

Disruptive technologies for improved reproductive management of sheep

Emmah Goldsmith

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Statement of Originality

Other than the assistance mentioned in the acknowledgements, the studies contained within this thesis were planned, executed, analysed and written by the author, and have not been previously submitted for any degree to a University or Institution.

Emmah Goldsmith

BAnVetBio (Hons I)

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Throughout the years of this thesis, the Australian wool industry has been impacted by catastrophic floods, drought and a global pandemic. Now, more than ever, we must look for ways to build the efficiency and sustainability of agricultural industries.

Though this thesis is but a small contribution to the mission, it has moved me to continue in this space for years to come.

Summary

Previous research has reported that almost a third of all rams are ‘non-performers’ that have little to no sexual interest in ewes (Fitzgerald and Perkins, 1991; Hulet et al., 1964). This represents a significant cost to the Australian sheep industry through less lambs on the ground, extension of lambing windows, delays to first oestrus in ewe lambs and the inheritance of low libido in ram lambs (Bench et al., 2001; Hulet et al., 1964; Price et al., 1996; Stellflug et al., 2006).

Serving capacity tests are the current industry method for measuring ram libido though due to their high labour cost, they are rarely used outside of research. The test involves isolating a single ram in a pen with up to four ewes and monitoring their mating and courtship behaviours for at least twenty minutes. The serving capacity test (SCT) has remained largely unchanged since its development in 1971 and several studies have demonstrated that the SCT score is not always linked to in-paddock libido, suggesting an unfamiliar testing environment is to blame (Allison, 1978; Chaill et al., 1975; Kilgour and Whale, 1980; Mickelsen et al., 1982).

The advent of precision livestock farming (PLF) presents an opportunity to fill this knowledge gap by remotely monitoring behaviour. On-sheep sensor research has grown exponentially during the last decade and has recently focused on the identification of mating behaviour in rams (Alhamada et al., 2017a; Fogarty et al., 2018; Mozo et al., 2019). Despite this emerging body of research, commercially ready technology for woolgrowers is non-existent. The translation of findings from research-

grade sensors to sensors that monitor behaviour in real-time is difficult in extensive production systems (Bailey et al., 2021; Masters, 2021).

The overarching aim of this thesis was to develop reproductive monitoring technology for extensive sheep industries. The following objectives were used to achieve this aim:

- Develop a computationally simple algorithm to detect ram mounting behaviour in raw accelerometer data.
- Integrate the algorithm into a sensor that has commercial potential in the Australian sheep industry.
- Utilise this sensor to measure ram reproductive behaviour during joining and investigate the relationship of libido, semen quality and other physiological parameters on the siring ability of rams.

The initial development and validation of an algorithm to detect mounting behaviour in rams and wethers was undertaken in Chapter 2. This was conducted using research-grade accelerometers, which allowed for the exportation and manipulation of raw data post-factum. A moving average converge-divergence (MACD) algorithm was applied to raw along the (vertical) z-axis to smooth noise. A high-pass filter was then applied to identify peaks produced by mounting events. In a controlled pen environment, the performance of the MACD+ was outstanding, at 100% sensitivity and 96% precision. When this was applied to data collected in-paddock, performance (particularly precision) decreased. False positives were incurred from high-velocity behaviours, such as running and headbutting, that were not observed in the pen environment. Extra conditions were added to the MACD (MACD+) to filter mounting peaks from other high-velocity behaviours. This significantly improved the performance of the

algorithm, returning a precision of 94% and a sensitivity of 92%. The MACD+ was also 91% precise and sensitive for wethers. The performance of the MACD+ made it a suitable candidate for integration into a commercial-type sensor.

In Chapter 3, we transferred the MACD+ algorithm developed in Chapter 2 to a sensor with commercial potential in the Australian sheep industry (Smart Tag; Australian Wool Innovation Pty Ltd). Unlike the research-grade accelerometers used in Chapter 2, the Smart Tag identifies mounting behaviour on the sensor in real-time and transmits mounting alerts through a wireless network to an external server. This was a crucial step not only in this thesis but in the broader research field. The performance of the MACD+ declined following its transfer to the Smart Tag, however these losses were tolerable. Mounting events were identified in real-time with 88% sensitivity and 86% precision.

The technology was applied in a commercial setting in Chapter 4 to investigate the factors that determine a ram's siring ability. Smart Tags were used to quantify the libido of rams (n=17) by taking the average number of mounts performed per day. The semen quality, testosterone level and scrotal circumference of rams were also measured, and parentage testing of lambs was undertaken. It was found that the libido of rams was significantly correlated with siring ability. This was attributed to the highest-performing ram and the lowest-performing ram siring high and low numbers of lambs, respectively. Outside of these extremes, the effect of libido on siring ability was more nuanced. The role of semen quality, scrotal circumference and testosterone in siring ability could not be clearly defined in this study and requires further investigation beyond this thesis. Nonetheless, it is clear that the Smart Tag could be

used as a decision support tool to identify rams with low libido and thus poor siring ability.

This thesis details the development, validation and application of a novel sensor to detect the libido of rams in real-time. It is the first of its kind with commercial potential in extensive sheep production systems. While further research is required to ascertain the cost-benefit of the technology and its suitability across regions and breeds, it demonstrates enormous potential to improve reproductive efficiency and to herald in commercially available on-sheep sensors.

List of abbreviations

°C	degrees celsius
Acc	acceleration
AIC	akaike information criterion
ASBV	Australian Sheep Breeding Values
BCS	body condition score
BSE	breeding soundness evaluation
CIDR	controlled internal drug releasing device
CSV	comma separated values
CV	coefficient of variation
DNA	deoxyribonuclease acid
dp	datapoint
ELISA	enzyme-linked immunosorbent assay
FN	false negative
FP	false positive
<i>g</i>	g-force
GNSS	global navigation satellite system
GPS	global positioning system
Hz	Hertz
IU	international unit
L	litres
m	metres
m (prefix)	milli ($\times 10^{-3}$)

MACD	moving average convergence-divergence
MCU	microcontroller unit
MLP	Merino Lifetime Productivity
PLF	precision livestock farming
QDA	quadratic discriminant analysis
RFID	radio frequency identification
ROTE	random over-sampling technique
s	seconds
SC	scrotal circumference
SCT	serving capacity test
SEM	standard error of the mean
SMOTE	synthetic minority over-sampling technique
SNP	single nucleotide polymer
T	testosterone
TN	true negative
TP	true positive
μ (prefix)	micro milli ($\times 10^{-4}$)
V?	volts
VIF	variance inflation ratio

List of figures

Figure 1.1: Rams wearing a mating harness with a blue crayon attached to the harness of the ram in the foreground. Blue marks on the ewes' rump indicate that she has been joined (Ehrhardt, 2020).

Figure 1.2: The typical orientation of an accelerometer on a quadrupedal vertebrate (Whitford and Klimley, 2019).

Figure 1.3: Schematic diagram of a capacitive accelerometer, where g is the force of acceleration (Venkatanarayanan and Spain, 2014)

Figure 1.4: Representation of features used in the algorithm developed by Mozo et al. (2018) to detect mounting behaviour in rams and a brief explanation of algorithm functioning. Features: Orange, kyz; Cyan, yz moving average; Red, angle; Green, angle average; Grey, derivative of x with respect to time; a, kyz variation; b, angle increase; c, angle decrease; d, mount length; e, consecutive mounts interval; Q₁, first mount; Q₂, second mount (Mozo et al., 2018)

Figure 1.5: Diagram of the Alpha-Detector system. (a) a leather harness containing an Alpha Detector, (b) the Alpha Reader (AR), (c) a ram wearing the Alpha-Detector during mating (Alhamada et al., 2016a).

Figure 2.1: The attachment points of the accelerometer to the withers (a) and neck (b) of a ram via a harness and collar, respectively, with the x-axis corresponding to the lateral plane of the ram. Illustration created by Dr K. R. Pool.

Figure 2.2: An example of a mounting event (shaded grey) in MACD transformed data (black) and raw z-axis data (grey) retrieved from a harness-attached accelerometer.

Figure 2.3: The behaviour class of false positives for each male type under the MACD and MACD+.

Figure 3.1: The Smart Tag (a) fitted posterior to the withers of a ram via a harness.

Figure 3.2: The average peak height (g) of the MACD during mounts $\pm$ SEM.

Figure 3.3: The proportion of behaviours falsely classified as mounting events (FP) by the Smart Tags, including headbutting (yellow), courting (light blue), running (dark blue), dismount (grey) and being headbutted by another ram (orange).

Figure 4.1: The Smart Tag (a) fitted posterior to the withers of a ram via a harness.

Figure 4.2: The average number of mounts performed by all rams across each day of the trial. Error bars represent the SEM.

Figure 4.3: A correlation matrix for the variables included in the study where, 1 = percent change in testosterone, 2 = percent change in scrotal circumference, 3 = body condition score, 4 = sperm concentration, 5 = colour and consistency, 6 = percent normal sperm, 7 = average number of mounts per day, 8 = post-joining testosterone, 9 = post-joining scrotal circumference, 10 = pre-joining testosterone, 11 = pre-joining scrotal circumference, 12 = number of lambs sired, 13 = subjective motility, 14 = semen volume, and 15 = wave motion.

Figure 4.4: The interaction between the mean number of mounts performed by a ram per day and the number of lambs sired.

Figure 4.5: The interaction between the scrotal circumference of a ram prior to joining and the number of lambs sired.

Figure 4.6: The interaction between the change in scrotal circumference of a ram from pre-joining to post-joining and the number of lambs sired.

Figure 4.7: The interaction between ejaculate volume and the number of lambs sired by a ram.

List of tables

Table 1.1: The criteria used by Van Metre et al. (2012) during the breeding soundness evaluations of rams.

Table 1.2: Commercially available on-animal sensors to monitor reproductive behaviours in livestock.

Table 2.1: Descriptions of the behaviours of rams and wethers recorded in trials 1 and 2 to develop algorithms detecting mounting behaviour.

Table 2.2: The total number of mounts for each ram during Trial 1.

Table 2.3: The frequency of mounting events for rams and wethers joined with 20 ewes in a paddock in Trial 2. Significant differences are denoted by superscript letters.

Table 2.4: The overall performance of the MACD and MACD+ algorithms in the detection of mounting events performed by rams and wethers. Optimal thresholds are defined by the maximum F1 score and are bolded.

Table 2.5: The performance of the MACD and MACD+ algorithms for individual rams and wethers in the detection of mounting events.

Table 2.6: The performance of the MACD and MACD+ algorithms in the detection of mounting behaviour of rams and wethers under individual optimal thresholds.

Table 3.1: Descriptions of observed behaviours in rams.

Table 3.2: The classification performance of the Smart Tags, including correctly identified mounting events (TP), mounting events that were not identified (FN), other

behavioural events that were falsely classified as mounting events (FP), and the sensitivity, precision and F1 score.

Table 3.3: The classification performance of the Smart Tags, including correctly identified mounting events (TP), mounting events that were not identified (FN), other behavioural events that were falsely classified as mounting events (FP), and the sensitivity, precision and F1 score.

Table 4.1: Descriptive statistics of variables measured from mature Merino rams (n=19) describing the mean, standard error of the mean and the range of the data.

Table 4.2: The average number of mounts performed per day by individual rams and the number of lambs they sired.

List of publications

The work described in this thesis has resulted in or contributed to the following published chapters or abstracts.

Chapter 2

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I designed the study, collected and analysed the data and wrote the drafts of the manuscript. L.R. Mercorelli co-analysed the data. J.P. Rickard, L.A. González and S.P. de Graaf provided reviews and edits of the manuscript drafts.

Student: Ms Emmah Goldsmith

I believe the statements above to be a true reflection of my contribution to the research chapters which have constituted this thesis.

Emmah Goldsmith, 17th January 2023

Supervisor: Professor Simon de Graaf

As supervisor for the candidate upon which this thesis is based, I can confirm that the authorship attribution statement above is correct.

