$	$11.4^{c} \pm 0.26$
Seminiferous tubule:				
Circumference, µm	$468.9^{c} \pm 37.80$	$621.9^{b} \pm 37.80$	$624.3^{b} \pm 37.80$	$911.70^{a} \pm 39.85$
Epithelium depth, µm	$26.9^{b} \pm 2.64$	$30.6^{b} \pm 2.64$	$33.2^{b} \pm 2.64$	$56.0^{a} \pm 2.78$

[a, b] LSMeans with different superscripts within rows are significantly different ($P \leq 0.05$)
ICS6 = lambs receiving their second vaccination six weeks before slaughter
ICS4 = lambs receiving their second vaccination six weeks before slaughter
B = lambs physically castrated on day 2 using a Burdizzo clamp
R = intact ram controls

Figure 7.7. Trimmed testes cut in half for surface colour measurement indicating the differences in size and colour between treatments. Testes 8 and 30 were from lambs immunocastrated with a six or four-week interval between second vaccination and slaughter, respectively. Testes 34 was collected from an intact ram, while testes 11 to 20 represent the entirety of the Burdizzo-castrated (B) treatment group.

7.3.3 Androgen serum concentrations

Concentrations were under the detection limit for 5α-androstanedione (5α-dione; 0.25 ng/mL), 5α-dihydrotestosterone (DHT; 0.25 ng/mL) and 11-ketoandrostenedione (11KA4; 0.01 ng/mL) for all serum samples. Testosterone (T) concentrations varied over the trial period for intact males (Figure 7.8). However, from D29 to D50, serum T concentrations for R were higher than all castration treatments ($P < 0.001$).

From D1 to D8, an intensive blood withdrawal was performed to establish the effect of primary vaccination for ICS6 and Burdizzo-castration, performed on D2 (Figure 7.9). The T concentrations for ICS6 after primary vaccination increased from D3 to D5 ($P = 0.04$) and then decreased ($P < 0.001$) from D5 to D8 and remained unchanged for the duration of the trial (Figure 7.8) with significant changes seen after second vaccination (Figure 7.10). However, T concentrations for R where low until D15, at which point ICS6 serum T concentrations were

lower (P < 0.001) than R and remained so until slaughter (Figure 7.8). The B lambs' serum T concentrations decreased (P = 0.01) from D1 to D3 (Figure 7.9) and remained unchanged for the study (Figure 7.8).

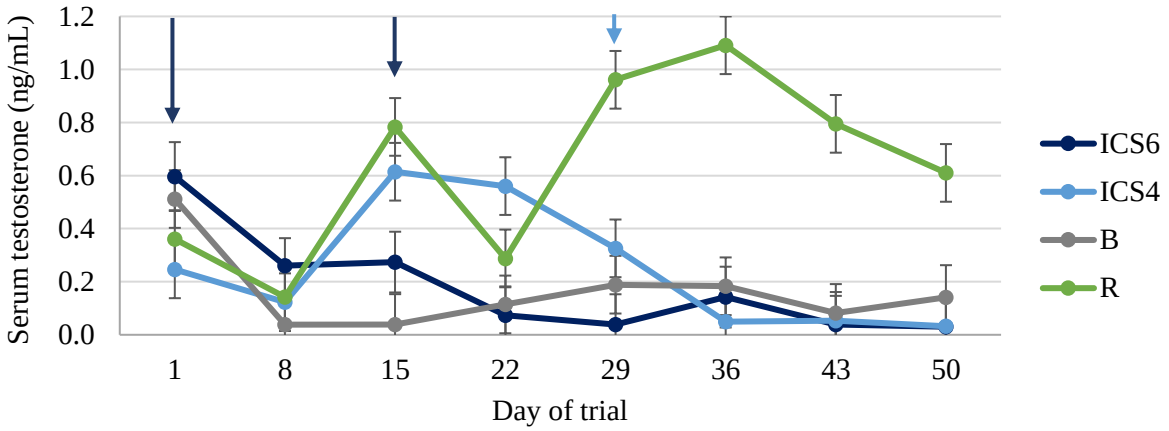
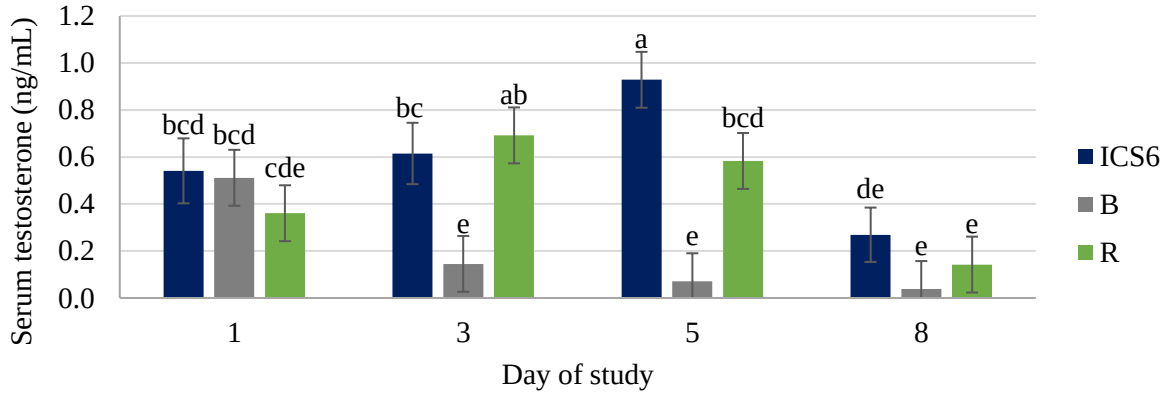


Figure 7.8. The average (±SE) serum testosterone concentration (ng/mL) measured in lambs immunocastrated with either six (ICS6) or four (ICS4) weeks between second vaccination and slaughter, Burdizzo-castrated lambs (B) and intact rams (R). Vaccinations are indicated by colour-coordinated arrows.

Figure 7.9. The average (± SE) serum testosterone concentrations (ng/mL) of lambs receiving their first immunocastration vaccination at D1 (ICS6), lambs Burdizzo-castrated on D2 and intact rams (R) for the intensive blood sampling period (D1-8). Letters indicate significant differences between means at a significance level of 5 %.

The ICS4 lambs received their first vaccination on D15, after which T concentrations decreased (P = 0.02) on D17, followed by an increase (P = 0.03) (Figure 7.10) such that there was no



overall change in T between D15 to D22 (Figure 7.8). Serum T then decreased ($P < 0.001$) from D22 to D36 and remained unchanged for the duration of the study (Figure 7.8).

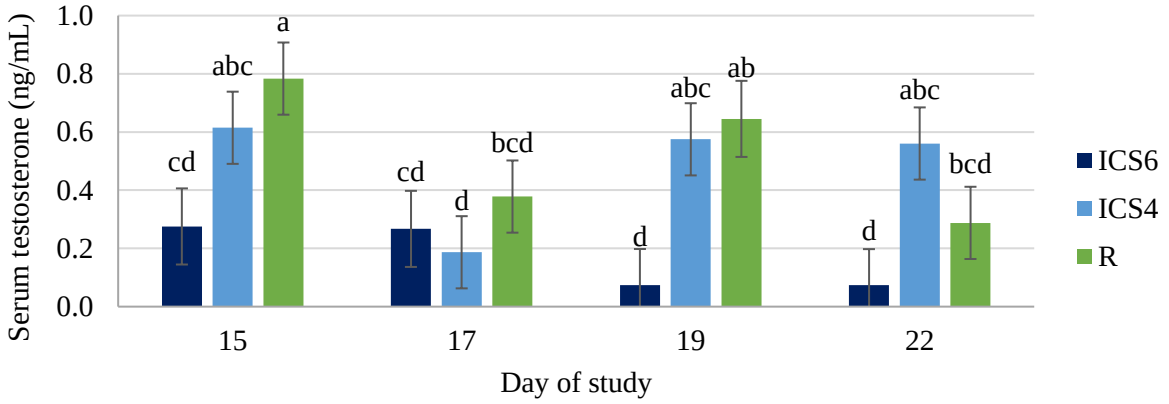


Figure 7.10. The average (± SE) serum testosterone concentrations (ng/mL) of lambs immunocastrated with their second vaccination at D15 (ICS6), lambs immunocastrated with their first vaccination at D15 (ICS4) and intact rams (R) for the intensive blood sampling period from D15 to D22. Letters indicate significant differences between means at a significance level of 5 %.

In the intensive blood sampling after the second vaccination, ICS4 showed no significant changes for T concentrations (Figure 7.11). Serum T concentrations for ICS4 and B were lower than R on D29 ($P < 0.001$) and D3 ($P < 0.001$), respectively (Figure 7.8). Thus, both ICS6 and ICS4 treatments had serum T concentrations lower than R within two weeks after their primary vaccinations.