Simon de Graaf, 17th January 2023

Table of Contents

Statement of Originality	ii
Acknowledgements.....	iii
Summary	v
List of abbreviations.....	ix
List of figures.....	xi
List of tables	xiv
List of publications.....	xvi
Authorship attribution statement	xviii
Chapter 1 – Review of the literature	1
1.1 Abstract	1
1.2 General Introduction.....	2
1.3 Current methods used to predict the reproductive performance of rams	5
1.3.1 Pre-joining inspection – the ‘five Ts.’	6
1.3.2 Breeding soundness evaluations	8
1.3.3 Parentage testing.....	11
1.3.4 Serving capacity tests.....	12
1.3.5 The ram mating harness	15
1.4 Precision livestock farming.....	16
1.4.1 The types of on-animal sensors commonly used in PLF research	18
1.4.2 The use of on-animal sensors in the sheep industry.....	22
1.4.3 Sensors to monitor reproductive behaviour in rams	26
1.4.4 Sensors to monitor reproductive behaviour in ewes	32
1.4.5 Commercial adoption of on-animal sensors in the livestock industries.....	34
1.5 Conclusion	38
Chapter 2 – The use of accelerometers for the remote detection of mounting behaviour in rams and testosterone-treated wethers.....	45
2.1 Abstract.....	45
2.2 Introduction.....	46
2.3 Materials and Methods.....	48
2.3.1 Trial 1: Development of algorithm in a controlled environment.....	49

2.3.2 Trial 2: Validation of the algorithm in a field environment and addition of other conditions.....	54
2.4 Results	58
2.4.1 Trial 1: Development of algorithm in a controlled environment	58
2.4.2 Trial 2: Validation of the algorithm in a field environment and addition of other conditions.....	59
2.5 Discussion	66
2.6 Acknowledgements	72
Chapter 3 – Monitoring the sexual behaviour of rams in real-time through a wireless sensor network.....	73
3.1 Abstract.....	73
3.2 Introduction	74
3.3 Materials and Methods.....	76
3.3.1 Trial 1: Pen trial with research-grade Smart Tags.....	78
3.3.2 Trial 2: Paddock trial with production-grade Smart Tags.....	80
3.4 Results	82
3.4.1 Trial 1: Pen trial with research-grade Smart Tags.....	82
3.4.2 Trial 2: Paddock trial with production-grade Smart Tags.....	83
3.5 Discussion	85
3.6 Acknowledgements	89
Chapter 4 – The application of on-animal accelerometers (Smart Tags®) to investigate the link between ram libido and sire ability	90
4.1 Abstract.....	90
4.2 Introduction.....	91
4.3 Materials and methods.....	94
4.3.1 Semen collection.....	94
4.3.2 Assessment of ram semen parameters	95
4.3.3 Fitting of Smart Tags and automated detection of mounting activity	97
4.3.4 Parentage testing.....	98
4.3.5 Statistical analysis	98
4.4 Results.....	99
4.4.1 Descriptive statistics.....	99
4.4.2 Libido.....	100
4.4.3 Semen quality	102

4.4.4 Ram scrotal circumference	103
4.4.5 Testosterone	103
4.4.6 Siring ability	103
4.4.7 Predictors of siring ability and correlations between variables.....	104
4.5 Discussion	108
4.6 Acknowledgements	114
Chapter 5 – General Discussion	115
References	125

Chapter 1 – Review of the literature

1.1 Abstract

Sources of reproductive inefficiencies often go undetected in extensively managed sheep. This is especially true for rams, of which nearly a third of the population have been reported to have little to no sexual interest in ewes. Rams with low libido extend the lambing window, necessitate the recruitment of surplus rams to the joining flock and pass on poor reproductive performance to offspring. Methods currently used to measure ram libido are labour-intensive and predictive at best. The advent of precision livestock farming technologies has paved the way for more precise methods of monitoring sexual behaviour in male sheep. Thus far, two studies have utilised on-animal sensors to monitor mounting behaviour in rams, with both reporting high precision and sensitivity. Despite the potential these studies have demonstrated, the technology remains in its infancy and is not yet suitable for commercial applications in the Australian sheep industry. By comparison, dozens of on-animal sensors are available to the dairy industry. The intensive nature of dairy farming means more frequent contact with animals and existing infrastructure to support precision livestock technologies, and is therefore not directly comparable to the sheep industry. It has, however, highlighted that an integrated monitoring system that measures an animal's overall health and welfare in addition to reproductive performance would endure more commercial success than a sensor developed solely to monitor reproductive behaviour. In addition to improving reproductive efficiency, a reproductive monitoring technology must be robust, economical and energy-efficient

to succeed in an extensive landscape. These limitations should be considered during the development of the hardware and an algorithm to identify mating behaviour in rams.

1.2 General Introduction

Increasing the production efficiency of the Australian sheep industry is more critical now than ever before as pressure mounts in the race against climate change.

Producers must look for ways to reduce greenhouse gas emissions while simultaneously coping with extreme weather events on the frontline (Wheeler et al., 2021). The impact of climate change was exemplified during the 2017-2019 Australian drought, which saw the national flock fall to a record low of 63.7 million head in 2020 after producers were forced to offload core breeding stock (Meat & Livestock Australia, 2020). Maximising reproductive efficiency is key as the industry works to rebuild the national flock. Not only will it expedite the drought recovery process, but it is also a demonstrable path towards mitigating greenhouse gas emissions, both increasing overall production efficiency and the rate at which favourable genes are transferred (Henry et al., 2012).

As sheep are polygynous breeders, rams disseminate valuable genetics more widely than ewes. Producers typically join rams to ewes at a rate of 2% (Australian Wool Innovation, 2019), although a single ram can service 100 ewes over a joining period of five weeks and return pregnancy rates of close to 90% (Fowler, 1982). For this reason, rams are the main route for genetic selection. There are three characteristics that a

ram must possess in order to pass on his genes successfully: fertility, the physical ability to copulate and the sexual motivation to do so. A considerable proportion of rams lack one or more of these qualities.

More than a quarter of rams are 'low performers' that have little to no sexual interest in ewes. The definition of a low-performing ram is one that fails to mate with ewes during short periods of exposure, usually 30 minutes in length (Hulet et al., 1964; Snowden et al., 2002). Low performers have been reported at a rate of 29.6% (Hulet et al., 1964) and 28.6% (Fitzgerald and Perkins, 1991). It is important to note that the proclivity of a ram to mate with a ewe exists on a spectrum. Approximately 8% of rams fail to express any sexual interest in ewes, even after long periods of exposure. The remainder of the rams classed as low performers display varying degrees of interest in ewes and often have a longer latency to mate (Hulet et al., 1964; Perkins and Roselli, 2007).

The impact of low-performing rams on a farm's productivity extends beyond the sunk cost for the ram itself. Low-performing rams are more likely to repeatedly mate with an individual ewe than high-performing rams, meaning they cover fewer ewes over the same period (Price et al., 1996) and extend the lambing window (Hulet et al., 1964). In a competitive breeding environment, submissive rams sire three times fewer lambs than dominant, high-performing rams (Stellflug et al., 2006). The omnipresence of low performers also means that producers must recruit more rams to the breeding flock and increase joining rates to ensure that all ewes are covered.

Further to the immediate impact of poor-performing rams on reproductive efficiency, research has shown that sexual performance is moderately heritable with estimates of

0.22 ± 0.04 (Snowder et al., 2002) and 0.33 ± 0.62 (Kilgour, 1985). Bench et al. (2001) demonstrated that the male offspring of high-performing rams performed significantly more ejaculations and mounts without ejaculation in serving capacity tests than ram lambs of low-performing rams. The sexual performance of ewe lambs born from low-performing rams was also affected, with the first oestrus occurring on average eight days later than ewe lambs born from high-performing rams (Bench et al., 2001). Ram mating success, as measured by the proportion of lambs sired, was reported to have a moderate heritability of 0.30 ± 0.09 (Juengel et al., 2019), though this is a product of fertility as well as libido.

In addition to a lack of sexual motivation, a significant proportion of rams are plagued by low fertility. A comprehensive study conducted by Van Metre et al. (2012) in the United States found that 29.6% of 14 667 breeding soundness evaluations from 11 804 rams recorded a 'failed' score. Of these, 43.8% failed due to poor semen quality (13.0% of the sample population), while the remainder failed on emaciation, obesity, abnormalities or inflammation of the genitalia and ulcerative posthitis. Arrebola-Molina et al. (2020) found similar results and reported that poor semen quality was the primary reason for 13.5% of rams failing a breeding soundness evaluation across 45 farms in Spain. Substandard semen quality, azoospermia and necrozoospermia presented in 11.1% of the ram population (n = 966). Other reasons for failure included genital abnormalities (2.1%) and conformation (0.3%). The removal of failed rams on these 45 farms increased the number of lambs born by 51% in the subsequent breeding season (Arrebola-Molina et al., 2020).

The overlap between low-performing rams and low-fertility rams is unclear. However, given the reported rates of poor sexual performance and substandard sperm quality, over one-third of rams may be unfit for joining. This high level of reproductive inefficiency not only affects the number of offspring and the window over which they are born but is likely prolonged over the next generation too. Unfortunately, the current methods to assess the siring ability of rams leave much to be desired, so it is difficult to identify sources of reproductive wastage in the flock.

This review discusses the limitations of the current methods used to predict siring ability, with a focus on libido. The second part of the review assesses the suitability of on-animal sensors to monitor the mating behaviour of rams during joining.

1.3 Current methods used to predict the reproductive performance of rams

The extensive nature of sheep farming in Australia means that low-performing and sub-fertile rams often go undetected. Extensively managed sheep farms are characterised by lower levels of input relative to the number of sheep farmed, low contact between farm staff and animals, and the size of the land they are farmed on. The average flock size in Australia was the largest globally at 2 857 head in 2015 (ABS 2016). Australian farms are also markedly larger than the rest of the world's, at an average of 4 331 ha (Lowder et al., 2016). At this scale of production, identifying sources of reproductive wastage during joining is an onerous task. One response to this has been increasing the joining percentage, i.e. the proportion of rams joined to

ewes. However, as outlined above, this perpetuates the cycle of poor sexual performance and sub-fertility.

Due to the difficulty of monitoring individuals, reproductive performance is often measured at the level of the flock. The proportion of pregnant ewes to the number of ewes joined (pregnancy rate) and the proportion of lambs weaned to the number of ewes joined (weaning rate) are the primary performance indicators used by producers to assess the reproductive efficiency of their flocks. The reproductive performance of rams can be assessed prior to joining, but the methods are predictive at best and occur *ex-situ*. These methods have remained essentially unchanged since their respective developments during the latter half of the 20th century. It is unclear how extensively these ram reproductive assessments are used, though the labour and monetary cost of some methods suggest that they are not widely undertaken. The following section of the review will evaluate their validity as well as their practicality in a production setting.

1.3.1 Pre-joining inspection – the ‘five Ts.’

A typical pre-joining inspection follows the ‘five Ts’ principle, where the torso, toes, teeth, tossle (penis and prepuce) and testes of a ram are inspected. It is fast and relatively easy to perform after minimal handler training. The pre-joining inspection primarily evaluates a ram’s physical ability to mate with a ewe. Of the three factors that contribute to a ram’s siring ability, poor physical condition is the easiest to identify but less prevalent than subfertility or poor sexual performance (Arrebola-

Molina et al., 2020; Van Metre et al., 2012). There are no peer-reviewed studies that review the efficacy of the 'five Ts' method and its correlation to sire ability, however the importance of each of the 'five Ts' will be described below.

Torso

It is recommended that rams have a body condition score of 3.5-4 (on a scale of 1-5) at the start of the breeding season as significant weight loss occurs during joining. Poor nutrition is also linked to significant decreases in sperm concentration, motility, normal morphology, DNA integrity and libido (Guan et al., 2014; Parker and Thwaites, 1972). Overnutrition similarly impacts libido and semen quality. Body condition scores above 4 are associated with reduced libido (Sargison, 2009).

Teeth

As described above, joining is an energy-intensive period for a ram, and those that have broken mouths or periodontal disease are unable to meet the required nutritional intake (Coop and Abrahamson, 1973).

Toes

The feet of a ram should be free of footrot, scald or other diseases that may cause lameness, and the legs should be structurally sound (Gouletsou and Fthenakis, 2010). Rams afflicted by lameness will spend more time recumbent and may not be physically capable of mounting ewes.

Tossle

The prepuce and penis are examined for injuries, lesions or other signs of disease.

Ulcerative posthitis (pizzle rot) is a disease that commonly affects rams. It typically does not affect sperm quality, but it will impact a ram's willingness to mate (Brook et al., 1966).

Testes

The scrotum, testes and epididymis should be examined for any signs of inflammation, abnormal morphology or size via palpation. Abnormalities impact the ability of the testes to thermoregulate and will affect the morphology and fertilising ability of the sperm. Epididymitis, primarily caused by *Brucella ovis*, is the most common disease that affects breeding rams and is characterised by an enlargement of the epididymis (Tibary et al., 2018).

It is also important to measure the circumference of the scrotum. Increased scrotal circumference (SC) has been linked to increased flock fertility by increasing the number of lambs born per ewe ($p < 0.05$) (Hulet et al. 1965; David et al. 2015). A higher SC is also correlated to increases in several semen quality parameters such as motility, volume and concentration, all of which are positively linked to fertility (David et al. 2015; Arrebola-Molina et al. 2020).

1.3.2 Breeding soundness evaluations

A breeding soundness evaluation (BSE) is a more rigorous assessment of a ram's reproductive capability. A BSE evaluates a ram's physical ability to mate and predicts

its fertility. It is recommended that a BSE is conducted prior to the purchase of a ram and before selecting ram lambs for the breeding flock. If the BSE is used as a pre-joining check, it is recommended it occurs 50 days prior to joining as this is the length of spermatogenesis in the ram and will allow for therapy should it be needed (Ridler et al., 2012). The BSE exam is not standardised or accredited in rams as it is in bulls (Chenoweth and McPherson, 2016; Norman, 2021) but typically involves a basic assessment of semen quality (volume, concentration, motility, morphology, incidence of white blood cells) and a screening test for *Brucella ovis* in addition to the ‘five Ts’. It is typically conducted by a veterinarian, especially if semen is collected via electroejaculation (Tibary et al., 2018). Rams are given scores for each assessable trait, which are then compiled to rate the ram as an ‘excellent’, ‘satisfactory’, ‘questionable’ or ‘unsatisfactory’ breeder (Table 1.1).

Table 1.1: The criteria used by Van Metre et al. (2012) during the breeding soundness evaluations of rams

Parameters	Categories of classification on BSE			
	Unsatisfactory potential breeder (fail)	Questionable potential breeder (fail)	Satisfactory potential breeder (pass)	Excellent potential breeder (pass)
Body condition	1 (emaciated), 5 (obese)		2.4	3
Physical abnormalities	Present	Absent	Absent	Absent
Epididymitis	Present	Absent	Absent	Absent

Parameters	Categories of classification on BSE			
	Unsatisfactory potential breeder (fail)	Questionable potential breeder (fail)	Satisfactory potential breeder (pass)	Excellent potential breeder (pass)
Ulcerative posthitis	Active and severe, and/or narrowing or distortion of preputial orifice; and/or internal form present	Active and mild to moderate	Healed or no lesions	Healed or no lesions
Semen collected by electroejaculation	No	Yes	Yes	Yes
Progressive sperm motility (%)	0	10–30	31–69	70–100
Normal sperm morphology (%)	≤29	30–50	51–79	≥80
Leukospermia: # white blood cells in semen/100× field	≥10	0–9	0–9	0–9
<i>Brucella ovis</i> indirect ELISA	Positive	Indeterminate	Negative	Negative
Scrotal circumference (cm):				
Rams aged 6–13 mo	<26	27–29	30–35	≥36
Rams aged ≥14 mo	<29	30–32	33–39	≥40

Surprisingly, there are few studies that have investigated the relationship between BSE score and sire ability, and those that have are conflicting. Removing rams that have failed the BSEs from breeding flocks has been shown to increase the lambing rate by

51% (Arrebola-Molina et al., 2020). A recent study by Alexander (2021), however, demonstrated that rams that failed the BSE (ranked 'questionable' or 'unsatisfactory') sired a similar number of lambs to the rams that passed the BSE. Similarly, rams that failed to sire lambs were not discernible by BSE status. An earlier study by Alexander et al. (2012) also found no correlation between BSE scores and the number of lambs sired. These findings are reflected in bulls, where it was demonstrated that BSE score is not a reliable predictor of fertility (Waldner et al., 2010). Even if a BSE deems a ram physically fit and able to reproduce, and this is a true reflection of his fertility, this does not mean that he will have the motivation to do so.

1.3.3 Parentage testing

While parentage testing is not a predictive measure of ram performance, it is the gold standard for the assessment of siring ability. Parentage testing has become increasingly affordable since the advent of a single nucleotide polymorphism (SNP) assay (Kijas et al., 2012). SNP markers are abundant in the sheep genome and can withstand high throughput genotyping tests, increasing the speed and the ease at which parentage testing can be conducted (Clarke et al., 2014). Prior to SNP assays, determining pedigree cost upwards of \$50 AUD per lamb. This price has more than halved since then to \$21 AUD (Granleese et al., 2019). Since parentage testing has become more affordable, the variation in the siring ability of rams has become increasingly apparent to producers (Heaton et al., 2014).

This variation was highlighted in the Merino Lifetime Productivity (MLP) project, an ongoing project that began in 2015 to assess the performance of sires and dams at five sites across Australia. The sires (n=134) used in the project were balanced for their performance in MERINOSELECT, Australia's Merino genetic evaluation system, but were widely used AI sires or had performed well at show, indicating they were in the upper echelon of sires nationwide (Australian Wool Innovation and Australian Merino Sire Evaluation Association, 2006). All sires underwent breeding evaluation and semen assessment prior to their involvement in the project, however, their libido was not assessed. Clear variability in siring ability existed between rams, with some sires producing twice as many lambs as others, despite being passed on semen evaluation (Australian Wool Innovation and Australian Merino Sire Evaluation Association, 2020). Although BSEs and semen evaluations are important predictors of siring ability, parentage testing studies such as this suggest that a predictive model for siring ability would be much improved by measuring libido during joining.

1.3.4 Serving capacity tests

The serving capacity test (SCT) was developed by Mattner, Braden and George in 1971 and is the current method of measuring ram sexual performance. In the original protocol, rams were introduced to a group of 3-4 ewes in oestrus in a 4 m x 4 m pen. The number of services (mounts with intromission and ejaculation) were tallied over a 30-minute period by two observers. Rams were deemed sexually inactive if they did not demonstrate an interest in the ewes and did not attempt to mate with them. Low

libido rams were those that performed less than four ejaculations within 30 minutes, while high libido rams performed an average of five to six services. Libido, as scored in the SCT, was positively correlated with the number of lambs sired (Mattner et al., 1971). Several variations of the SCT have been trialled since its development. The structure of the test is not standardised in the industry, nor is the number of services or mounts that deem a ram high-performing.

Since the development of the serving capacity test, there has been much debate as to whether it is an accurate reflection of a ram's libido in-paddock. A number of studies that followed the development of the test by Mattner et al. (1971) failed to link SCT score with in-paddock performance (Chaill et al., 1975; Kilgour and Whale, 1980; Kilgour, 1985). Kilgour and Whale (1980) demonstrated that the number of services performed by rams ($n=7$) during a 20-minute SCT was a poor predictor of the number of ewes serviced in-paddock ($r=0.30$). This was compared to two hour-long serving capacity tests, for which the mean number of services across both hour-long tests was significantly correlated with the number of ewes serviced ($r=0.88$). The number of services for high-performing rams was higher in the final 40 minutes of the SCT than in the first 20 minutes, implying that a period of habituation is required. A subsequent study by Kilgour (1985) again demonstrated that SCTs of 1 hour or 3 hours measured ram sexual behaviour with a high level of repeatability, but tests of 20 minutes did not. Studies that conducted SCTs of thirty minutes or less failed to show a correlation between SCT score and sexual performance in the paddock (Allison, 1978; Chaill et al., 1975; Mickelsen et al., 1982).

Other than an unfamiliar testing environment, variation in the attractiveness of ewes and the proximity of other rams have been noted as factors contributing to the unreliability of serving capacity tests. Tilbrook and Lindsay (1987) demonstrated that oestrous ewes differ in attractiveness to rams. While attractiveness was perceived similarly by different rams, it is likely that the variation between ewes is liable to affect the repeatability of a ram's test score and the validity of test scores between rams. The social hierarchy of a ram relative to rams held nearby also affects the reliability of the SCT. The sexual performance of subordinate rams is suppressed in the presence of dominant rams. When the audience was removed, the number of mounts and ejaculations for subordinate rams nearly quadrupled (Lindsay et al., 1976).

Not only are SCTs unreliable, but they are also time-consuming and labour-intensive. As the literature states, an SCT should be conducted over the course of an hour to allow a ram to habituate to the unfamiliar environment. It is also advisable that SCTs are repeated for each ram at least twice to provide an accurate measure of libido and that rams are given two introductory tests that do not contribute to the final SCT score (Kilgour, 1985; Stellflug and Berardinelli, 2002). Given that two observers are required, the labour and opportunity cost of SCTs quickly becomes exorbitant. It is unclear how often SCTs are practised in industry, but anecdotal evidence and the high labour cost suggest that this assessment is typically only used by researchers.

1.3.5 The ram mating harness

The ram mating harness is the only tool available to monitor in-paddock joining. The harness features a coloured crayon that sits against the ram's brisket and marks the wool on the rump of ewes that have been mated (Figure 1.1). The mating harness is most commonly used as an oestrus detection tool in research and to confirm the success of oestrus synchronisation protocols in artificial breeding programs (Lindsay, 1966; Maddison et al., 2016; Romano et al., 2000). Vasectomised rams or androgenised wethers are fitted with the harness and introduced to a group of oestrus-synchronised ewes. The time of oestrus onset can be narrowed down to a 12- or 24-hour window, depending on how often the flock is mustered. The mating harness is rarely used in industry as an oestrus detection tool due to the labour this level of monitoring would demand. It is possible that it could provide a rudimentary measure of libido if breeding rams were assigned different crayon colours over joining (Kilgour, 1980). The selection of colours is limited, however, and crayon marks are prone to fading within two weeks (Radford et al., 1960).



Figure 1.1: Rams wearing a mating harness with a blue crayon attached to the harness of the ram in the foreground. Blue marks on the ewes' rump indicate that she has been joined with (Ehrhardt, 2020).

Current methods for monitoring the reproductive performance of rams, in particular their sexual performance, leave much to be desired. The gold standard for measuring sexual performance is to directly monitor a ram's behaviour during joining.

Historically this has been unfeasible due to the difficulty of monitoring extensively farmed livestock, but technology could be the path forward. The advent of on-animal sensors, and with them precision livestock farming, presents a promising opportunity to monitor the behaviour of individual sheep in real-time.

1.4 Precision livestock farming

Precision livestock farming (PLF) is a management practice that is based on continuous real-time monitoring of individuals with the aim of improving animal health, welfare and productivity (Berckmans, 2017). PLF technologies take many

forms, including advanced systems such as robotic milking and automatic feeders in the dairy industry and technologies in their infancy, such as activity sensors in sheep (Fogarty et al., 2018; John et al., 2016). On-animal sensors are a PLF technology that offer insight into an animal's behaviour *in situ*. This eliminates the stressors of in-person monitoring and the variability that can arise when testing for behaviour in an unfamiliar environment. It also allows for continuous monitoring with finer granularity than could be achieved by a human observer (Brown et al., 2013).

There are several different types of on-animal sensors, and the technology chosen will depend on what dimension of the animal is being monitored, such as its behaviour, physiology or geolocation. For example, determining an animal's location would require a sensor that is able to position the animal in space. Monitoring social interactions, however, would require a sensor that is able to determine the proximity of one animal to another. Commonly used sensors include Global Navigation Satellite Systems (GNSS), proximity sensors, accelerometers, heart rate monitors, pedometers and heat sensors (Fogarty et al., 2018).

The next section of this review will discuss in detail the specifications and applications of three types of sensors due to their frequency in research and their candidacy as a reproductive monitoring tool. These sensors are accelerometers, Global Navigation Satellite System (GNSS) sensors and proximity sensors.

1.4.1 The types of on-animal sensors commonly used in PLF research

Accelerometers

Accelerometers are sensors that measure the force of acceleration of an object as it moves through time and space. Acceleration is measured in gravitational units (g), with $1\text{ }g = 9.8\text{ m/s}$. These measurements are made along one or more planes relative to the object upon which the accelerometer is stationed. Tri-axial accelerometers are commonly used in livestock research and measure acceleration along the x-, y- and z-axes, also commonly referred to as sway, surge and heave (Figure 1.2).

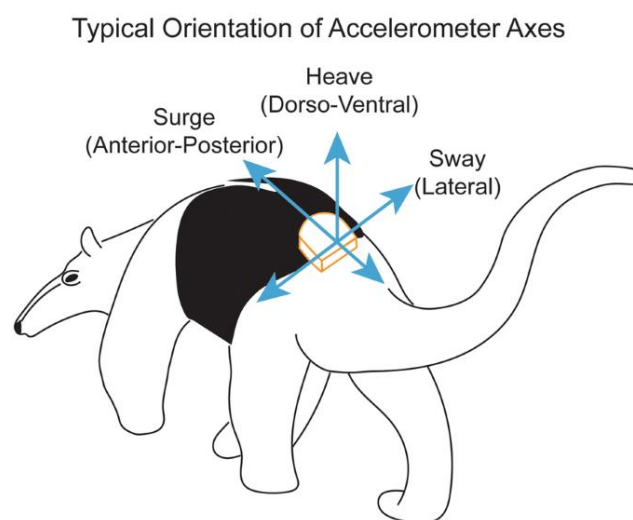


Figure 1.2: The typical orientation of an accelerometer on quadrupedal vertebrate (Whitford and Klimley, 2019).

There are three different types of accelerometers: piezoelectric, piezoresistive and capacitive. Capacitive accelerometers are the most common type of accelerometer in behavioural research as they have greater sensitivity, stability and resolution than piezoelectric or piezoresistive accelerometers (Wong et al., 2007). They use change in electrical capacitance to measure acceleration. When an object accelerates, the seismic mass of the accelerometer moves in response and the distance between the fixed and

flexible electrodes are measured (Figure 1.3). Measured acceleration is the sum of acceleration due to movement by the object the accelerometer is fixed to (dynamic acceleration) and acceleration due to the Earth's gravitational field (static acceleration).