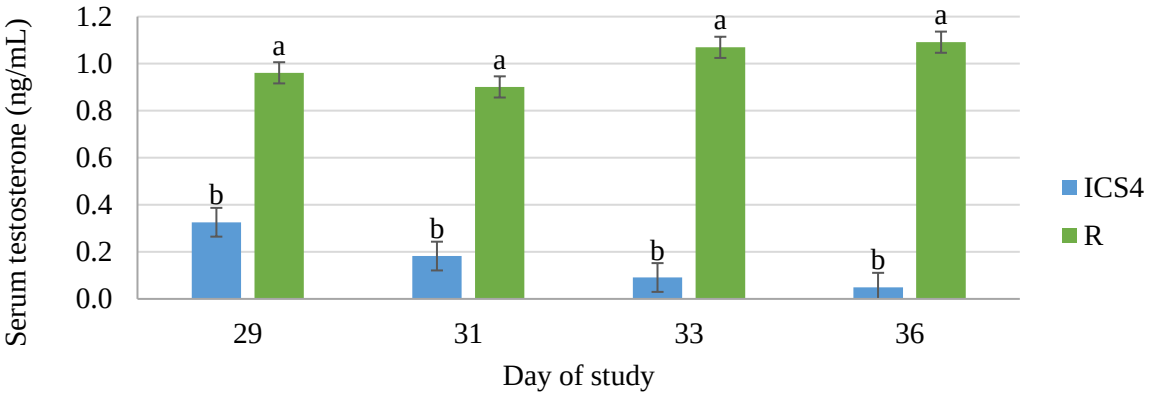
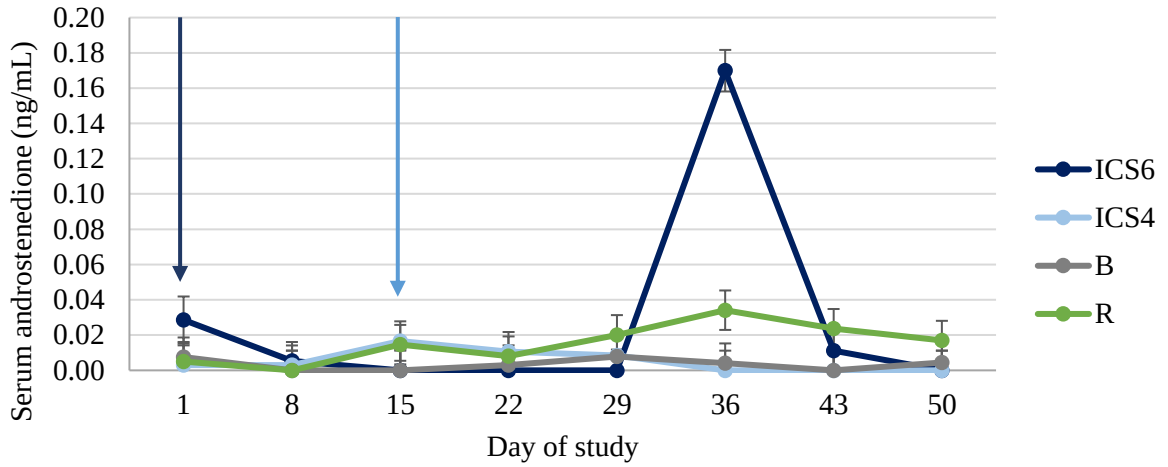


Figure 7.11. The average (± SE) serum testosterone concentrations (ng/mL) of lambs immunocastrated with their second vaccination at D29 (ICS4) and intact rams (R) for the intensive blood sampling period from D29 to D36. Letters indicate significant differences between means at a significance level of 5 %.

Serum androstenedione (A4) did not differ between treatments other than a spike in A4 at D36 for ICS6 (Figure 7.12). However, A4 concentrations were low for all treatments for the duration of the trial (Figure 7.12). Even though androstenedione is secreted by both the testes and adrenal glands, the factors that govern the production thereof are different. Therefore, within



the context of this study it will be difficult to reason why there was a peak in A4 production, thus it is likely a result of experimental error.

Figure 7.12. The average (± SE) serum androstenedione concentrations (ng/mL) measured in lambs immunocastrated with either six (ICS6) or four (ICS4) weeks between second vaccination and slaughter, Burdizzo-castrated lambs (B) and intact rams (R). Vaccinations are indicated by colour-coordinated arrows.

7.4 Discussion

The influence of immunocastration on testes development and histology has been investigated in ram lambs using various vaccines, all showing success in decreasing testes growth and seminiferous tubule development (Kiyma et al., 2000; Ülker et al., 2002; Ülker et al., 2005). Although Improvac® decreases testicular growth for at least three months after the second

vaccination (Janett et al., 2003), the effect of decreased scrotal circumference on the reproductive capacity of immunocastrated lambs over time has not been established. The two-week interval used in Chapter 5 (5.3.1) showed the fastest effect on testosterone concentrations and remained effective until slaughter four weeks later. It is thus important to establish when this decrease in testosterone starts to influence sperm production while keeping in mind clearance of sperm storage in the epididymis.

Semen collection for this study was performed two weeks after Burdizzo-castration to allow for wound healing, as well as to focus on the period after second vaccination for the immunocastrated lambs, as this is when the large decrease in serum testosterone has been recorded (Chapter 5, 5.3.1). Semen colour scores for semen collected in this study varied, all scores were less than four (Figure 7.2) indicating fair to unacceptable colour scores (Table 7.1). The Burdizzo-castrated lamb semen samples showed the largest influence of treatment on colour scores, producing watery samples throughout the trial, with some animals not producing any samples by the end of the trial. While the accessory sexual glands which produce the seminal plasma that constitutes the bulk of a semen sample, are not physically damaged by Burdizzo-castration, the functionality of these glands depends on testosterone secretion (Gofur et al., 2014). Decreased accessory gland development was reported when recombinant ovalbumin-GnRH vaccines were used for immunocastration of goats (Ülker et al., 2009). Thus, the decrease in colour scores of immunocastrated lambs and the lack of semen samples produced from some Burdizzo-castrated animals is likely due to the decrease in secretory activity of the accessory sexual glands in response to decreased testosterone concentrations (Gofur et al., 2014).

Semen motility scores on the day of second vaccination (D15) for ICS6 were considered fair, with 45 to 65 % motile sperm, which decreased until four weeks after second vaccination

(D43), when no visible sperm mass motility was seen. Similarly, mass motility scores decreased for ICS4 samples from the second vaccination until the end of the trial, three weeks after second vaccination. Thus, although serum testosterone concentrations begin to decrease after the primary immunocastration vaccination for both vaccination schedules with Improvac®, either a lower testosterone concentration (as seen after second vaccination) is required to influence sperm motility or perhaps a lag period exists between onset of reduced testosterone concentrations and decreased sperm motility. The intact rams had both poor colour scores and motility scores for the trial indicating that although Dohne Merino ram lambs over 40 to 60 % of their mature body weight (~ 40 kg) are considered post-pubertal, semen quality may still be suboptimal.

Acceptable motility scores for rams is > 30 %, while the acceptable standard for sperm cell concentration is between 2.5 to 6 x 10^9 sperm cells per mL raw semen (Bedford-Guaus, 2017). Thus, all lambs had sub-optimal semen concentrations throughout the trial. However, the intact rams showed improvement in the proportion of total live sperm of normal morphology from 54.5 % at the start of the collection period (D15) to 75 % five weeks later. The percentage of normal live sperm in intact rams at the end of the trial was verging on the "exceptional" standard of 80 % normal live sperm for breeding rams (Bedford-Guaus, 2017). However, the sperm viability in intact rams for this study was lower than the normal live sperm percentages (97.7 ± 1.37 %) reported for post-pubertal Dohne Merino rams in Chapter 3. The immunocastrated lambs produced samples characterized by a decrease in the percentage of total live sperm and an increase in abnormal dead sperm throughout the trial. Sperm samples obtained from the ICS6 lambs indicated a more consistent decrease in the percentage total live sperm, whereas ICS4 lambs experienced a spike in percentage of total live sperm the week after second vaccination (D36). This peak in total live sperm may be due to clearance of stored

sperm within the epididymis, with testosterone concentrations being significant enough to maintain them for one week after second vaccination until electro-ejaculation. The increase seen in total live sperm for B may indicate incomplete shearing of the spermatic chords and thus questionable efficacy.

The percentage of dead sperm increased steadily for ICS6 samples until the end of the trial where up to 80 % of the total sperm cells were dead. For semen samples to be considered acceptable, more than 50 % of the sperm cells need to be of normal morphology (Bedford-Guaus, 2017). The ICS4 lambs already showed less than 50 % normal live sperm percentages one week after their first vaccination, followed by a peak one week after their second vaccination and a subsequent decrease to below 50 % again. This peak in normal live sperm may be the clearance of sperm stored in the epididymis, with immunocastration influencing spermatogenesis in the subsequent samples. Unfortunately, the semen quality and sperm quality between primary and secondary vaccination for ICS6 was not quantified; however, the percentage of normal live sperm decreased after second vaccination, contrary to ICS4 which showed increased percentages the following week. From two weeks after second vaccination, ICS6 lambs had less than 50 % of the sperm cells with normal morphology and being alive. However, due to the overall low sperm concentration and poor motility scores, the fertility potential of immunocastrated lambs is questionable.

The lambs used in this study are older relative to the typical commercial finishing and slaughter age. This decision was made to ensure that the rams had a better functioning reproductive system to measure these effects. These rams also entered the trial at the age when Dohne Merino ram lambs are selected out of the breeding flock, should they have any cull-defects, and are too old for physical castration before finishing for slaughter. However, if younger lambs are used, spikes seen in live sperm may be prevented by interrupting the

reproductive system before puberty. As the vaccination schedules used in this study were successful in interrupting the reproductive capacity of lambs older than six months of age, immunocastration may successfully prevent younger rams attaining puberty.

The ICS6 lambs had scrotal circumferences smaller than R two weeks after second vaccination (D29), whereas ICS4 showed smaller scrotal circumferences within the week after second vaccination (D31; Chapter 6, Section 6.3.4). At slaughter, scrotal circumferences were similar for all immunocastrated and Burdizzo-castrated lambs, thus decreased trimmed testes weights did not differ between these two castration treatments. However, both immunocastrated and Burdizzo-castrated lambs had lighter testes weights compared to intact rams. Testes weights of intact rams were nearly 70 % of that recorded for mature rams in Chapter 3 and although seminiferous tubule size was almost equivalent, testes cut surface colours were comparable between the pubertal lambs in this study and mature rams in Chapter 3.

The testes cut surface colour was evaluated in Chapters 3 and 7 as this may be an indication of decreased tissue activity and thus vaccination success in immunocastrates (Lealiifano et al., 2011). However, testes cut surface colour differed between castration treatments. The Burdizzo-castrated lambs had a high variation in colour values due to tissue necrosis as suspected, but overall the reddest and most yellow testes. This tissue necrosis and cortisol concentrations indicate the compromised welfare of Burdizzo-castrated animals, despite the intensive use of pain mitigation. This castration method was ineffective in especially the older rams in this study, and therefore the use of Burdizzo-castration should be reconsidered. The ICS6 lambs had lighter testes colour than ICS4 and R but was equivalent to ICS4 for redness and yellowness. Both immunocastration treatments had redder and more yellow testes than intact rams, which was also reported for immunocastrated lambs in Chapter

5 (5.3.2). Immunocastration has also been shown to cause more yellow testicles in swine compared to intact controls; however, they are also less red and thus lambs and swine appear to differ in this regard (Lealiifano et al., 2011). In Chapter 5 (5.3.2), the two and three-week inter-vaccination periods had a more pronounced influence on seminiferous tubule epithelium depth than the four-week vaccination interval. When the two-week inter-vaccination period was combined with either a four or six-week interval between second vaccination and slaughter in this study, no differences were seen in seminiferous tubule epithelium thickness but ICS6 had the most pronounced reducing effect on seminiferous tubule circumference.