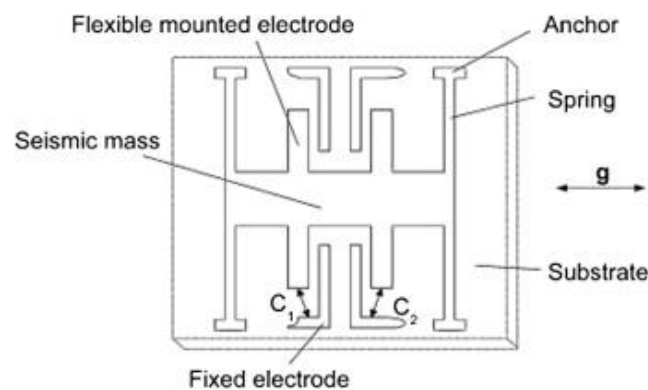


Figure 1.3: Schematic diagram of a capacitive accelerometer, where g is the force of acceleration
(Venkatanarayanan and Spain, 2014)

Accelerometers produce high-resolution data and can be programmed to record from 0.5 Hz to 10 000 Hz, depending on the specific model used (Brown et al., 2013). A tri-axial accelerometer configured to a sample rate of 1 Hz, for example, would produce three data points per second. Higher sampling frequencies require a higher power output over the same period, resulting in lower battery life. The trade-off between data resolution and temporal resolution is an important consideration for researchers and should be determined with the target behaviour in mind. The Nyquist theorem dictates that the sampling frequency should be twice that of the signal frequency. For animal behaviour research, the sampling frequency of accelerometers should be programmed to at least twice the frequency of the most rapid body movement in a behaviour (Brown et al., 2013). For example, the stroke cycle frequency of the little

penguin (*Eudyptula minor*) during swimming is 3.6 Hz (Sato et al., 2006). By applying the Nyquist theorem, an accelerometer sampling frequency should be at least 7.2 Hz in order to distinguish swimming from other behaviours of the little penguin.

The ability to precisely measure changes of acceleration in three different dimensions at such a fine temporal scale means that accelerometers are frequently used to study behaviour in animals. States of rest, walking and foraging can be differentiated from one another by the distinct acceleration patterns of each behaviour. Chapter 1.4.2 will discuss examples of accelerometer research in sheep.

Global Navigation Satellite Systems (GNSS)

The primary use of GNSS is to determine an object's geographical location. GNSS consists of a constellation of satellites and a receiver on the earth's surface, to which the satellites transmit a signal. The distance between the receiver and the various satellites that make up the constellation can be determined by calculating the time it takes the signal travelling from the satellite to reach the receiver. Trilateration is used to determine the position of the receiver on the earth with respect to each of the satellites in the constellation. It should be noted that many studies refer to Global Positioning Systems (GPS) rather than GNSS. GPS is a common type of GNSS owned by the United States government that uses a particular constellation of satellites (Kaplan and Hegarty, 2017). Other types of GNSS include Galileo (owned by the European Union), GLONASS (owned by the Russian government) and BeiDou (owned by the Chinese government) (Ren et al., 2016).

If the sampling rate of the GNSS device is increased, the speed and velocity of the animal can be calculated, allowing the observer to make inferences about behaviour. There are a multitude of studies that have used GNSS devices to monitor the individual behaviour of sheep (Dobos et al., 2014; Fogarty et al., 2015; Manning et al., 2014). GNSS has also been used to measure social interactions between sheep (Keshavarzi et al., 2023). Despite the breadth of research, the balance between battery life and sampling frequency is a constraint on the technology's commercial success as a behavioural monitoring tool (Mason and Sneddon, 2013; Swain et al., 2011). GNSS devices are also sensitive to interference, which may take the form of cloud cover, vegetation cover and obstructing topography (Cain et al., 2005).

Proximity sensors

In recent years, proximity sensors have been used in PLF to measure social interactions (Ozella et al., 2020; Paganoni et al., 2021, 2020). The most common type of proximity logger uses wireless personal area network technology, such as Zigbee or Bluetooth Low Energy, to communicate with other devices (Wang et al., 2006). The devices transmit radio packets at fixed temporal intervals and proximity is determined by the Received Signal Strength Indicator through the receiver device (Wilson-Aggarwal et al., 2019).

This technology can also be used to determine geolocation within a known area. By creating a wireless mesh network with fixed nodes, trilateration can be used to determine the location of an animal in relation to the strength of signals it receives from the stationed nodes (Yang et al., 2020). Proximity sensors are less accurate and

less flexible than GNSS devices in determining location, however, they do so at a fraction of the cost and power (Kiani, 2018).

1.4.2 Developing applications of on-animal sensors for sheep

The use of on-animal sensors in sheep research has increased significantly in the last fifteen years. A systematic review that mapped the use of on-animal sensors in the sheep industry reported that there were 28 studies in the field published between 2011-2015, almost double that of the preceding five-year period and more than five times the number of studies published between 2001-2005 (Fogarty et al., 2018). Since the publication of the review, the industry's interest in the use of on-animal sensors has continued to flourish.

Accelerometers have become increasingly popular amongst researchers in the years since the review by Fogarty et al. (2018), outnumbering the studies that use GNSS devices. Researchers appear to be increasingly interested in direct measurements of behaviour and a sheep's daily energy budget rather than relying on the inferences that can be made of an animal's behaviour using GNSS or proximity sensors alone.

Accelerometers offer a more granular insight into a sheep's behaviour, are more accurate than proximity sensors and less power consumptive than GNSS devices.

The majority of sensor research in the sheep industry has focused on the identification of state behaviours rather than event behaviours (Fogarty et al., 2018). State behaviours are those that occur over long periods of time and are expressed in terms of their duration. By contrast, event behaviours are short in nature and are recorded

by frequency rather than duration (Bateson and Martin, 2021). Walking, grazing and standing are all examples of state behaviours in sheep. Urination, suckling and mating are classed as event behaviours. State behaviours are arguably easier to classify due to their sheer ubiquitousness in data sets.

Initial studies that used accelerometers to monitor sheep behaviour aimed to validate the use of the technology rather than evaluate health, welfare or productivity (Alvarenga et al., 2016; Barwick et al., 2018; Giovanetti et al., 2017; Radeski and Ilieski, 2017). Authors that are particularly interested in nutrition tend to differentiate between grazing, ruminating and resting (or other activities) (Decandia et al., 2021; Giovanetti et al., 2017). Other authors classify walking, grazing, standing and lying behaviours (Alvarenga et al., 2016; Barwick et al., 2020; Radeski and Ilieski, 2017). Classification models are commonly used to analyse behavioural data from accelerometers. Broadly speaking, they are models that use information from a training data set to apply classification labels to unseen data (Valletta et al., 2017). Barwick et al. (2018) used a Quadratic Discriminant Analysis (QDA), a classification model, to predict behaviour in ewes. When applied to data from an ear-tag deployed accelerometer, the QDA predicted grazing, standing and walking behaviours with accuracies of 94%, 96% and 99%.

Classification models can identify state behaviours with a high level of accuracy but they leave much to be desired in the identification of event behaviours. Abell et al. (2017) aimed to predict mounting behaviour in bulls from accelerometers attached to the ear, withers and neck. Three types of classification models were applied: a decision tree, random forest and random tree. The sensitivity of the classifiers ranged from

70.1% (random tree; ear-mounted accelerometer) to 80.8% (random forest; wither-mounted accelerometer). Although the predictions made by the classifiers captured an admissible portion of the observed mounting events, the vast majority of events predicted as mounting were false positives. The highest level of precision achieved by the classifiers in the mounting behaviour classification was 2.5% (random forest and random tree; neck-mounted accelerometer). This contrasted against the extremely high level of precision achieved by classifiers in the lying and standing behavioural classifications: 98.9% (random forest; wither-mounted accelerometer) and 91.1% (random forest; neck-mounted accelerometer), respectively. Lying and standing combined accounted for 90% of all observations while mounting only represented 0.6%. The class imbalance in this model has clearly produced a bias against mounting as a minority class. Class imbalance is an intrinsic issue in event behaviour classification due to the short, infrequent nature of these behaviours.

Lush et al. (2018) discovered a similar bias towards state behaviours when random forest models were used to identify urination events in ewes. Urination was the least well-classified behaviour in an ethogram that otherwise consisted of state behaviours (foraging, walking, running, standing, lying). Despite a clear signature of a urination event in the raw accelerometer records, the behaviour was predicted with a sensitivity of 72.2% using a sliding window of five seconds. This was compared to lying and foraging, which achieved sensitivities of 99.9% and 97%, respectively. Similar to Abell et al. (2017), lying and foraging were the two most frequently observed behaviours at a total of 8345 s and 7595 s, while observations of urination totalled 127 s. Although a sensitivity of 72.2% could be considered successful, this further highlights the disparity

between event and state behaviours when classification models are applied. The unique and often clear acceleration signals that characterise event behaviours are concealed by the sheer ubiquitousness of state behaviours. The imbalance between these behaviours in the training data set results in a model that is inherently biased. Down-sampling majority classes or up-sampling minority classes through techniques such as SMOTE and ROTE are avenues through which severe class imbalance can be mitigated (Fernández et al., 2018; Post et al., 2020). If the event behaviour is of singular interest, however, it is beneficial to do away with classification models altogether. Lush et al. (2018) remarked that urination had a distinct signature in raw accelerometer data and may be better identified with template matching. Template matching is the process of searching a dataset for a pre-defined signature, or 'template', of a behaviour. The template is slid across the entirety of the data set and the similarity of the template to the corresponding data at any given point is computed (Walker et al., 2015). Lush et al. (2018) concluded that this might be a more appropriate method of classification when an event behaviour is of singular interest; however, they also cautioned that the rigidity of template matching could present a problem if the duration of the behaviour is variable.

With regards to monitoring the reproductive performance of rams, mating behaviour is certainly of singular interest. For target behaviours such as these, researchers have deviated from classification models and turned towards specifically crafted algorithms.

1.4.3 Sensors to monitor reproductive behaviour in rams

The use of on-animal sensors as a reproductive monitoring tool in the sheep industry is a recent area of research, particularly where it concerns monitoring ram mating behaviour. Despite the eruption of sensor research in sheep, only two studies to date (Alhamada et al., 2017a; Mozo et al., 2019) have used sensors to detect mating behaviour in rams. Though they are in their infancy, there is huge potential for on-animal sensors to become a new method of reproductive monitoring.

Mozo et al. (2019) were the first authors to identify ram mounting behaviour in accelerometer records. Tri-axial accelerometers sampling at 12.5 Hz were attached to the withers of Rasa Aragonesa rams (n=7) via a harness. In the training phase of the trial, three rams were separately introduced to two ewes in oestrus in a pen for one hour each. Accelerometer data collected during this training phase was annotated and used to create transformed features to assist with mounting identification: the time since power-up; Kalman filtered values from the x-, y- and z-axes; the vector sum of the y- and z-axes; derivative of the x-axis; the angle of the accelerometer from the ground; the 10-minute moving average for angle; and the 5-minute moving average for the Kalman filtered yz angle. The training phase continued with observations of the same three rams in a paddock when they were exposed to 60 ewes in oestrous over five days to assist with algorithm development.

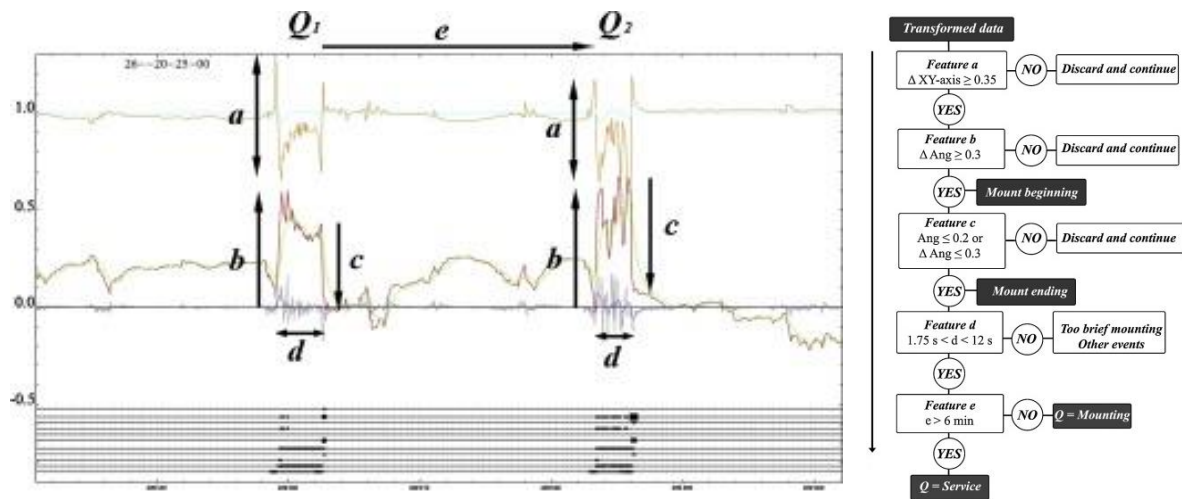


Figure 1.4: Representation of features used in the algorithm developed by Mozo et al. (2018) to detect mounting behaviour in rams and a brief explanation of algorithm functioning. Features: Orange, kyz; Cyan, yz moving average; Red, angle; Green, angle average; Grey, derivative of x with respect to time; a, kyz variation; b, angle increase; c, angle decrease; d, mount length; e, consecutive mounts interval; Q1, first mount; Q2, second mount (Mozo et al., 2018)

A mounting event can be broken down into three phases: the mount, intromission (with or without ejaculation) and dismount. This produces a clear and unique signature of a mounting event in accelerometer records, with a sharp increase in acceleration during the mount, a plateau during intromission and a sharp decrease upon dismount. Mozo et al. (2018) took advantage of this signature in their final algorithm and applied thresholds to the aforementioned features to describe the three phases of the mounting event (Figure 1.4). The time from mount to dismount was used to disqualify mounting events that were too brief in length, while the time between a dismount and mount was used to determine if a mounting event was a service (a mount that ends in an ejaculation). A six-minute refractory period was assumed to separate services from attempted matings. Other than citing the software package used, Mozo et al. (2018) did not indicate how the analysis of the data was performed. It is possible that template matching was a method of analysis.

The algorithm developed by Mozo et al. (2019) accurately identified mounting events in rams with an overall sensitivity of 77.9%. Services were identified with higher sensitivity at 94%. Of all events classed as mounts or services, 16.4% were false positives. Precision was not reported separately for these two behaviours. The sources of false positives were not discussed but given the high pass filters that were applied for acceleration features, it is likely that these false positives resulted from high activity behaviours, such as running or agonistic behaviours. While the global performance of the algorithm was sound, the authors highlighted high variability in detection performance between rams. Sensitivity in mount detection varied from 54.3% to 90.6% between individual rams, while sensitivity in service detection ranged from 79.2% to 100%. There was marked variation in the precision of the algorithm between rams too, ranging from 60.9% to 91.2%. The authors hypothesised that variation in the sizes of rams contributed to the wide range of performance. They remarked that the ram with the lowest sensitivity for services was larger in size, though this was not supported by data. In theory, larger rams would require less upward effort to mount ewes than smaller rams that would mount at an angle more perpendicular to the ewe's dorsal plane in order to achieve intromission. Differences in body size and conformation between individuals have also been linked to variability in the classification performance of accelerometer data for posture discrimination in sows (Ringgenberg et al., 2010).

Overall, Mozo *et al.* (2018) were able to identify mating activity in rams with a high degree of accuracy. With an improvement in the variation of performance between individual rams, this algorithm would function well as a research tool. It is not yet

designed for commercial application, however, because all analysis of the data occurred post-hoc once the data had been extracted from the accelerometers. Given the number of calculations that would need to occur in real-time to identify mating as it occurred, it is unclear how well this would translate to a commercial tag.

The sole other study that has investigated the use of sensors to monitor ram mating behaviour utilised pneumatic sensors rather than accelerometers. The Alpha-Detector is a novel mating detection system that was developed by Alhamada et al. (2016). The system consists of three key features: an Alpha-Detector, an RFID transponder and an Alpha-Receptor (Figure 1.5). Prior to joining, the Alpha-Detector is fitted to the brisket of a ram via a harness and transponders are adhered to the tails of ewes. The pneumatic sensor in the Alpha-Detector is activated when a ram mounts a ewe, which then triggers the Alpha-Detector's integrated RFID antenna to initiate a scan of nearby transponders. The mounted ewe's RFID is recorded, along with the date and time. Following joining, a handheld device (Alpha-Receptor) is used to retrieve the recorded data from the Alpha-Detector.

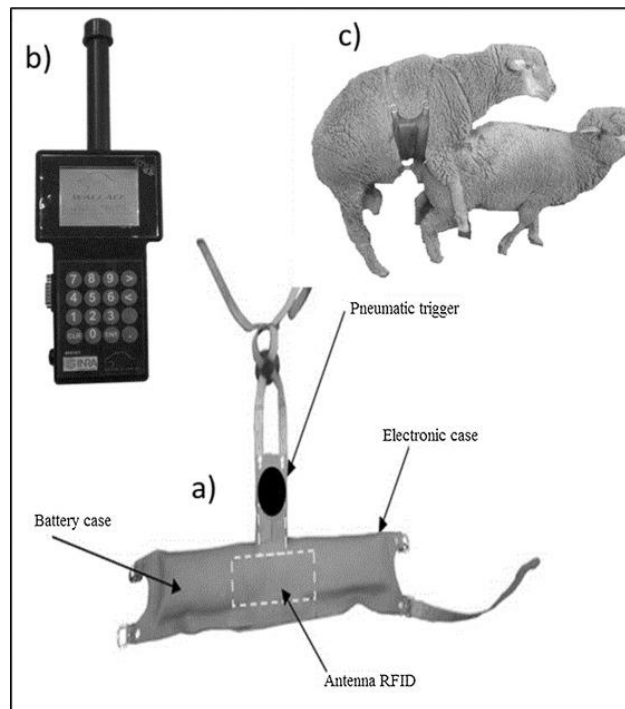


Figure 1.5: Diagram of the Alpha-Detector system. (a) a leather harness containing an Alpha Detector, (b) the Alpha Reader (AR), (c) a ram wearing the Alpha-Detector during mating (Alhamada et al., 2016a).

The Alpha-Detector identified mating events in sheep with high accuracy. Two rams were introduced to 12 oestrous-synchronised ewes in a barn for a total of four days during their first cycle and five days during their second cycle. Of the 414 mounts that were observed during the trial, 352 were identified by the Alpha-Detector, equating to a sensitivity of 85%. The authors noted that a large portion of the false negatives stemmed from ewes that were not in standing oestrus and would move away from the ram when he attempted to mount them. After omitting these attempted mounts from the results, the Alpha-Detector identified mounts with a sensitivity of 94%. In a subsequent study, 12 rams were equipped with Alpha-Detectors and underwent a series of six serving capacity tests. A total of 1191 mounts were observed, 1026 of which were identified by the Alpha-Detectors, returning a sensitivity of 94%. No false

positives were recorded in either study. While the authors stated that there were significant differences between the number of mounts performed by each ram, they did not report on inter-individual variability in the performance of the Alpha-Detector.

A system that can determine the identity of the ewe that a ram has mated with has applications beyond that of monitoring ram sexual performance in-paddock. In fact, the primary purpose of the Alpha-Detector is to detect ewes in oestrus. Alhamada et al. demonstrated across two separate studies that the Alpha-Detector was able to identify 100% of ewes in oestrus (Alhamada et al., 2017b, 2016). Lambing dates were recorded by Alhamada et al. (2016b) and it was stated that these dates confirmed the time of conception predicted by the Alpha-Detector, however, the data to support this was not supplied. The most compelling characteristic of the Alpha-Detector is its ability to identify mating pairs. Even in the dairy industry, where on-animal sensor research is comparatively advanced, multi-sensor systems that map one animal's behaviour in relation to another are uncommon.

Despite its accuracy in monitoring both ram libido and detecting oestrus in ewes, there are limitations to the Alpha-Detector system that would undermine its success as a commercial product in the Australian sheep industry. Firstly, the authors stated that the battery of the Alpha-Detector “usually lasts more than a week”. This would require the breeding flock to be mustered five times during joining to recharge the battery in the Alpha-Detectors. This process is highly disruptive to the breeding flock and demands extra labour from the producers. In addition to the poor battery life, the

attachment of transponders to the tails of ewes is likely to discourage producers whose income depends upon the condition of wool.

These two studies have demonstrated that the detection of mounting behaviour in rams can be achieved in rams with a moderate to high level of precision and sensitivity. Despite their success, a considerable re-engineering of the technology would be required before they could be deemed commercially successful in the Australian sheep industry.

1.4.4 Sensors to monitor reproductive behaviour in ewes

A commercial sensor to monitor the reproductive behaviour of rams would ideally detect their interactions with ewes as well. The use of sensors to monitor the behaviour of ewes during oestrus and joining is a relatively new area of research, as it is with rams. Aside from the oestrus detection studies by Alhamada et al. (2017b, 2016), research in this area does not involve isolating a target behaviour. Instead, sensors are used to monitor secondary signs of oestrus, such as increased activity and male-seeking behaviour.

The earliest automated oestrus detection study in ewes employed GNSS devices to monitor the activity of ewes during oestrus (Fogarty et al., 2015). The GNSS devices were deployed on two groups of ewes (n=20; n=18) that were synchronised to display oestrus one week apart. Devices sampled four times over the course of a minute at three-minute intervals. Diurnal activity was higher for non-oestrus ewes, while ewes in oestrus were significantly more active during the early hours of the morning. Further

to the difference in the activity between oestrus and non-oestrus ewes, the authors found that the speed of movement of oestrus ewes was an important determinant of the number of mounts received. With every 0.05 m/s increase of a ewe's speed between the hours of 8 am and 9 am, there was a 28-fold increase in the number of mounts received. Although the authors did not develop a classifier for oestrus detection, it is likely that oestrus could be successfully identified using activity thresholds, as it is in dairy cattle (Reith and Hoy, 2018).

Proximity sensors have also been used to monitor the expression of oestrus behaviour and predict lambing date in ewes (Paganoni et al., 2020). The number of interactions between rams and ewes during joining was measured in a study by Paganoni et al. (2020). Bluetooth-based proximity sensors were attached to rams ($n=2$) and ewes ($n=71$) via collars prior to a 26-day joining period. The description of the sampling rate of the sensor was ambiguous, with the authors stating that the sensors sampled at 30 Hz and that sensors scanned for nearby devices every minute. The range of the sensors was approximated at 1-15m, although this would vary depending on the level of interference. The ratio between the number of interactions between the ram and the individual ewe and the average number of daily interactions between the ram and all ewes was used to estimate the day of conception. The day that returned the highest ratio for each ewe was estimated to be her date of conception, providing that the ratio was above 1.5. If there were two days within a three-week period where a ewe's ratio was greater than two, the latter day was deemed to be her conception date. The date of lambing was calculated as 150 days from the date of conception. All ewes lambed within five days of their predicted lambing date and 82% lambed within two days. For

the computational simplicity of this algorithm and the energy efficiency of the technology relative to GNSS (Katevas et al., 2016), proximity sensors are a strong candidate for monitoring ram-ewe interactions alongside a mounting detection sensor.

1.4.5 Commercial adoption of on-animal sensors in the livestock industries

Despite the influx of on-sheep sensor research in recent years, there is only one on-animal sensor that is commercially available and tailored to sheep producers (Table 1.2). The SmartShepherd tag monitors interactions between ewes and lambs to determine maternal pedigree and became available for purchase in 2018. The tag is a proximity sensor that uses Bluetooth Low Energy technology to measure the signal strength between the ewe's tag and the tags of lambs nearby. It has been reported to have an accuracy of 96% with 48 hours of use (MLA, 2017), though it has not been externally validated in peer-reviewed literature. The system is comprised of tags attached via collars and a handheld receiver to retrieve data from the tags. There is no infrastructure required and data recording and transmission do not rely on an internet connection. As of July 2021, over 200 000 sheep had been collared with SmartShepherd tags (SmartShepherd, 2021). The simplicity and accuracy of the SmartShepherd system has no doubt bolstered its success.

Commercially available on-animal sensors are far more widely available to cattle producers, particularly those in the dairy industry. This section of the review will explore the application of commercial on-animal sensors in the dairy industry to both

highlight the limitations faced by extensive production systems and to postulate the future of on-animal sensors in the sheep industry.

A recent review of the use of sensor technologies in the dairy industry identified 129 technologies available from 67 providers (Stygar et al., 2021). Over half of these technologies were accelerometer-based. As the authors were solely interested in welfare monitoring, sensors that monitored reproduction or methane emissions were excluded from this figure. The scale of the on-animal sensor market in the dairy industry is apparent from this review alone. The contrast in the availability of PLF technology in the dairy industry compared to the sheep industry boils down to the difference in production systems. The intensive nature of dairy farming lends itself to more frequent contact with livestock, existing infrastructure for milking which can be used to support on-animal sensor technologies and better network connectivity. It is also possible that dairy farmers are more inclined to invest in on-animal sensors due to their higher level of capital investment per animal than sheep farmers and the greater value of cattle in comparison to sheep (Adenuga et al., 2020).

There are dozens of commercially available sensors that monitor the reproductive behaviour of cows (Table 1.2), many of which are used to detect oestrus. Accurate oestrus detection is particularly important to the dairy industry as it reduces calving intervals, thereby increasing the annual milk yield per cow (Pfeiffer et al., 2020). This, in conjunction with the reported shortening of the oestrus period and an increase in herd numbers, has attracted the industry to oestrus detection technologies (Kerbrat and Disenhaus, 2004; Lucy, 2007; Saint-Dizier and Chastant-Maillard, 2012).