Serum testosterone concentrations measured in this study were comparable to those reported in lambs by Kiyma et al. (2000), Ülker et al. (2005) and Gökdal et al. (2010), with all concentrations being under 2 ng/mL for intact rams. The lambs immunocastrated with a six-week interval between second vaccination and slaughter also showed a more consistent decrease in serum testosterone concentration over the trial period compared to those lambs vaccinated with a four-week interval. The results for both immunocastration treatments confirmed that the primary vaccination had an influence on testosterone concentration, as reported in Chapter 5, and that a two-week inter-vaccination period prevented the subsequent increase in testosterone secretion after primary vaccination seen when using longer intervals (Chapter 5). However, this contrasts with what has been reported in swine, with a noticeable effect on testosterone only seen after secondary vaccination with Improvac® (Claus et al., 2007). Immunocastration decreased serum testosterone to concentrations comparable to physical castrates within one week after their respective second vaccinations, which is faster than the 14 days after the second vaccination where Bopriva® decreases testosterone concentrations of cattle to those equivalent to physical castrates (Amatayakul-Chantler et al., 2013). In cattle, immunocastration is considered effective when testosterone concentrations

reach concentrations below 5 ng/mL (Bopriva^{TM} Veterinary Guide, 2010). However, D'Occhio and Brooks (1982) showed that low concentrations of testosterone are needed in sheep to display mounting behaviour (0.32 to 0.65 ± 0.01 ng/mL) and mating with intromission and ejaculation (1.26 ± 0.13 ng/mL). The ICS6 lambs reached serum testosterone concentrations under 0.3 ng/mL within one week after primary vaccination, while ICS4 lambs reached this concentration one week after second vaccination. The likely reasoning for the difference in time to reach basal testosterone concentrations between immunocastration treatments may be due to the increase in testosterone seen in ICS4 lambs before primary vaccination. This increase in testosterone may also explain why a high percentage of normal live sperm where still found in ICS4 semen samples after secondary vaccination. Considering the low testosterone concentrations exhibited in immunocastrated ram lambs, mounting behaviour should not be exhibited allowing them to be kept together with ewes without the stress of such behaviours and subsequent detrimental effects on welfare and consequently meat quality.

7.5 Conclusion

Dohne Merino rams between 45 to 53 kg live weights may be considered pubertal regarding the degree of reproductive activities in the testes. *Post-mortem* evaluation of the testes of Burdizzo-castrated lambs confirmed suspicions of physical stress while its efficacy remains questionable. Immunocastration was successful in interrupting the reproductive functioning and testosterone production of pubertal ram lambs, and can thus be considered an alternative to Burdizzo-castration. The use of a two-week inter-vaccination period with a six-week interval between second vaccination and slaughter appears to be preferred regarding decreased sperm viability and serum testosterone concentrations. However, in a commercial production system, it may be beneficial to administer the first vaccination earlier, before puberty, or keep older

rams separate from ewes until two weeks after the second vaccination if ram fertility is a concern. For behavioural control, testosterone concentrations indicate that lambs will be under the threshold for mounting and sexual behaviours within one week after the primary vaccination. Although extending the interval between second vaccination and slaughter interrupted reproductive functioning for the duration of the trial, its effect on carcass and slaughter traits needs to be investigated.

7.6 References

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CHAPTER 8

Influence of extending the pre-slaughter interval after second vaccination on the carcass performance and meat quality of immunocastrated lambs

Abstract

The influence of varying intervals between second vaccination and slaughter of immunocastrated rams on carcass characteristics, carcass cutting yield and meat quality of the *Longissimus thoracis* muscle (LT) was investigated using forty Dohne Merino ram lambs (45.4 ± 3.68 kg) randomly allocated to four treatment groups. The booster vaccination intervals investigated were six (IC6) and four (IC4) weeks prior to a fixed slaughter age, with two weeks between primary and secondary vaccination. The third group included animals physically castrated with a Burdizzo (B) on the second day of the trial. An intact group of rams (R) was maintained as a control for the duration of the 57-day growth trial. At slaughter, offal weights were recorded, and reported as a percentage of the live weight. Cold carcass weight was recorded after 24 hours of storage at 4°C, and carcasses were then processed into commercial prime cuts. The respective cuts were weighed, and reported as a percentage of the cold carcass weight. A thoracic three-rib cut was removed from both sides of the carcass (ninth rib to edge of twelfth rib), photographed and the LT muscle area and backfat depth was determined using ImageJ. The LT muscles were removed and used to determine pH, CIE cut surface colour, drip loss, cooking loss and Warner-Bratzler shear force values. Both Burdizzo-castration and immunocastration resulted in an increase in backfat thickness and decreased CIE a* cut surface meat colour values. No treatment effects were observed for the other meat quality parameters as well as cutting yields. Immunocastration of rams can be used as a method to manipulate carcass fatness without negatively influencing carcass weight, cutting yield and meat quality. Further investigation into the sensory acceptability of meat produced by immunocastrated lambs is warranted to establish if differences in meat surface colour, which may influence consumer acceptance, are observed.

8.1. Introduction

The castration of ram lambs intended for slaughter assists with management by reducing sexual and aggressive behaviour, while allowing for marketing at an earlier age due to faster fat deposition. Although intact rams have higher growth rates and superior feed efficiency (Sales, 2014), they also produce less fat and have a lower edible carcass yield (Seideman et al., 1982). Intact rams produce meat with lower tenderness (Field, 1971), with meat from these rams tending to have an undesirable meat colour (Sales et al., 2014) and flavour (Crouse et al., 1981). However, standard methods of physical castration negatively influence the welfare of lambs (Melches et al., 2007). Even though Burdizzo-castration has been identified as the best procedure to use in older rams, observed cortisol concentrations and testes tissue necrosis indicate physiological stress experienced by castrates (Chapters 6 & 7).

Immunocastration of peri-pubertal (Chapters 4 & 5) and pubertal lambs (Chapters 6 & 7) using various vaccination schedules did not negatively influence growth performance and was successful in interrupting reproductive functioning. This interruption of reproductive functioning was also reported in the studies of Kiyma et al. (2000), Ülker et al. (2002), Ülker et al. (2003) and Gökdal (2010). Although the effects on extending the interval between second vaccination and slaughter of immunocastrated lambs indicated that a six-week period may be preferred regarding testosterone suppression and decreased sperm viability, the influence on fat deposition, slaughter performance and meat quality needs to be established.

Varying the period between second vaccination and slaughter from two, three, four or six weeks has shown to influence backfat thickness by up to 2 mm in swine (Lealiifano et al., 2011). Fatness of lamb meat cuts is a dominant selection criterion for consumers (Jeremiah et al., 1993) and thus carcass grading/classification and subsequently price is largely determined by fat covering. However, the feeding of lambs during the finishing period to stimulate the

deposition of subcutaneous fat is expensive, but so is trimming of excess fat in the preparation of retail cuts. Fatness of meat cuts is also considered important for the sensory eating experience of red meat (Mancini & Hunt, 2005). Therefore, manipulation of lamb carcass fatness using immunocastration may prove beneficial, resulting in cuts that will meet the demands of consumers.

Contrary to the production of pork, lamb production systems often rely on external factors such as rainfall and grazing quality that determines the age at weaning and ultimately the fattening period. In South Africa, there is variation in the age at which lambs are weaned, with breeds/maturity type, type of production system and fodder types which may influence the overall lamb production period between birth and slaughter. Thus, a commercial immunocastration vaccination schedule for ram lambs not only needs to be flexible and effective, not only in terms of the interval between first and second vaccination, but also between second vaccination and slaughter. Although the interval between first and second vaccination is flexible regarding the parameters measured in Chapters 4 and 5, the interval between second vaccination and slaughter could potentially have a more pronounced influence on carcass characteristics and meat quality. Variable sex differences exist in lamb meat quality (Hopkins & Mortimer, 2014; Sales, 2014), and thus the meat quality of immunocastrated lambs relative to that of both Burdizzo-castrated lambs and intact rams needs to be investigated.

The objective of this study was therefore to determine the effect of extending the interval between second vaccination and slaughter on the cutting yield, fatness and meat quality of immunocastrated ram lambs, compared to that of both physically castrated and intact males.

8.2. Materials and Methods

Ethical clearance was obtained from the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00073) and animal husbandry was in accordance specifications of the South African National Standards 10386: 2008.

8.2.1 Animals, castration techniques and slaughter

Dohne Merino ram lambs (n = 40; 45.4 ± 3.68 kg) used for this study were maintained as discussed in Chapter 6 (Section 6.2). Treatments groups included a control consisting of intact rams (R), Burdizzo-castrated lambs (B), and lambs immunocastrated with either a four (ICS4) or six-week (ICS6) interval between second vaccination and slaughter. Immunocastration was performed using Improvac® (2 mL per dose) injected subcutaneously on the shoulder, with a two-week interval between the primary and secondary vaccination for both immunocastration treatments (Figure 7.1). Burdizzo-castration was performed on Day 2 of the study (Figure 7.1) with a clamp application time of 30 seconds per testis after the administration of Metacam® according to veterinary recommendations (20 mg Meloxicam/mL at 0.25 mL/10 kg body weight).

8.2.2 Slaughter and sampling

All sheep were slaughtered according to standard practices (refer to Chapter 3 for details) after a 57-day growth period, when lams reached an average live weight of 53.7 ± 4.8 kg. Rams were weighed before being loaded and transported to a commercial abattoir (73 km on tarred road) on the morning of slaughter. The lambs were kept separate from other commercial sheep in shaded lairage at the abattoir and allowed *ad libitum* access to water. Slaughter commenced that same afternoon, with electrical stunning (four seconds at 200 V and 250 AMP) followed

by exsanguination within 60 seconds using a spearcut. Six carcasses were electrically stimulated at a time (one minute at 11 Volts and 250 AMP) after a six-minute bleeding time. Carcasses were then dressed, and various offal items were collected on the slaughter line and weighed as described in Chapters 3 and 4. The offal items included the head, feet, skin, gastrointestinal tract (GIT; esophagus, rumen, reticulum, omasum, abomasum, small intestine, large intestine, caecum, colon and rectum), GIT fat, red offal (heart, lungs, liver and trachea), and the scrotum containing the testes.