Traditionally herds would be manually observed for signs of oestrus. Unlike sheep,

cows will mount other females; thus standing heat can be used as an indicator of oestrus in herds without bulls or teaser males. The advent of tail paint allowed producers to do away with manual observations. Using a similar concept to the ram mating harness, cows that have their tail paint partially or completely removed are deemed to be in oestrus and are drafted for artificial insemination. Electronic pneumatic sensors such as the HeatWatch system were developed as an advanced alternative to tail paint and pinpoint the precise time of the first mount rather than estimating a window of oestrus onset, similar to the Alpha-Detector in sheep. The accuracy of this sensor is undermined by the reliability of standing heat as an indicator of interest, considering that only 37% to 58% of cows were reported to stand for mounting in recent studies (Kerbrat and Disenhaus, 2004; Mayo et al., 2019; Roelofs et al., 2005; Van Eerdenburg et al., 2011; Zebari et al., 2018). Interestingly, both Roelofs et al. (2005) and Van Eerdenburg et al. (2011) found the most reliable sign of oestrus to be mounting behaviour, however, no commercially available sensor monitors mounting behaviour in cows. As mentioned in section 3.2.1, Abell *et al.* (2017) applied classification models to accelerometer data collected from bulls which resulted in a maximum precision of only 2.5%. No further studies have been conducted concerning the detection of mounting behaviour in cattle using on-animal sensors.

Activity monitors are the most common type of oestrus detection technology available. Rather than monitoring a single behaviour, these sensors monitor the suite of behaviours that a cow performs over the course of a day, including feeding or rumination, walking and recumbency. On the day of oestrus, cows reduce the time spent lying and feeding and increase the time spent walking. Monitoring changes in

the activity budget also have the added benefit of identifying cows that do not present standing heat. Zebari, Rutter and Bleach (2018) found that silent oestrus could be detected by monitoring for decreases in feeding behaviour on the day of oestrus. The greatest advantage of activity monitors, however, is their ability to simultaneously monitor the health, welfare and productivity of cows based on their daily activity budgets. Sensors such as the CowManager, eSense, IceQube are all marketed as integrated monitoring systems for health, welfare and productivity, as well as detecting oestrus with an accuracy of over 80% (Dolecheck et al., 2015; Macmillan et al., 2020) (Table 1.2). As a farmer's decision to adopt a technology largely depends on the return on investment, technologies that maximise both the depth and breadth of economic benefits will reap more success.

There is strong potential for an on-animal sensor to improve the reproductive efficiency of Australian sheep flocks (Corbet et al., 2018; O'Neill et al., 2014; Selvam and Archunan, 2017; Shahriar et al., 2016), however it must be designed with the end-point user in mind. For a commercial product to succeed in an extensive industry, it needs to be robust, economical and energy efficient. Recent investigations into on-sheep sensors as a PLF technology have not proceeded past their use as a research tool. Many of these studies use technologies with a store-on-board memory and apply complex classification models to the data post-hoc. Computational cost needs to be considered on a device that is required to process, analyse and transmit data in real-time. This is especially true for a sensor to monitor reproductive performance as joining lasts for up to five weeks.

The interoperability of the technology is also important, as complex data flows from multiple products are not useful for a producer (Bahlo et al., 2019). Recent studies that utilised on-animal sensors to detect event behaviours in livestock suggest that algorithms specifically designed to target a behaviour of interest are more accurate (Abell et al., 2017; Alhamada et al., 2017a; Lush et al., 2018; Mozo et al., 2019) but the commercial success of holistic monitoring systems in the dairy industry warrants consideration. Sheep graziers are less inclined to adopt new technology than those in intensive livestock industries due to lower input and output per head and the larger numbers of animals per farm (Odintsov Vaintrub et al., 2021). The likelihood of a reproductive monitoring technology becoming commercially successful would be boosted by the ability to monitor state behaviours. As it has already been demonstrated that ewes experience a similar increase in activity during oestrus as cows do (Fogarty et al., 2015), it is likely that activity monitors could serve as an oestrus detection technology for the sheep industry as well. A system that combines this with specifically designed mount detection technology to monitor libido in rams would allow producers to identify areas of reproductive wastage in their flock and maximise reproductive efficiency.

1.5 Conclusion

Until recently, it has been notoriously difficult to monitor the reproductive performance of sheep during joining. On-animal sensors have enormous potential to identify low-performing rams in real-time and replace outdated methods of estimating ram reproductive performance. This has been highlighted by the recent success of two

novel mating detection technologies, the Alpha-Detector (Alhamada et al., 2017a) and the algorithm developed by Mozo et al. (2019), though neither are yet suitable for commercial application in the Australian sheep industry. Despite the performance of these mounting detection technologies and dozens of studies on the use of on-sheep sensors to monitor activity, rumination, disease and oestrus behaviour, only one sensor is commercially available to sheep producers.

By contrast, there are dozens of on-animal sensors available to the dairy industry, many of which monitor oestrus. The culmination of higher input per head, frequent contact with livestock and more advanced infrastructure has led to the acceleration of the on-animal sensor market in the dairy industry. Integrated monitoring systems are common, suggesting that producers are more inclined to adopt a technology if it can monitor an animal's overall activity budget, and thereby health and welfare, as well as reproductive performance. There are additional constraints that need to be considered when developing an on-animal sensor to monitor extensively farmed animals including poor connectivity, less frequent opportunities for data retrieval and less investment per head. Thus, for a reproductive monitoring technology to succeed in the sheep industry, it must be robust, economical and energy efficient. The early stages of product development should consider these limitations during the selection of technology and the design of the algorithm used to detect mating.

Consideration of the limitations outlined above and the lack of PLF technology available to the Australian sheep industry informed the direction of the research within this thesis. The overall aim of this thesis was to develop a sensor to monitor the

mating behaviour of rams in real-time, with the potential for commercial application on-farm. The following objectives were used to achieve this aim:

- Develop a computationally simple algorithm to detect ram mounting behaviour in raw accelerometer data.
- Integrate the algorithm into a sensor that has commercial potential in the Australian sheep industry.
- Utilise this sensor to measure ram reproductive behaviour during joining and investigate the relationship of libido, semen quality and other physiological parameters on the siring ability of rams.

Table 1.2: Commercially available on-animal sensors to monitor reproductive behaviours in livestock

Species	Name	Company	Country	Sensor type	Purpose	Position	Externally validated
Cattle	Alert'Vel	ALB Innovation	France	Accelerometer	Calving	Tail	No
Cattle	Smart'Vel	Evolution international	France	Accelerometer	Calving	Tail	No
Cattle	Moocall Calving Sensor	Moocall Ltd.	Ireland	Accelerometer	Calving	Tail	(Horváth et al., 2021; Voß et al., 2021)
Cattle	Aficollar	Afimilk Agricultural Cooperative Ltd.	Israel	Accelerometer	Heat detection	Collar	No
Cattle	AfiAct II	Afimilk Agricultural Cooperative Ltd.	Israel	Accelerometer	Heat detection	Leg	(Chanvallon et al., 2014; LeRoy et al., 2018; Mayo et al., 2019; Wojcik and Olszewski, 2015)
Cattle	HeatSeeker II	BouMatic LLC	United States	Accelerometer	Heat detection	Collar, Leg	No
Cattle	RealTime SmartTag	BouMatic LLC	United States	Accelerometer	Heat detection	Collar	No
Cattle	Cowlar	Cowlar	United States	Accelerometer	Heat detection	Collar	No
Cattle	Smarttag / All in One	CRV international B.V.	Netherlands	Accelerometer	Heat detection	Collar, Leg	No

Cattle	MooMonitor+	Dairy Master	Ireland	Accelerometer	Heat detection	Collar	(Moore et al., 2021)
Cattle	Track A Cow	ENGs Systems	Israel	Accelerometer	Heat detection	Leg	(Dolecheck et al., 2015; Mayo et al., 2019)
Cattle	Gyuhō (cow step) SaaS	Fujitsu	Japan	Accelerometer	Heat detection	Leg	(Hojo et al., 2018)
Cattle	CowScout	GEA Farm Technologies, Inc.	Denmark	Accelerometer	Heat detection	Collar, Leg	(Madureira et al., 2020)
Cattle	Rescounter III	GEA Farm Technologies, Inc.	Denmark	Accelerometer	Heat detection	Collar, Leg	No
Cattle	Breeder Tag	Genius Breeding Ltd.	United Kingdom	Accelerometer	Heat detection	Leg	No
Cattle	Smart Collar	HerdInsights	Ireland	Accelerometer	Heat detection	Collar	No
Cattle	IceQube	IceRobotics Ltd.	United Kingdom	Accelerometer	Heat detection	Leg	(Dolecheck et al., 2015; Mayo et al., 2019)
Cattle	Qwes ISO LD (+ variations)	Lely	Israel	Accelerometer	Heat detection	Collar	No
Cattle	HeatPhone	Medria Inc.	France	Accelerometer	Heat detection	Collar	(Chanvallon et al., 2014)

Cattle	Milkrite InterPuls Neck Tag/Pedometer	milkrite InterPuls	Italy	Accelerometer	Heat detection	Collar, Leg	No
Cattle	Moocall Heat	Moocall Ltd.	Ireland	Accelerometer	Heat detection	Collar	No
Cattle	Smarttag Neck	Nedap livestock management	Netherlands	Accelerometer	Heat detection	Collar	(Roelofs et al., 2017; Van Erp-Van der Kooij et al., 2016)
Cattle	SmartTag	Pearson International LLC	Ireland	Accelerometer	Heat detection	Collar, Leg	No
Cattle	eSense™/cSense Flex tag	SCR Engineers Ltd. (Allflex Europe SA)	Israel	Accelerometer	Heat detection	Ear, Collar	(Indira et al., 2020; Macmillan et al., 2020)
Cattle	SCAR H-LD (+ variations)	SCR Engineers Ltd. (Allflex Europe SA)	Israel	Accelerometer	Heat detection	Collar	No
Cattle	Alpro	DeLaval International AB Inc.	Sweden	Accelerometer	Heat detection		(Peralta et al., 2005)
Cattle	Heat Box	Gènes Diffusion	France	Accelerometer	Heat detection		No
Cattle	Heatime-RuminAct	SCR Engineers	France	Accelerometer	Heat detection	Collar	(Borchardt et al., 2021; Chanvallon et al., 2014)
Cattle	CowManager Sensor	Agis Automatisering BV	Netherlands	Accelerometer, temp	Heat detection	Ear	(Dolecheck et al., 2015)

Cattle	SMARTBOW	Smartbow GmbH (Zoetis Services LLC.)	Austria	Accelerometer, temp	Heat detection	Ear	(Schweinzer et al., 2019)
Cattle	Smart Rumen Bolus	Moonsyst Industrial Technologies Ltd.	Hungary	Accelerometer/temp/pH	Heat detection	Bolus	No
Cattle	smaXtec classic / pH Plus Bolus	smaXtec Animal Care Inc.	Austria	Accelerometer/temp/pH	Heat detection	Bolus	(Roelofs et al., 2018; Vicentini et al., 2018)
Cattle	LiveCare	uLikeKorea Co., Inc.	South Korea	Accelerometer/temp/pH	Heat detection	Bolus	(Kim and Ahn, 2016)
Cattle	Crysta-Heat	Fullwood Ltd	United Kingdom	Pedometer	Heat detection	Leg	No
Cattle	HeatWatch II	CowChips, LLC	United States	Pressure	Heat detection	Tail	(Peralta et al., 2005)
Cattle	Vel'Phone	Medria Inc.	France	Temperature	Calving	Vagina	(Choukeir et al., 2020; Horváth et al., 2021; Ricci et al., 2018)
Cattle	HerdStrong	DVM Systems Co.	United States	Temperature	Heat detection	Bolus	No – not for oestrus
Cattle	Cow Call	Cow Call	Ireland	Temperature/light	Calving	Vagina	No
Sheep	SmartShepherd	SmartShepherd	Australia	Proximity	Mothering up	Collar	No

Chapter 2 – The use of accelerometers for the remote detection of mounting behaviour in rams and testosterone-treated wethers

The experiments herein are published as:

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<https://doi.org/10.1016/J.COMPAG.2022.107129>

2.1 Abstract

It has been estimated that up to a third of all rams have little to no sexual interest in ewes. Despite the prevalence of this issue, it is challenging to monitor the reproductive behaviour of individual sheep in an extensive farming environment. In this study, we developed a single-feature algorithm to detect in-paddock mounting activity from the accelerometer records of rams and androgenised wethers. Tri-axial accelerometers were first deployed on the necks and withers of the rams (n=15) in a controlled pen test to determine the optimal attachment point for detecting mounting events. A Moving Average Convergence Divergence (MACD) algorithm was applied to accelerometer data with a threshold of 0.3 g. Validation and performance evaluation of the algorithm was based on observed video footage. The MACD was more sensitive and precise when applied to data collected from the withers than the neck (100% vs 89%; 98% vs 86%, respectively). Following the pen test, the MACD was applied to data collected in-paddock from accelerometers attached to the withers of rams (n=6) and

androgenised wethers (n=8). A MACD+ was simultaneously evaluated, which included additional conditions that must be satisfied for the detected peak to be deemed a mounting event. For both algorithms, a series of thresholds were tested. The threshold that returned the highest F1 score (the harmonic mean of precision and sensitivity) for the respective algorithm and male type was selected as the optimal threshold. At their respective optimal thresholds, the MACD+ was marginally more precise than the MACD in-paddock for both wethers (91% vs 84%, respectively) and rams (94% vs 84%, respectively). The sensitivity of the MACD in detecting in-paddock mounting behaviour was 91% in rams and 86% in wethers. The MACD+ was similarly sensitive at 91% for both rams and wethers. This research demonstrates the success of this algorithm, particularly the MACD+, in detecting mounting activity. Following integration into a commercial sensor and further validation, this algorithm would allow producers to identify rams with low libido and improve reproduction and productivity.