All animals had no permanent incisors and were thus classified as 'A' lambs according to the guidelines of the Agricultural Product Standards Act, 1990 (ACT No. 119 of 1990). The carcasses were suspended by means of both hind legs at the Achilles tendon for 24 hours at 4°C, after which the carcasses were weighed to determine the cold carcass weight (CCW). Carcasses were then processed into commercial prime cuts that were weighed and expressed as a percentage of the cold carcass weight. The commercial cuts included: the neck, hind legs, hind-shanks, back, belly, shoulders, and fore-shanks (Figure 8.1). The neck was sectioned from the carcass between the last cervical vertebra and the first thoracic vertebra. The fore shanks were sectioned off from the shoulder at the joint between the humerus and the radius, while the hind shanks were sectioned off at the joint between the femur and the tibia. The shoulders were removed from the rest of the carcass at the sixth rib and the hind legs were sectioned from the back and belly before the seventh lumbar vertebra. The belly was sectioned from each side of the back by cutting below the vertebrae, *psoas major* and *psoas minor* muscles, straight towards the distal region of the back.

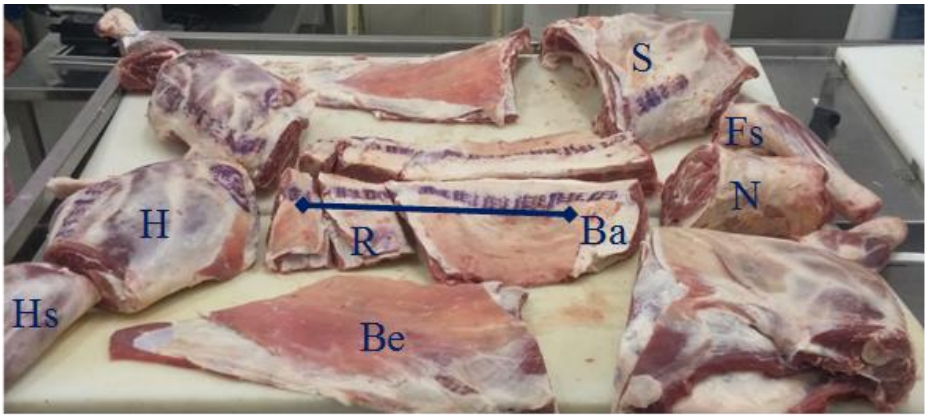

Figure 8.1. The dissection of a lamb carcass into commercial prime cuts for cutting yield determination. Prime cuts included the neck (N), back (Ba), belly (Be), shoulder (S) and fore-shank (Fs), hind leg (H) and hind-shank (Hs). The three-rib-section (R) was removed for meat quality assessment.

8.2.3 Physical meat quality assessment

At 24 hours *post-mortem*, a three thoracic-ribs section was removed from the "back" prime cut and split in half through the spinal vertebrae. This rib cut was removed by cutting along the cranial side of the ninth rib bone and again along the cranial side of the twelfth rib so that the three-rib section included the ninth, tenth and eleventh rib. Both the left and right three thoracic-rib sections were placed on stable surface allowing for proper reflection and photographed with the twelfth rib facing upwards (Figure 8.2). These photographs were calibrated using ImageJ2 software according to the ruler placed within each photograph and used to determine the LT muscle cross-sectional area and backfat thickness using ImageJ2 (Schindelin et al., 2015).

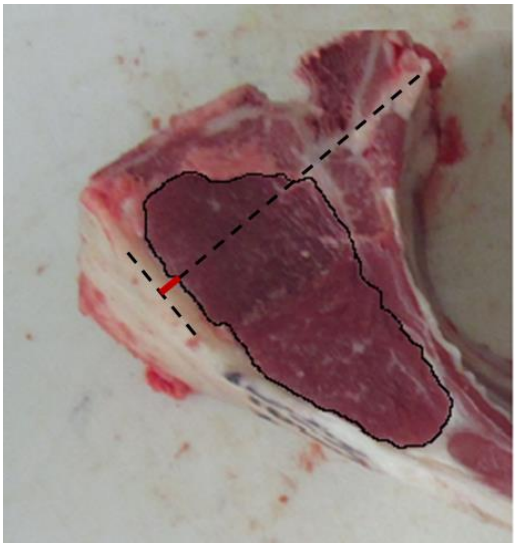

Figure 8.2. Determination of the fat depth on the *Longissimus thoracis* muscle on the cranial side of the ninth rib. The edge of the subcutaneous fat was marked (dashed line), and a perpendicular line drawn up to the edge of the rib to determine the location of fat depth measurement (red line). LT muscle was outlined, and muscle surface area was determined using ImageJ2.

The *Longissimus thoracis* (LT) section was removed from both three-rib cuts and used for pH determination 24 hours *post-mortem* (pH_{24}), physical meat quality assessment and cut surface colour analysis. After pH_{24} was measured (Crison PH25 pH metre, Allela, Barcelona) on the left LT section, both LT sections were cut into 2 cm thick steaks (Figure 8.3), perpendicular to the long axis of the muscle fibres as described by Needham & Hoffman (2015). The first and second steak (A & B) cut from the left LT at the caudal end was bloomed for 45 minutes after which the surface CIE Lab* colour system colour was measured at three random locations over the two steaks (Color-guide 45°/0° colourimeter, BYK-Gardner GmbH, Gerestried, Germany). The third steak (C) cut from the left LT was used for drip loss determination while three steaks (D, E & F) cut from the caudal end to cranial end of the right LT was used for cooking loss analysis and subsequently shear force measurement according to Honikel (1998). Steak C was weighed and suspended at 4°C from a wire within a closed impermeable plastic bag, ensuring

that the meat was not touching the sides. After 24 hours, the steaks were removed, blotted and weighed to determine the drip loss as a percentage of the initial steak weight.

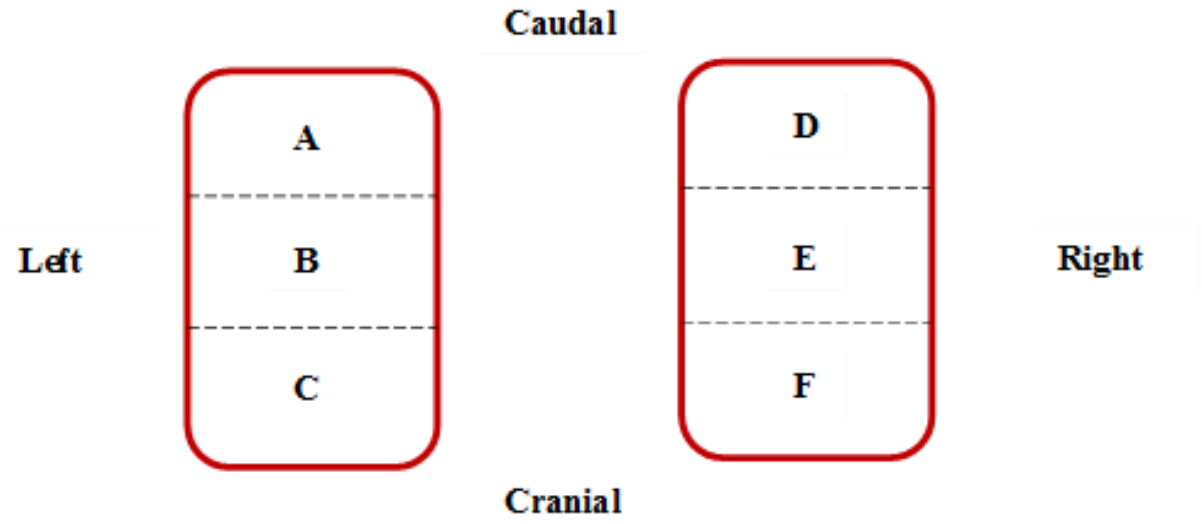

Figure 8.3. Sectioning of the left and right *Longissimus thoracis* muscles from the caudal side of the eleventh rib to the cranial side of the ninth rib for meat quality assessment. Steak A and B was used for CIE Lab colour measurement, C for drip loss, and D to F for cooking loss.

The three steaks (C, D & E) per animal were weighed, placed within a thin plastic bag in a waterbath at 80 °C, ensuring that the opening was above the surface. All samples were cooked in one batch for 60 minutes, after which the cooking loss was removed, and the meat samples were cooled at 4 °C within their individual plastic bags. Each steak was then blotted dry, weighed and the cooking loss expressed as a percentage of the initial steak weight. Subsequently, the cooking loss steaks were used for shear force determination using an Instron Universal Testing Machine (Instron UTM, Model 2519-107) fitted with a 1 mm thick Warner-Bratzler blade (0.508 mm cutting edge radius; 5 kN capacity; 200 mm/ minute crosshead rate). Two rectangles (1 x 1 x 2 cm) were cut from each steak, thus totalling six sub-samples per animal. Each sub-sample was cut with the muscle fibres parallel to the length of the rectangle

such that the blade sheared the muscle fibres at a right angle. The peak force (N) to shear each 1 cm^2 sub-sample was recorded.

8.2.4 Statistical analysis

Statistical analysis was performed using STATISTICA 64 Version 13.2 (StatSoft Inc.). Residuals were tested for normality and possible outliers established. Homogeneity of the data was ensured using Levene's test. The one-way Analysis of Variance (ANOVA) approach was used to determine the differences between treatment effects for the various measurements. When the assumption of homogeneity was met, Fisher's Least Significant Difference was the chosen post hoc test. For non-homogenous data, Games-Howel post hoc tests were used for the comparison of treatment means. Significant differences were reported at a significance level of 5 % and results are reported as least square means (LSMean) ± standard error of the mean (SEM).

8.3. Results

8.3.1 Slaughter performance, offal yields and prime cutting yields

Immunocastration vaccination interval and Burdizzo castration did not influence average slaughter live weight, cold carcass weight or prime cut percentages. However, larger GIT percentage yields were recorded for the intact rams ($P = 0.03$) and immunocastrated lambs (IC6, $P = 0.03$; IC4, $P = 0.004$), when compared to the Burdizzo-castrated lambs (Table 8.1). Lambs immunocastrated with a six-week interval before slaughter had a higher GIT fat percentage than the intact rams ($P = 0.02$) and the lambs vaccinated four weeks before slaughter ($P = 0.008$) respectively, with the Burdizzo-castrated lambs yielding the same GIT fat percentage as IC6 lambs. The proportion of the live weight represented by the scrotum and testes was lower ($P < 0.01$) in all castration treatments, when compared to the intact lambs. The skin percentage recorded for R lambs tended to be greater ($P = 0.08$), when compared to the respective castration

treatments (Table 8.1). The hind leg contribution to carcass weight tended to be higher (P = 0.09) in IC4 and IC6 lambs, when compared to intact and physically castrated lambs (Table 8.1).

Table 8.1. The effect of vaccination interval between second vaccination and slaughter on the slaughter performance, carcass traits and cutting yields of immunocastrated Dohne Merino ram lambs, compared to Burdizzo-castrated lambs and intact lambs.

Parameter	Vaccination schedule				*P*-value
	IC6	IC4	B	R	
Live slaughter weight, kg	53.9 ± 0.76	52.0 ± 1.76	52.9 ± 1.99	56.1 ± 0.80	0.25
Cold carcass weight, kg	23.12 ± 0.62	21.7 ± 0.86	22.8 ± 0.92	23.4 ± 0.44	0.38
Offal Items:					
Head, %	5.4 ± 0.11	5.5 ± 0.09	5.4 ± 0.10	5.5 ± 0.07	0.72
Feet, %	2.4 ± 0.05	2.4 ± 0.05	2.4 ± 0.05	2.3 ± 0.03	0.68
Skin, %	8.3 ± 0.17	8.7 ± 0.15	8.8 ± 0.24	9.1 ± 0.23	0.08
GIT, %	25.8^{a} ± 0.41	26.4^{a} ± 0.90	23.8^{b} ± 0.49	25.8^{a} ± 0.45	0.02
GIT fat, %	0.48^{a} ± 0.05	0.32^{b} ± 0.04	0.41^{ab} ± 0.04	0.34^{b} ± 0.02	0.03
Red offal, %	4.3 ± 0.07	4.6 ± 0.18	4.4 ± 0.25	4.4 ± 0.06	0.79
Scrotum & testes, %	0.6^{b} ± 0.02	0.6^{b} ± 0.02	0.6^{b} ± 0.06	1.1^{a} ± 0.04	< 0.01
Prime yields:					
Hind legs, %	27.3 ± 0.25	27.0 ± 0.43	26.2 ±0.44	26.7 ± 0.27	0.09
Hind shanks, %	5.9 ± 0.10	5.9 ± 0.11	5.8 ± 0.19	5.8 ± 0.05	0.70
Shoulders, %	27.9 ± 0.28	28.2 ± 0.50	28.7 ± 0.34	28.2 ± 0.40	0.33
Fore shanks, %	4.6 ± 0.16	4.5 ± 0.14	4.7 ± 0.14	4.8 ± 0.11	0.52
Back, %	18.2 ± 0.17	17.6 ± 0.62	18.4 ± 0.42	17.8 ± 0.23	0.46
Belly, %	8.1 ± 0.12	8.3 ± 0.11	8.0 ± 0.21	8.2 ± 0.12	0.65
Neck, %	4.9 ± 0.15	5.4 ± 0.16	5.2 ± 0.16	5.3 ± 0.22	0.20

[a, b] LSMeans with different superscripts within rows indicate significantly differences (*P*-values as specified in the Table)
ICS6 = lambs receiving their second vaccination six weeks before slaughter
ICS4 = lambs receiving their second vaccination six weeks before slaughter
B = lambs physically castrated on day 2 using a Burdizzo clamp
R = intact ram controls

8.3.2 Longissimus thoracis muscle area, fat thickness and objective meat quality

Treatment did not influence the LT muscle area as determined in this study (Table 8.2). There was a decline in subcutaneous backfat thickness, with the B lambs having the thickest (P < 0.01) subcutaneous backfat, followed by immunocastrated lambs and then intact rams (Table 8.2). Although no differences were observed for pH_{24}, the LT muscle samples of IC4 lambs were characterised by higher (P = 0.005) L* values when compared to the IC6 samples (Table 8.2), but did not differ from samples obtained from B and R lambs. The ICS6 lamb meat surface colour

L* values did not differ from the values recorded for R rams (Table 8.2). Intact rams had the highest a* values, followed by ICS4, B and ICS6 ($P < 0.001$; Table 8.2). No differences were seen for b*, drip loss, cooking loss and Warner-Bratzler shear force for all treatments (Table 8.2).

Table 8.2. The effect of vaccination interval between second vaccination and slaughter on the *Longissimus thoracis* muscle area, backfat depth, cut surface colour and instrumental meat quality of immunocastrated Dohne Merino ram lambs compared to Burdizzo-castrated lambs and intact lambs.

Parameter	Vaccination Schedule				*P*-value
	IC6	IC4	B	R	
Muscle area, mm^2	184.9 ± 15.6	164.3 ± 13.7	173.3 ± 8.24	175.0 ± 7.21	0.85
Subcutaneous fat depth, mm	3.2 ± 0.23	3.3 ± 0.32	4.3 ± 0.48	2.1 ± 0.21	< 0.01
pH_{24}	5.7 ± 0.02	5.7 ± 0.03	5.7 ± 0.02	5.7 ± 0.03	0.31
CIE Lab colour:					
*L**	38.3^{b} ± 0.19	40.9^{a} ± 0.47	39.6^{a} ± 0.19	39.2^{ab} ± 0.75	0.005
*a**	16.5^{d} ± 0.033	24.0^{b} ± 0.28	20.3^{c} ± 0.30	26.8^{a} ± 0.34	< 0.001
*b**	12.3 ± 0.18	13.0 ± 0.38	12.9 ± 0.24	12.4 ± 0.31	0.20
Drip loss, %	1.0 ± 0.05	1.0 ± 0.07	1.1 ± 0.04	1.0 ± 0.05	0.17
Cooking loss, %	26.7 ± 0.50	26.4 ± 0.59	27.0 ± 0.61	27.7 ± 0.50	0.44
Shear force, N	48.0 ± 1.84	45.7 ± 3.88	40.2 ± 2.05	45.1 ± 2.23	0.11

[a, b] LSMeans with different superscripts within rows are significantly different (*P*-values as specified in the Table)
ICS6 = lambs receiving their second vaccination six weeks before slaughter
ICS4 = lambs receiving their second vaccination six weeks before slaughter
B = lambs physically castrated on day 2 using a Burdizzo clamp
R = intact ram controls
pH_{24} = pH measured 24 hours *post-mortem*

8.4. Discussion

Differences between sexes for carcass and meat quality have been established in numerous studies in sheep, well summarised by Hopkins and Mortimer (2014), and compared using meta-analysis by Sales (2014). However, studies on carcass characteristics and meat quality traits of immunized ram lambs are scarce. Physical castration of rams indicated a tendency for wethers to have a lighter carcass weight than intact rams, with greater backfat depths and a smaller *Longissimus thoracis et lumborum* (LTL) muscle area (Sales, 2014). These observations were supported by the current study, where no difference in LT muscle area was observed between

R rams, B lambs, and IC4 and IC6 lambs as pertaining to muscle area. However, the study duration of 57-days is short compared to the duration from early physical castration of lambs shortly after birth to slaughter. Should lambs be immunocastrated shortly after birth, the effects on growth and carcass weight would need to be quantified due to an early disruption of the reproductive system.

Intact rams were leaner than Burdizzo-castrated lambs with an intermediate backfat depth reported in the IC4 and IC6 rams. Although no differences in fatness have been reported for immunocastrated cattle under a range of conditions (Adams et al., 1993; Adams et al., 1996; Huxsoll et al., 1998; Cook et al., 2000; Aïssatet al., 2002; Ribeiro et al., 2004; Amatayakul-Chantler et al., 2013; Miguel et al., 2014), varying degrees of carcass fatness were reported in immunocastrated swine carcasses compared to intact or castrated controls (Dunshea et al., 2001; Zamaratskaia et al., 2008; Pauly et al., 2009; Gispert et al, 2010). In swine, immunocastrates also have intermediate carcass traits to intact males and surgical castrates, depending on the vaccination schedule (Bonneau et al., 1994). Immunocastrated Karakas lambs did not differ from intact males in terms of subcutaneous fat weight but experienced less fat deposition than elastrator band castrated lambs (Ülker et al., 2002). However, the plane of nutrition will also influence the degree to which fat deposition is affected, as low levels of nutrition can suppress sex differences in growth rate and thus potentially fat deposition between rams and wethers (Prescott, 1969). All the lambs in this experiment had sufficient feed and so this factor was deemed to have minimal influence on the results. No differences were seen between sexes in the current study for LT muscle area, which corresponds to the findings of Ülker et al. (2002), Daley et al. (1995), and Kiyma et al. (2000).

Intact rams also produce a higher percentage of discarded tissues, which include skin, head and reproductive tissue, compared to castrated lambs (Gatford et al., 1996). Similarly,

intact rams in this study tended to have heavier skins than castrated lambs. However, Ülker et al. (2002) found no differences between immunocastrated, elastrator-castrated and intact Karakus lambs regarding offal weights in their study. The same observation was reported for offal weights of native Turkey rams immunocastrated earlier at 10 weeks of age (Ülker et al., 2003). However, it would be preferable to evaluate both offal and wholesale cut weights as a percentage of the live weight and carcass weight, respectively, to account for the effects of live weight and carcass weight on offal weight and wholesale cut weights respectively. Unlike the peri-pubertal lambs in Chapter 4 (4.3.2), no differences were seen in red offal percentages and offal percentage means closely resembled those of the post-pubertal lambs in Chapter 3 (3.3.4). All castration treatments decreased scrotal and testes percentages to nearly half that of the intact rams, in accordance to the results reported for scrotal circumference (Chapter 6) and trimmed testes weights (Chapter 7).