2.2 Introduction

It is estimated that between a quarter and a third of all rams are 'non-performers' that display little to no sexual interest in ewes (Alexander et al., 2012; Fitzgerald and Perkins, 1991; Hulet et al., 1964). This could result in fewer lambs being born, extend the lambing window and necessitate the recruitment of surplus rams to the breeding flock (Alexander et al., 2012). The sexual performance of rams also has a moderate heritability (Snowder et al., 2002), with low sexual performance passed onto male offspring and time to first oestrus delayed in female offspring (Bench et al., 2001).

Despite the importance of ram reproductive performance, measuring such traits is labour-intensive and inaccurate (Kilgour and Whale, 1980; Lindsay et al., 1976; Mickelsen et al., 1982).

The most common method for evaluating ram libido is a serving capacity test (Mattner et al., 1971). There are several variations of this test, but fundamentally they involve introducing a ram into a pen with up to five ewes and recording the number of mating and courtship behaviours performed over thirty minutes (Kridli and Said, 1999; Snowden et al., 2002). Studies have shown that the foreign testing environment, sexual attractivity of the ewes, the proximity of humans and male hierarchy influence the reliability of the test (Kilgour, 1993). Stellflug and Berardinelli (2002) advised that six repetitions of a serving capacity test were required to ensure a reliability of 95%. Therefore, technologies for the automated monitoring of sexual behaviour could provide an accurate and practical means to monitor reproductive performance in rams.

Accelerometers provide insight into an animal's behavioural repertoire without the stressors of in-person monitoring and artificial environments (Brown et al., 2013). There has been an influx of accelerometer research in sheep in recent years for this reason (Fogarty et al., 2018). The majority of this research has focused on the classification of state behaviours, which occur over long periods of time and are measured by the duration for which they occur, such as grazing, walking and recumbency (Barwick et al., 2018; Fogarty et al., 2020; Walton et al., 2018). Event behaviours, by contrast, are much shorter in duration and are typically measured by the number of times they occur rather than duration. Due to their short, sudden

nature, they can be difficult to detect remotely. Few studies have utilised accelerometers to detect event behaviours in sheep (Kuźnicka and Gburzyński, 2017; Lush et al., 2018), and only one study has used accelerometers to monitor reproductive behaviour in rams (Mozo et al., 2019). In addition, no studies have used accelerometers to detect mounting behaviour in androgenised wethers, which are employed in artificial breeding programs and research studies to detect ewes in oestrus.

The overarching goal of the present study was to develop an algorithm that could be integrated into a commercial sensor and used by producers to monitor the reproductive behaviour of rams in real-time. For an on-sheep sensor to be commercially successful in the Australian sheep industry, it must be energy efficient, accurate and economical. An algorithm to detect mounting behaviour in sheep should therefore be computationally simple to be compatible with low-cost microprocessors and limited energy inputs.

The aim of the present study was to develop, validate and evaluate the success of a computationally simple algorithm to detect mounting behaviour from the accelerometer records of rams and wethers.

2.3 Materials and Methods

This study took place at the University of Sydney's Animal Reproduction Unit, Cobbitty, NSW, Australia (Longitude 150° 39' 57" E, Latitude 34° 01' 02" S). All procedures herein were approved by the University of Sydney Animal Ethics

Committee, 2018/1309. Two trials were carried out to fulfil the objectives of this study, with the first under controlled conditions to develop the algorithm to detect mounting events and the second in field conditions to evaluate the algorithm.

2.3.1 Trial 1: Development of algorithm in a controlled environment

Animals & Instrumentation

Mature merino rams (n=15) were each fitted with two tri-axial accelerometers (ActiGraph GT3X-BT; ActiGraph, LLC; Pensacola, USA) prior to the trial. The accelerometers were attached to the neck using a collar and posterior to the withers via a harness (Figure 2.1). In both locations, accelerometers were positioned such that the x-axis corresponded with the lateral axis of the ram. The accelerometers weighed 19 g and were programmed to record at a rate of 30 Hz. This was the minimum sampling rate available on the accelerometers used in the study.

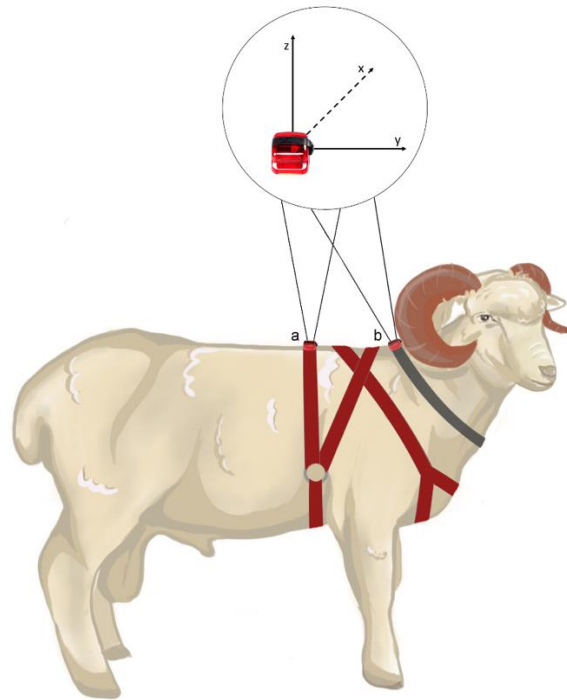


Figure 2.1: The attachment points of the accelerometer to the withers (a) and neck (b) of a ram via a harness and collar, respectively, with the x-axis corresponding to the lateral plane of the ram. Illustration created by Dr K. R. Pool.

A ewe in oestrus was secured in a headstall in an open 2 x 2 m pen. A technician was stationed by her flank with an artificial vagina in order to intercept the ram prior to intromission. Rams were held in a separate pen 10 m away and were introduced to the ewe one at a time. Once the ram was released in the pen with the ewe, they were given three minutes to perform courting and mating behaviours. The technician intercepted all mounts with an artificial vagina. Observers stationed outside the pen filmed the ram's behaviour. This was repeated twice over the course of the day for each ram, with at least two hours between each session. Following this period, rams were moved to an adjacent pen before another ram was immediately introduced.

Development of the single-feature algorithm

Accelerometers were removed from the rams immediately following the trial and data were retrieved from the store-on-board memory of the accelerometers using the Actilife software. Data sets were trimmed to begin when the ram was released from the holding pen and ended after the allotted three minutes. The raw accelerometer data was manually annotated with behaviours observed in the recorded videos (Table 2.1) by three operators. Operators were trained in data annotation and all instances of event behaviours were cross-checked by the lead operator.

A moving average convergence-divergence (MACD) algorithm was applied to the raw z-axis data in Python. The MACD is a variation of a trading indicator used in the finance industry (Appel, 2005) that smooths data by subtracting a fast simple moving average from a slow simple moving average. The z-axis was selected as mounting is distinguished from other behaviours by movement through the vertical dimension, as opposed to the lateral (x-axis) and longitudinal (y-axis) dimensions. The equation for the MACD was as follows:

$$Acc_N^z = \frac{Acc_t^z + Acc_{t-1}^z + \dots + Acc_{t-N+1}^z}{N}$$

Where Acc is the moving average acceleration over the last N timesteps, A is acceleration in the z-axis as measured on the ram mounted accelerometer and t is most recent time.

$$MACD_{10-90}^z = Acc_{10}^z - Acc_{90}^z$$

$$MACD = MACD_{10-90}^z$$

Where $MACD$ is the difference in 10 datapoint Acc of the z-axis (short moving average) and 90 datapoint Acc of the z-axis (long moving average).

The transformed data was run through a peak detection package in Python (`scipy.findpeaks`) to visualise peaks in the collar and harness-collected data associated with mounting. A vertical threshold of 0.3 g was applied such that any peak greater than 0.3 g was deemed to be a mounting event (Figure 2.2). A minimum horizontal distance threshold of 150 dp, equivalent to five seconds, was set to minimise the likelihood of a dismount peak being captured by the algorithm. An upper threshold of 1 g was also set. Thus:

Mounting event = $MACD > 0.3\text{ g}$ and $MACD < 1\text{ g}$, with no identified in the last 150 dp.

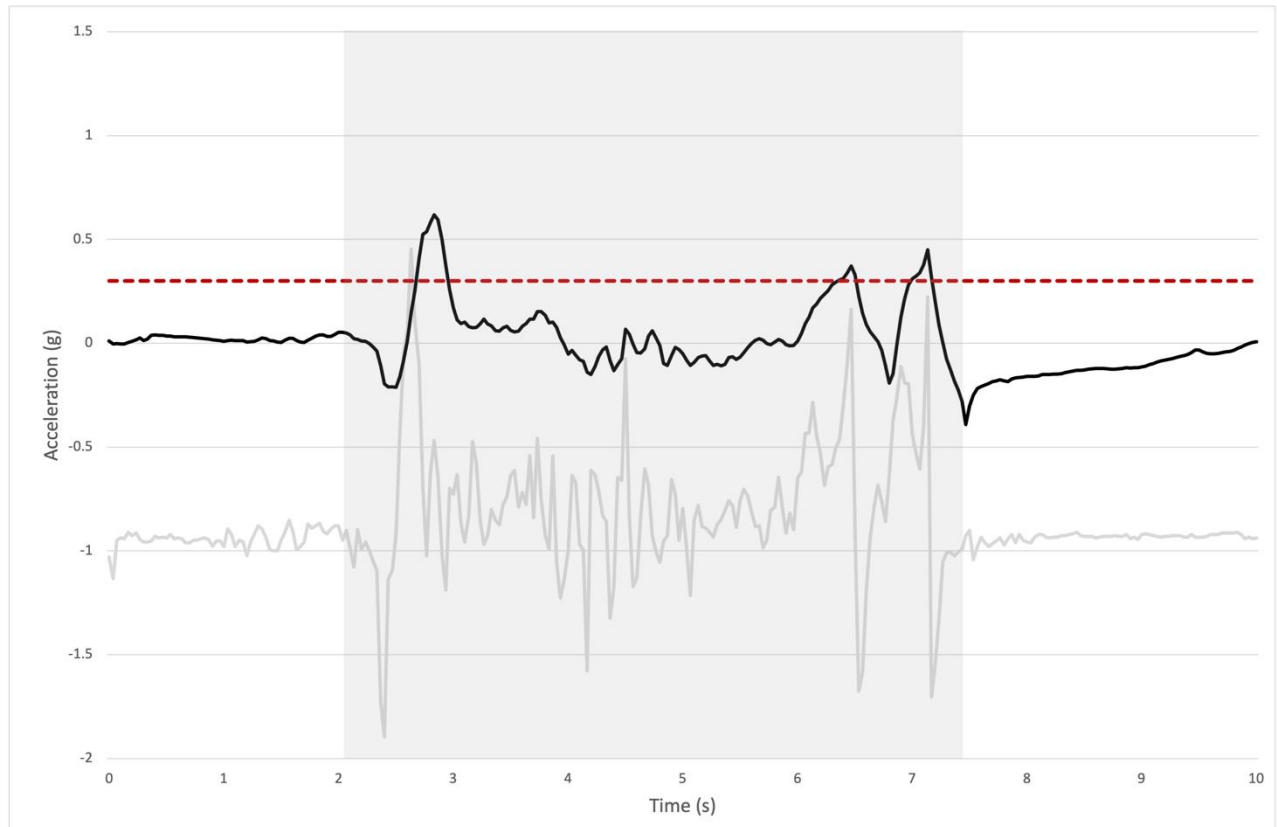


Figure 2.2: An example of a mounting event (shaded grey) in MACD transformed data (black) and raw z-axis data (grey) retrieved from a harness-attached accelerometer.

The video footage was used to determine whether the predicted mount was also an observed mount and to identify any observed mounts that were not detected by the MACD. The number of observed mounting events correctly identified (true positives; TP), the number of non-mounting events that were incorrectly identified as mounting events (false positives; FP), and the number of observed mounting events that were not identified by the algorithm (false negatives; FN) were recorded for both the collar and harness-collected data and their respective thresholds. If two peaks were registered for a singular mounting event, the second peak was labelled as a FP. Sensitivity, precision and F1 score (the harmonic mean of sensitivity and precision) were calculated and used to determine the optimal attachment point for the

accelerometer and select a threshold that maximised both sensitivity and precision.