The differences seen in GIT and GIT fat percentages between treatments may be a result of providing a higher plane of nutrition to the lambs compared to the trial in Chapter 4, allowing differences to materialise which would not have been seen otherwise. The lower GIT percentages for physically castrated lambs may thus represent decreased gut fill. Although, meta-analysis has shown that wethers do not differ from rams in feed intake but do take longer to reach slaughter weight and thus require a longer duration of feeding (Sales, 2014). Therefore, a feeding trial needs to be conducted to further investigate the effects of castration status on feed intake using a high plane of nutrition in a feedlot environment. The increased GIT fat percentages may also indicate a shift in metabolic functioning from that of an intact ram to that of a physically castrated ram. However, should an even higher plane of nutrition be provided, the effects on fat deposition may be more pronounced and thus the slaughter interval used should be carefully considered.

Commercial prime cut yields are indicators of differential growth and in rams of different castration statuses, testosterone is largely responsible for differences in muscular and skeletal growth during puberty (Harper, 1969). Rams show more development in the neck and shoulder cuts than physical castrates (Kemp et al., 1970) with differences in musculature noticeable at approximately five months old (Hammond, 1932). In cattle, however, hindquarter development appeared to be more pronounced in immunocastrated bulls than in steers and bulls (Miguel et al., 2014). In swine, immunocastration increased the LTL percentage compared to that observed for entire males (Gispert et al., 2010). Immunocastrated pigs also had lighter heads, feet, hams and shoulder cuts than boars (Bonneau et al., 1994). Although, Ülker et al. (2002) reported that immunocastrated lambs had greater chest widths than intact rams but not physical castrates, no differences were found in wholesale cut weights between sexes. Likewise, no differences were seen in the commercial prime cut yield percentages of the lamb carcasses in this study for the respective castration treatments. The lack of differentiation in hindquarter and forequarter development between the immunocastrated, Burdizzo-castrated and intact lambs is likely due to the short duration of the study and perhaps the fact that they were castrated at a physiological stage considered peri/post-puberty. Thus, subcutaneous fat deposition seems to respond faster than muscle development to decreased androgen secretion in post-pubertal rams.

Although fatness of the meat is important for eating quality, meat colour is one of the first quality attributes a consumer considers when buying meat. Testosterone has been shown to have negative correlations with CIE b* (yellowness) colour of the LTL muscle (Fahmy et al., 1999) and thus meat colour is influenced by castration status. Immunocastration has improved beef colour attributes compared to intact bulls, showing greater redness (a*), lightness (L*) and b* (yellowness) values thus having a possible advantage regarding retailing

(Miguel et al., 2014). However, lambs in this study showed varying L* values for cut meat surface colour, with ICS4 and B having lighter meat than ICS6 with R being intermediate. Although, the differences in L* values were minor, the differences seen between R and ICS6 for a* values motivate further investigation into whether consumers may be able to perceive these differences in the cut surface colour a* values for the respective treatments. Extending the interval between second vaccination and slaughter decreased a* values resulting in less red meat compared to all other treatments; however, the a* values for ICS6 more closely resemble that reported by Cloete et al. (2012) for Dohne Merinos of similar live slaughter and carcass weights.

Meat pH is important for flavour, aroma and colour properties of meat as well as stability in terms of shelf-life. Intact male rams are more likely to produce meat with a higher pH (Cloete et al., 2012) but this is seldom translated into meat quality issues (Hopkins & Mortimer, 2014). In cattle, meat pH was higher in intact bulls, but cooking loss values did not differ between immunocastrates, bulls and steers (Miguel et al., 2014, Ribeiro et al., 2004; Amatayakul-Chantler et al., 2013). However, the effects of immunocastration on beef tenderness are conflicting (Cook et al., 2000; Ribeiro et al., 2004; Amatayakul-Chantler et al., 2012; Amatayakul-Chantler et al. 2013; Miguel et al., 2014). Meat pH did not differ between lambs in this study 24 hours *post-mortem* and was similar to values reported by Cloete et al. (2012) and is in accordance with acceptable pH ranges. The pH values for meat samples obtained from Dohne Merino rams reported in the study of Cloete et al. (2012) did not differ between 24 to 48 hours *post-mortem*. Similar values for objective meat quality tests were found between the current study and the study of Cloete et al. (2012), even though in the latter study, meat quality was assessed 48 hours *post-mortem*.

Although ageing of lamb meat may improve tenderness (Young et al., 2006), lamb meat is not aged in South Africa and is distributed from the abattoir cooling rooms to retailers as early as 24 hours *post-mortem*. Cloete et al. (2012) reported Warner-Bratzler shear force values for meat from intact rams that were twice as high as what was reported for the meat from intact rams in the current study, despite using the same meat cooking methodology before shear force analysis. Thus, the age differences between the sheep used for the two studies had a large effect on meat tenderness, despite similar carcass weights and other meat quality parameters.

Cooking loss of meat is an important factor to consider in terms of overall liking and flavour scores of lamb meat (Hopkins et al., 2006). Meat from physically castrated lambs tend to have lower cooking losses and less tough meat than intact rams (Sales, 2014), however, no differences were seen for drip or cooking losses between immunocastrated, Burdizzo-castrated and intact rams in this study. Both meat drip and cooking losses over all treatments within this study were similar to that reported by Cloete et al. (2008) and Cloete et al. (2012) for Merino-type and Dohne Merino sheep respectively.

Meat shear force represented the largest source of variation when flavour and juiciness scores are considered (Hopkins et al., 2006). In this study, meat shear force obtained using the Warner-Bratzler was 44.7 ± 8.89 N, with no differences observed between treatments. Australian consumers consider shear force values of below 49 N as being acceptable (Shorthose et al., 1986); however, a more recent study by Hopkins et al. (2006) indicated that lamb LTL meat needs to have a shear force of 27 N or less to achieve a consumer failure rate less than 10 %. Although the meat from the current study was electrically stimulated, it would be considered acceptable according to Shorthose et al., (1986). Processing the lamb meat further by ageing for 5 days may improve tenderness to that within the range deemed optimal by Hopkins et al. (2006). However, shear force measurements correlate well with sensory meat tenderness when

trained sensory panels are used (Safari et al., 2001) but not necessarily with consumer panels due to the high variation in results (Hopkins e al., 2006). Furthermore, consumer acceptability of meat quality depends on a range of factors, including nationality, and thus it would be beneficial to evaluate the consumer acceptability of lamb meat with varying shear force measurements within South Africa.

8.5. Conclusion

Immunocastration resulted in lamb carcasses with an intermediate backfat depth, without negatively influencing the other carcass traits. The lack of differences for slaughter performance and carcass cutting yields can potentially be ascribed to the short interval between treatment application and slaughter and motivates the use of immunocastration as an acceptable alternative to both physical castrated and intact male lamb production. Nutrition may influence these findings, and thus the effects of varying planes of nutrition should be quantified. The effect of vaccination interval on fresh meat surface colour values needs to be evaluated using a sensory panel and/or a consumer panel to see whether the change in meat surface colour influence consumer perception and ultimately choice. The objective meat quality parameter values are in accordance with previously normal ranges reported for lambs of similar age and genotype. In conclusion, immunological castration of lambs may be used to manipulate carcass fatness of rams to meet market demands, without adversely influencing slaughter performance, cutting yields or meat quality.

8.7. References

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CHAPTER 9

Application of immunocastration in commercial Dohne Merino ram flocks before weaning: a technical note

Abstract

The influence of immunocastrating young ram lambs before weaning on the growth, incidences of reaction to vaccination, serum testosterone concentration and slaughter performance were determined. Immunocastration was performed using 2 doses of 2 mL Improvac® on the shoulder, administered subcutaneously using a Sterimatic® needle guard system that included a Stericap® fitted to the multi-dose injector. The first vaccination was administered to 50 lambs with an average weight of 16.0 ± 3.05 kg, and the second vaccination was carried out six weeks later, when the lambs weighed on average 20.5 ± 4.11 kg. Fifty lambs, with an average weight of 16.0 ± 3.05 kg, were physically castrated using elastrator bands, simulating the local standard commercial practice. Four data collection points were chosen according to the management plan for the commercial enterprise and sampling was minimised, where lambs were weighed, blood samples (n = 20) collected, testes examined to establish presence, and reaction to vaccination was established. Mortalities amounted to 8 % in the first six weeks of the trial and were unrelated to treatment. Despite the expected decreased growth rate due to chronic pain in elastrator-castrated lambs, no differences were reported for weight gain between the two castration methods for the duration of the trial. Both castration methods were successful in preventing testosterone secretion. Improvac® can thus be considered successful in eliciting an immune response and thus testosterone suppression in young lambs. Testes weights were drastically reduced at slaughter and thus is likely that normal development and functioning of the reproductive tract would be permanently interrupted. The Sterimatic® and Stericap® system was successful in providing an easy-to-use and safe system to use commercially, with no reactions to vaccination recorded at the injection sites. Early immunocastrated lambs performed similarly to elastrator castrated lambs with regards to growth, carcass weights and carcass fatness.

9.1 Introduction

In South Africa, ram lambs are castrated so that mixed-sex flocks may be maintained in extensive production systems, without the welfare concerns of aggressive behaviour and unwanted breeding amongst slaughter lambs. Although the effects of post-weaning immunocastration of Dohne Merino ram lambs has been investigated from a research point of view (Chapters 4 to 8), the potential of the protocol for application in industry needs to be validated.

As highlighted throughout the previous chapters, lamb production in South Africa involves a range of production systems. Management plans regarding sheep production need to be flexible to accommodate as best possible all activities on the farm and ensure that factors such as nutrition and climate influences are accommodated to ensure viable and sustainable production. It is therefore necessary to investigate the application of immunocastration under commercial production conditions, including ram lambs from different age groups. Although vaccinating ram lambs after weaning will potentially minimise the physiological stress that lambs and ewes experience while the lamb is young, some producers may still prefer to immunocastrate lambs before weaning. Immunocastrate lambs prior to weaning requires both the inter-dose period and the slaughter interval after second vaccination to be longer than the intervals investigated in Chapters 4 to 8.

Early immunocastration of ram lambs at two to three months of age (20 kg) with Improvac® (2 x 2mL with a three-week inter-dose period) was successful in suppressing testosterone secretion for three months after the second vaccination (Janett et al., 2003). Testosterone concentrations were low in control intact rams (0.1 – 0.9 ng/mL), with no differences recorded between intact and immunocastrated lambs. However, the effects of early immunocastration using Improvac® in ram lambs has not been compared to elastrator-castrated lambs, which is

the preferred physical castration method in industry for young lambs. The potential of a safety 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 in Chapters 4 to 8 in pre-weaning lambs in a commercial production system using a safety vaccinator system (Sterimatic®). The influence of an extended vaccination period as well as a slaughter interval, were studied in a commercial environment to ultimately assess the influence of immunocastration on the growth and serum testosterone concentrations of ram lambs, compared to elastrator-band castrated lambs under the same production conditions.

9.2 Materials and Methods

Ethical clearance was obtained from the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00073) and animal husbandry was in accordance specifications of the South African National Standards 10386: 2008.

9.2.1 Animals, castration & data collection

One hundred Dohne Merino ram lambs (average live weight 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 treatments, which included 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 not only consisted of commercial Dohne Merino production for meat and wool, but also an Angus cattle stud, wildlife breeding unit and various crops. Thus, an extended vaccination schedule had to be developed that could be accommodated in the daily management of the farm. Therefore, a six-week vaccination interval was chosen to coincide with dosing for internal parasites and vaccinating for enterotoxaemia (pulpy kidney). Fifty lambs were injected subcutaneously with 2 mL Improvac® in front of the shoulder region (Figure 9.1) using a Sterimatic® needle guard system (Sterimatic Worldwide Ltd, UK) fitted with a Stericap® (Figure 9.2; Sterimatic Worldwide Ltd, UK). The Stericap® is 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, and care was taken to ensure that the testes were below the ring before the applicator was removed (Figure 9.3).

Figure 9.1 The recommended vaccination site for subcutaneous injection of lambs using the Sterimatic® system chosen for the immunocastration of 50 commercial Dohne Merino ram lambs (16.0 ± 3.05 kg) raised extensively (source: http://www.fwi.co.uk/advertisement/sterimatic-how-to-guide-for-injecting-cattle-and-sheep.htm).

Figure 9.2 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 25mm metal hub needle, resulting in a 12mm 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® (source: http://www.fwi.co.uk/advertisement/sterimatic-how-to-guide-for-injecting-cattle-and-sheep.htm).

Figure 9.3 Illustration of the method used to elastrator-band castrate ram lambs, using rubber rings which are placed above the testes, but below the nipples, using an applicator (source: http://www.infovets.com/books/smrm/c/c104.htm).

To minimise disruption of handling activities, data recording was simplified and timed to be accommodated as part of the farm management program. Lambs were weighed at first injection/elastrator-band castration at Week 1 (W1), at second vaccination (W6) and 70 days later (W16). Blood samples were collected during W1, W6 and W16 from the same ten

randomly chosen animals per treatment after weighing (please refer to Chapter 3) to determine the potential of the extended vaccination interval to effectively interrupt/inhibit testosterone production. Serum samples were processed and subjected to ultra-performance convergence chromatography tandem mass spectrometry analysis to determine serum testosterone concentrations (please refer to Chapter 3; Quanson et al., 2016). Injection sites were monitored at W6 and W16 and scored using Table 6.2. Testes palpation was performed on 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.

9.2.2 Statistical analysis

An 8 % mortality rate was recorded after W1 from spinal infection after tail docking being the primary cause. 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 (StatSoft Inc.) 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 the random effect was animal. The ADG data and slaughter data were analysed using ANOVA. Fisher's LSD was the chosen post hoc test and differences between means are reported at a significance of 5 %.

9.3 Results & discussion

The results for the trial are summarised in Table 9.1. Serum testosterone concentrations were higher ($P < 0.001$) in EC lambs than IC prior to the application of either castration method in Week 1. Serum testosterone concentrations in all EC lambs were higher than 0.2 ng/mL, with three lambs having serum concentrations exceeding 1.0 ng/mL, thus experimental error is unlikely as would be seen should an outlier be recorded. Needle contamination within the UPC^2-MS/MS is also unlikely as a blank sample was inserted before and after the standards of varying testosterone-1, 2-d2 concentrations were analysed, prior to sample analysis. Therefore, the reason for the high mean testosterone concentration for EC lambs within W1 is unknown.

Testosterone concentrations decreased ($P < 0.001$) from W1 to W6, after elastrator-castration, and serum testosterone concentrations remained low for the duration of the trial. Immunocastration prevented an increase in testosterone secretion, the production of which can be expected in intact lambs with an average live weight of 35.6 ± 4.74 kg. In Chapter 5, an average serum testosterone concentration of 2.8 ± 0.34 ng/mL was reported in lambs with an average live weight of 35.0 ± 2.18 kg. In the current study, both immunocastration and elastrator castration were thus successful in suppressing testosterone secretion.

Early immunocastration using Improvac® in young ram lambs was successful in suppressing testosterone secretion, as similarly reported in rams vaccinated at 20 kg, which resulted in suppressed testosterone secretion for up to 12 weeks after second vaccination (Janett et al., 2003). The borderline non-detectable serum testosterone concentrations can potentially

be of adrenal gland origin and can be considered negligible. Thus, Improvac® was successful in eliciting an immune response within young lambs, as has been reported in swine (Brunius et al., 2011) and is likely to have also permanently interrupted normal development and functioning of the testes.

Although IC serum testosterone concentrations were no different to EC serum concentrations, testes were present in all lambs at the initiation of the trial and were observed at W16 in immunocastrated lambs. The testes of immunocastrated lambs were barely palpable and extremely small, compared to the average scrotal circumference (23.5 ± 2.20 cm) reported for lambs in Chapter 6. The testes of the lambs castrated with the elastrator-bands sloughed off before W6, which according to Melches et al. (2007), normally occurs around five weeks after castration. Elastrator castration was incorrectly applied to one lamb, which had one testis (90.508 g) present within the body cavity at slaughter. Immunocastration was ineffective in one lamb as well (101.898 g), possibly from only received one successful injection with Improvac®. The slaughter age and live weights (49.01 ± 4.85 kg) of the lambs within this trial was similar to that in Chapters 5 (52.4 ± 1.41 kg) and 7 (52.6 ± 4.73 kg), and thus one would expect the trimmed paired testes weights of an uncastrated/intact male to be approximately 258.5 ± 19.58 g to 279.3 ± 20.14 g, as reported in Chapter 5 and 7 respectively. Therefore, should the afore-mentioned immunocastrated lamb with larger testes be a non-responder to immunocastration, its testes weights should have been heavier than recorded. Two immunocastrated lambs had only one testis present at the time of slaughter, weighing 7.740 and 6.167 g respectively, and were omitted from the determination of the mean testes weights for the immunocastrated lambs. When immunocastration was performed after weaning, paired testes weights were between 76.7 ± 4.71 to 90.1 ± 7.34 g (Chapter 5) and 79.2 ± 4.71 to 92.4 ± 9.44 g (Chapter 7). However, administering the first vaccination prior to weaning resulted in

testes weights of 14.3 ± 1.25 g and thus not only successfully interrupted reproductive functioning, but the development of the testes before puberty (Table 9.1; Figure 9.4). Immunocastration was thus successful in interrupting testes growth and thus functioning 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 thus two months longer than when lambs were injected with their primary dose at two months of age (Janett et al., 2003).

Figure 9.4 Testes collected from Dohne Merino lambs immunocastrated prior to weaning and slaughtered 25 weeks after primary vaccination (~ 1 month of age).

While the injection site was not monitored intensively, no reactions were evident at the injection site after immunocastration vaccinations on the days of measurement indicating that the location and hygiene system was 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 vaccination taking a similar amount of time to perform as elastrator-band castration. Thus, the commercial unit 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 for the handlers to learn as injecting is frequently performed.

Although the immunocastrated lambs had a higher overall ADG (P = 0.04), no differences were observed in live weight and hot carcass weight (HCW) between the two castration methods (Table 9.1). 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 of dam 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 in Chapter 6. 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).

No differences were recorded for slaughter performance (HCW, dressing and offal percentages and grading (Table 9.1). 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 % and 67.4 % were A2. Despite the lack of differences in live weights and HCW, physical castration has been shown to reduce the welfare of ram lambs (Melches et al., 2007; Chapter 6) and thus both consumer and producer concern about animal welfare and the enforcement of legislation would be the largest motivation to abolish the practise.

Table 9.1 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.

Parameter	Elastrator-castrated				Immunocastrated			
	Week 1	**Week 6**	**Week 16**	**Week 25**	**Week 1**	**Week 6**	**Week 16**	**Week 25**
Testosterone, ng/mL	$0.87^{a} \pm 0.097$	$0.030^{b} \pm 0.087$	$0.034^{b} \pm 0.092$	-	$0.076^{b} \pm 0.087$	$0.052^{b} \pm 0.092$	$0.042^{b} \pm 0.092$	-
Reaction score, 0-5	-	-	-		0	0	0	
Testes present, Y/N	Y	N	N	N	Y	Y	Y	Y
Testes weight, g	-				14.3 ± 1.25			
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			$179.6^{b} \pm 3.50$				$190.0^{a} \pm 3.65$	
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 ($P \leq 0.05$)
Y = yes
N = no
ADG = average daily gain

9.4 Conclusion

Immunocastration was successful in suppressing testes development and testosterone production in ram lambs weighing less than 20kg and maintained in an extensive commercial production system. The use of tail docking should be re-examined, considering the high mortality rate resulting from this technique. The early immunocastration of ram lambs resulted in a similar growth and slaughter performance to that of elastrator castrated lambs. The negative effects of elastrator-band castration on the welfare of lambs has been well-documented and despite the lack of influence on growth, immunocastration has been indicated to be associated with the improved welfare of ram lambs castrated with Improvac®. However, the influence of immunocastration ram lambs maintained in a feedlot system where a high plane of nutrition is used, needs to be determined. The effect of early castration using Improvac® on testes functioning and behaviour of ram lambs also warrants investigation. The subcutaneous injection of Improvac® using the Sterimatic® and Stericap® system fitted to a multi-dose injector produced commercially acceptable results regarding reaction at injection sites, improved user safety, and efficacy.

9.5 References

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CHAPTER 10

General Conclusion and Recommendations

The physical castration of male livestock is used as a management tool and to manipulate carcass traits (Ladd et al., 1994; Cronin et al., 2003; Price et al., 2003); however, the practise is under scrutiny due to welfare concerns. While consumer pressure has been to enforce pain mitigation when physical castration is performed, the on-farm commercial application of this may be suboptimal. It is thus necessary to formulate and evaluate an alternative strategy to both the use of physical castration and intact male animals in meat production that can be easily accommodated in commercial South African lamb production systems.

Immunocastration shows potential for use in Dohne Merino ram lambs, a popular South African breed. To confirm the successful interruption of reproductive functioning of rams through immunocastration, parameters such as testes growth, testosterone concentrations, and semen quality need to be considered. Thus, using older, more developed, rams is preferred to quantify the effects of immunocastration on reproductive functioning. However, literature on the growth and sexual development of Dohne Merino rams is limited. Therefore, the sexual maturation of Dohne Merino lambs after puberty was characterised, providing standard parameter estimates for physiological indicators to aid in identifying successful immunocastration in subsequent trials. The scrotal circumference, testes cut surface colour values and seminiferous tubule circumference reported in this study provide a representation of rams entering sexual maturity with functional reproductive systems. The need to standardise season and time of day during which data collection is carried out, was confirmed by the variation in scrotal circumference and serum testosterone concentrations in the post-pubertal rams.

Currently, no commercial immunocastration vaccination schedules or protocols exist for ram lambs, and due to the variable response between species, an effective vaccination schedule needed to be designed that can be accommodated in management programs with minimal disruption of management activities. Although the immunocastration vaccine Improvac® is effective in a range of species, including sheep (Janett et al., 2003), aspects such as injection frequency as well as the administration interval and period from last administration to slaughter had to be established. Immunocastration vaccination schedules were thus investigated in both peri-pubertal lambs from approximately 35 to 49 kg as well as pubertal rams from approximately 45 to 53 kg, due to the potential greater influence on the welfare of older rams.

Two-, three- and four-week intervals between primary and secondary vaccinations were successful in disrupting reproductive functioning in peri-pubertal Dohne Merino rams until slaughter (four weeks after secondary vaccination), with no need for further vaccination and no negative influence on growth. Similarly, carcass traits were not influenced by the inter-vaccination period, and changes in offal yields were not observed in subsequent trials. Towards the end of this trial, live weights tended to differ between treatments, and thus extending the interval between second vaccination and slaughter may potentially result in ensuring a more pronounced effect on growth and may also result in differences in carcass traits, such as backfat thickness.

Changes in testes cut surface colour indicated a potential time-efficient way to effectively analyse for vaccination status on the slaughter line prior to carcass classification. Across all the studies, immunocastrated lamb testes cut surface colour showed a* values (3.0 to 4.9) at least twice that recorded for intact rams (0.7 to 1.4). Although immunocastration resulted in pronounced changes in testes colour, decreased testes weight, and seminiferous

tubule circumference and epithelium thickness, the thermoregulatory ability of the testes was not compromised.

The two-week interval between primary and secondary vaccination was identified as preferable, motivated by the low incidences of injection site reactions and the greatest decrease in serum testosterone production after the primary vaccination until slaughter. The decrease in serum testosterone after the primary vaccination of immunocastrates motivated measuring the semen quality of immunocastrates over time, to establish at which stage of the treatment period the reproductive capacity of the lambs was compromised. Reactions at the site of vaccine administration warrants adaptation of the vaccination protocol, with either distribution of the adjuvant or location of injection that need to be improved. The assessment of the injection site reaction resulted in the development of a more descriptive table for evaluation of site reactions during the subsequent trial. Stiffness in the forequarter was noted after vaccination within the first immunocastration trial, thus behavioural responses were recorded in conjunction with the measurement of serum cortisol concentrations during the subsequent trial.

Physically castrated lambs were included in the second immunocastration trial investigating extending the interval between second vaccination and slaughter to compare the effects of the two castration techniques. Extending the interval between second vaccination and slaughter from four to six weeks did not influence the growth performance of immunocastrated lambs, which had similar growth rates and prime cut yields, when compared to both Burdizzo-castrated and intact male ram lambs. Immunocastration improved the welfare of ram lambs compared to that of Burdizzo-castrated lambs. Behaviour and cortisol data indicated higher levels of stress and discomfort in Burdizzo-castrated lambs, despite pain mitigation using Metacam®; with abnormal behaviours continuing until a week after castration, indicative of the rams experiencing pain. Wound-healing was deemed complete two weeks after the Burdizzo

castration procedure, as judged by testes palpation and visual assessment. However, cortisol peaked during the third and fifth week of the trial indicating chronic physiological stress. Thus, pain mitigation was not effective in eliminating pain and discomfort experienced by Burdizzo-castrated lambs.

Reactions at the site of injection for immunocastrated lambs improved within the second immunocastration trial, and while stiffness in the forequarter was evident in some lambs, it did not influence normal behaviour. The subcutaneous administration of Improvac® on a flat surface area (shoulder) rather than behind the fore leg, where no friction or ground contact may occur, was likely the largest contributing factor to the decreased incidences of reactions at the injection site and is thus recommended for future applications.

The two immunocastration treatments did not produce the same cortisol responses, despite the use of the same inter-vaccination period, which could be due to the acclimatization of the animals to handling. Furthermore, the two immunocastration treatments did not result in similar serum testosterone concentrations or a decline in semen quality. The vaccination schedule using a two-week inter-vaccination/dose period, and a slaughter interval of six weeks after the second vaccination, was the most successful in decreasing the serum testosterone concentrations from primary vaccination. Androstenedione secretion followed similar patterns to that of testosterone across the studies, which could indicate its conversion into other androgen metabolites. However, results of some the analysis of some of the androgen metabolites identified from pathways in Figure 5.1 were under the detection limits for all serum samples.

The lambs injected with the second immunocastration vaccination dose six weeks before slaughter experienced a more consistent decline in sperm viability than all other castration treatments. All semen samples collected from the immunocastrated treatments

throughout this trial were considered fair to poor regarding mass motility, suggesting the disruption of spermatogenesis and sperm maturation. Intact rams experienced, as expected in the case of pubertal rams approaching sexual maturity, an improvement in the mass motility and viability of sperm as the trial period progressed. Although testes size and cut surface colour were influenced to the same extent by both immunocastration protocols, seminiferous tubule circumference was smaller in lambs immunocastrated using the extended six-week slaughter interval protocol, which demonstrates the more pronounced influence of this vaccination schedule on testes function.

Immunocastration resulted in carcasses with an intermediate backfat depth without influencing the LT muscle area, and it can thus be recommended that immunocastration may be used to manipulate subcutaneous backfat depths. Regarding meat quality characteristics, immunocastration and Burdizzo-castration resulted in a decrease in the redness of the LT meat cut surface colour. Previous studies on cattle reported contradictory effects of immunocastration on beef muscle colour (Amatayakul-Chantler et al., 2012; Amatayakul-Chantler et al., 2013; Miguel et al., 2014), and therefore this effect in lamb needs to be further investigated using a larger sample size. Thus, the vaccination schedule involving two doses of 2mL Improvac® given two weeks apart, the second of which is administered six weeks before slaughter, resulted in the most favourable overall performance with regards to growth, reproduction, reaction to vaccination, slaughter and carcass quality in Dohne Merino lambs.

Throughout the duration of the entire study, the importance of maintaining a high standard of hygiene when injecting lambs was emphasized, to ensure minimal injection site reactions. Furthermore, the identification of a system to ensure the safety of the person administering Improvac® was required. The Sterimatic® system was subsequently investigated and used to immunocastrate 50 extensively farmed Dohne Merino lambs in a commercial trial.

Using this system resulted in no reactions on the shoulder area where animals were injected, while successfully maintaining low testosterone concentrations without influencing growth or slaughter performance. Despite the limitations regarding data collection and nutrition, this trial presents a baseline for the refinement and adaptation of immunocastration to commercial lamb flocks in South Africa. This study also confirmed that early immunocastration is possible in ram lambs (~1-month-old), potentially permanently affecting the development and functioning of the reproductive system of Dohne Merino ram lambs.

Future research regarding the application of immunocastration in various production systems such as intensive feedlots, with a focus on feed intake, development of various fat depots, meat quality and sensory consumer acceptance will be beneficial. Due to the influence of immunocastration on subcutaneous backfat deposition, focus should be placed on the effects of immunocastration on the carcass composition, using techniques such as magnetic resonance imaging, to further investigate the effects on various fat depots and possible long-term effects on bone deposition, also taking into consideration the role of testosterone. The effects of immunocastration on the fatty acid profile, along with volatile fatty acids, of lambs is recommended along with sensory characterization compared to both physical and intact lambs. Although consumer acceptance of immunocastration in swine is positive in international consumers (Tuyttens et al., 2012), it is important to quantify consumer and farmer acceptance of immunocastration and the related products in South Africa.

Investigation into the application of the immunocastration vaccination schedule for sheep breeds of varying maturity types also needs to be done, particularly in feedlotting enterprises where weaned lambs of various breeds are purchased at auction for finishing. Analysis of the behaviour of immunocastrated lambs in feedlot systems would improve the understanding of the effects on sexual and aggressive behaviour, particularly under high

stocking rates. Any further investigations into the stress responses of immunocastrated lambs should incorporate an adaptation period to handling and data recording if serum cortisol concentrations are to be determined.

Due to the decrease in testosterone production, immunocastrated swine have been shown to have varying nutritional requirements and thus require adaptation of dietary feed formulations (Needham et al., 2016). The influence of the nutritional requirements of immunocastrated lambs receiving a concentrated diet within feedlots also needs to be carried out. The effects of growth-promoting pharmaceutical substances on the hormone profile, growth, carcass performance and meat quality of immunocastrated lambs should be considered, as this is still common practise in some industries.

The development of an extraction protocol for C-19 steroids from various androgenic tissues would provide further insight into the effects of such treatments on the numerous androgen pathways within rams. Investigation into the expression of FSH and LH receptors in the testes tissue may further explain the effects of suppressed GnRH and testosterone. Lastly, the effects of immunocastration on the mating and fertilizing capacity of rams of varying ages as well as the possible reversal of the immunocastration effect needs to be established should rams be maintained for longer periods to promote wool production yields. A large-scale commercial feedlot trial will also provide robust data regarding the discrepancies seen between trials for offal yields.

In conclusion, this project provides essential baseline data regarding how growth influence the reproductive development of Dohne Merino rams and provides guidelines regarding the commercial application and registration of Improvac® as a suitable pharmaceutical approach to the immunocastration in ram lambs slaughtered for meat production.

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