The definitions are as follows:

$$\text{Sensitivity} = \text{TP}/(\text{TP} + \text{FN})$$

$$\text{Precision} = \text{TP}/(\text{TP} + \text{FP})$$

$$\text{F1 score} = 2 * ((\text{Precision} * \text{Recall})/(\text{Precision} + \text{Recall}))$$

2.3.2 Trial 2: Validation of the algorithm in a field environment and addition of other conditions

Animal and instrumentation

Forty merino ewes were oestrus-synchronised by the administration of progesterone through a controlled internal drug releasing device (EAZI-BREED CIDR, Zoetis). The CIDRs were removed after 12 days and ewes were given an intramuscular injection of 400 IU of pregnant mare serum gonadotropin (PMSG; Pregnecol, Vetoquinol) to aid synchronisation. Sexual behaviour in wethers (n=8) was induced with an intramuscular injection of 400 mg testosterone (Ropel; Jurox Animal Health) at the time of ewe CIDR insertion and another 150 mg at ewe CIDR removal. At 45 hours post-CIDR removal, tri-axial accelerometers (BT-GT3X Actigraph, Pensacola, USA) were fitted to rams (n=6) and wethers as described for Trial 1. All male sheep were fitted with a unique bib for ease of identification during paddock observations.

Observations

Rams (n=6) and wethers (n=8) were introduced to separate flocks of ewes (n=20) immediately following accelerometer attachment. Observations took place over six hours in a 25 m x 75 m paddock. Two observers stationed at the edge of the paddock filmed the flock continuously. Mounting events were also manually recorded along with the ID of the male performing the event and the exact time it occurred.

Data were retrieved from the accelerometers in the form of comma separated values (CSV) files, which were annotated with behaviours observed in video footage (Table 2.1). Two classes of behaviour were determined: event behaviours and state behaviours. Event behaviours included mounting, running, shaking and agonistic behaviours (head-butting or head-butted). Standing, walking and recumbency were classed as state behaviours and were subsumed under one behavioural label.

Table 2.1: Descriptions of the behaviours of rams and wethers recorded in trials 1 and 2 to develop algorithms detecting mounting behaviour

Behaviour	Description
Mounting	Male bends knees and surges forward on his hind legs, connects his brisket with the ewe's rump (this may include intromission and ejaculation) and then dismounts and resumes a standing position
Running	Forward motion at a pace faster than a walk
Head-butting ¹	Forceful head-forward movement of aggression directed at another sheep
Head-butted ¹	Reception of a forceful head-forward movement of aggression from another male
Shaking	Rapid, vigorous movement of the entire body from side to side

Walking	Forward motion at a slow pace, where each hoof hits the ground independently
Grazing ¹	Foraging while standing or walking with head lowered to the ground
Lying ¹	Recumbent on the ground
Standing	Sedentary in an upright position supported by all four legs

¹Behaviours observed only during trial 2

Data analysis

All annotated data were processed for analysis in Python as in trial 1. The MACD algorithm developed in trial 1 was applied to the data from trial 2 to determine its accuracy. In addition, a new algorithm was created (MACD+) which included additional conditions added to the MACD to reduce false positives in the paddock data. This involved the creation of three extra features: a MACD of the x-axis ($MACD^x$), a 10 dp smooth moving average of the x-axis (Acc_{10}^x) and a 10 dp smooth moving average of the y-axis (Acc_{10}^y). For a peak to be passed under the MACD+, it must have exceeded the pre-determined threshold and satisfied at least three of the four following conditions:

1. Maximum of the next 15 $MACD^x < 0.8 g$
2. At least 36 of the next 45 in $Acc_{10}^y > 0$
3. Minimum of next 30 dp in $Acc_{10}^x > -0.5 g$
4. Maximum swing between positive and negative integers in the next 15 $Acc_{10}^y <$

The peak detection package was run as in Trial 1, with a minimum horizontal distance of 150 dp and an upper threshold of 1 *g*. An array of thresholds were tested to determine the optimal threshold for accurate mount detection. These thresholds ranged from 0.25 *g* to 0.65 *g*, increasing in 0.05 *g* increments.

A comma separated values file was generated with all detected peaks and their associated behaviour label to assess the performance of each algorithm for each of the nine thresholds. The behaviour label was used to classify detected events as true positives or false positives. These were compared against the number of all observed mounting events to determine the number of false negatives. Precision, sensitivity and F1 score were calculated as in Trial 1.

An optimal threshold was determined for rams and wethers as well as for each individual male by taking the threshold that returned the highest F1 score. A generalised linear mixed model was used to compare the heights of MACD peaks with male type as the fixed effect and animal ID as the random effect. The same model was used to compare the duration of mounting events. A likelihood ratio test was used to determine whether the random effect was significant.

2.4 Results

2.4.1 Trial 1: Development of algorithm in a controlled environment

A total of 96 mounting events were observed across the 15 rams over two replications (Table 2.2). The MACD algorithm identified mounting events in accelerometer data collected from harnesses with perfect sensitivity (100%) and extremely high precision (96%). The F1 score for harness data when the MACD algorithm was applied was 98%.

Table 2.2: The total number of mounts for each ram during Trial 1

Ram	Total mounts
A	9
B	12
C	7
D	3
E	3
F	4
G	6
H	16
I	10
J	4
K	4
L	8
M	3
N	3
O	4

The MACD was less accurate when applied to collar-collected data compared to the harness-mounted data using the same threshold of 0.3 *g*. Sensitivity decreased from

100% to 88% when collar data was used ($p < 0.01$). The algorithm was also less precise when applied to collar data at 80% ($p < 0.01$). The F1 score for the collar-collected data was 84%

2.4.2 Trial 2: Validation of the algorithm in a field environment and addition of other conditions

A total of 378 mounts were observed in rams and 170 in wethers (Table 2.3). There was no significant difference between rams and wethers for the average duration of a mount and the height of the peak a mount produced. For both mount duration and peak height, there was significant variation between individual males.

Table 2.3: The frequency of mounting events for rams and wethers joined with 20 ewes in a paddock in Trial 2. Significant differences are denoted by superscript letters.

	Total number of mounting events	Average duration $\pm$ SEM (s)	Average peak height $\pm$ SEM (g)
Rams	378	2.7 $\pm$ 1.57^a	0.54 $\pm$ 0.008^a
A	137	2.7 $\pm$ 1.50	0.54 $\pm$ 0.009
B	46	2.5 $\pm$ 1.31	0.40 $\pm$ 0.011
C	63	2.9 $\pm$ 1.41	0.71 $\pm$ 0.016
D	30	3.9 $\pm$ 2.26	0.68 $\pm$ 0.018
E	43	2.1 $\pm$ 1.13	0.55 $\pm$ 0.014
F	59	2.9 $\pm$ 1.67	0.40 $\pm$ 0.013
Wethers	170	3.8 $\pm$ 1.82^a	0.65 $\pm$ 0.012^a
A	18	5.5 $\pm$ 3.24	0.53 $\pm$ 0.060

B	12	4.3 ± 1.37	0.71 ± 0.028
C	13	2.9 ± 1.28	0.76 ± 0.021
D	67	3.9 ± 1.54	0.73 ± 0.014
E	23	3.0 ± 1.20	0.60 ± 0.020
F	10	3.1 ± 1.39	0.56 ± 0.036
G	8	3.6 ± 0.78	0.59 ± 0.033
H	19	3.4 ± 1.49	0.47 ± 0.019

Under the MACD, the maximum F1 score for rams was 87%, corresponding to an optimal threshold of 0.45 (Table 2.4). Wethers had a slightly lower maximum F1 score of 85% at a threshold of 0.5. At their respective optimal thresholds, the MACD was 91% precise for rams and 86% for wethers. Precision was lower overall and was 84% for both rams and wethers at their optimal thresholds. Agonistic behaviours (headbutting or being headbutted) represented 79% of the events that were incorrectly identified as mounting in rams (Figure 2.3). By comparison, 48% of events misclassified as mounting in wethers were agonistic. Running was equally misclassified as mounting in wethers at 48% as well.

Table 2.4: The overall performance of the MACD and MACD+ algorithms in the detection of mounting events performed by rams and wethers. Optimal thresholds are defined by the maximum F1 score and are bolded.

	Threshold (g)								
	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65
MACD									
<i>Ram</i>									
Sensitivity (%)	99	98	97	96	91	86	72	63	47
Precision (%)	58	68	76	80	84	88	90	92	92
F1 score (%)	73	81	85	87	87	87	80	75	62

<i>Wether</i>									
Sensitivity (%)	98	98	98	94	89	86	78	65	50
Precision (%)	60	62	67	73	79	84	87	88	89
F1 score (%)	74	76	80	82	84	85	83	75	64
MACD+									
Ram									
Sensitivity (%)	96	95	93	92	86	79	63	54	41
Precision (%)	78	88	91	94	96	97	98	99	99
F1 score (%)	86	91	92	92	91	87	77	70	58
Wether									
Sensitivity (%)	92	92	91	88	83	78	72	59	46
Precision (%)	87	89	91	90	91	91	91	93	95
F1 score (%)	89	90	91	89	87	84	81	72	62

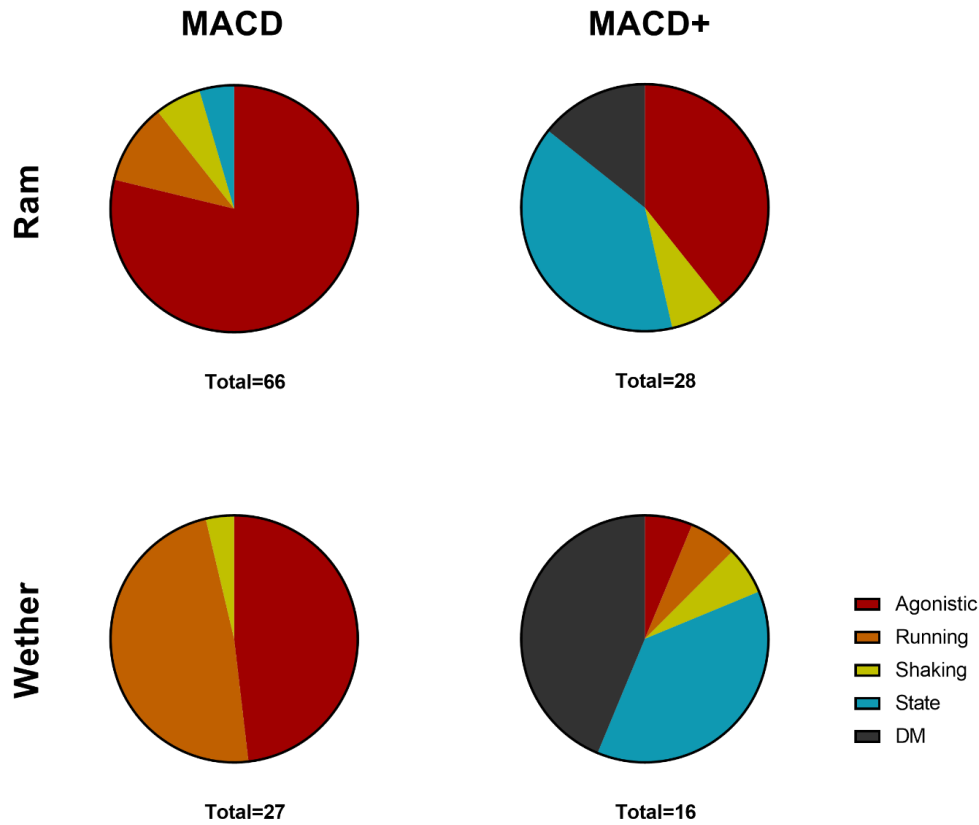


Figure 2.3: The behaviour class of false positives for each male type under the MACD and MACD+

When the additional conditions of the MACD+ were applied to data from rams, precision increased from 84% to 94%. The proportion of agonistic behaviours decreased to 39% of all false positives (Figure 2.3). Precision was also improved by applying the MACD+ to data from wethers, increasing from 84% to 91%. The double detection of mounting events accounted for 44% of all false positives, with state behaviours accounting for 38% and agonistic and running behaviours representing only 6% of total false positives each.

The maximum F1 scores were higher in the MACD+ than in the MACD due to the improvement in precision (Table 2.4). These scores were 92% and 91% for rams and

wethers, respectively, at thresholds of 0.40 g and 0.35 g. In addition to having higher F1 scores, the optimal thresholds were also lower overall under the MACD+.

Table 2.5: The performance of the MACD and MACD+ algorithms for individual rams and wethers in the detection of mounting events.

Animal ID	MACD			MACD+		
	Sensitivity (%)	Precision (%)	F1 score (%)	Sensitivity (%)	Precision (%)	F1 score (%)
Ram						
A	94	97	96	96	99	97
B	77	70	73	77	90	83
C	94	76	84	95	94	95
D	97	67	80	93	76	84
E	100	88	94	100	88	94
F	83	88	85	75	94	83
Wether						
A	94	100	97	94	100	97
B	100	71	83	100	92	96
C	100	100	100	100	93	96
D	90	91	90	96	83	89
E	91	78	84	70	100	82
F	89	67	76	100	90	95
G	86	54	67	100	100	100
H	37	88	52	74	100	85

Variability in the performance of the mounting detection algorithm between individual rams was the highest when the MACD was applied (Table 2.5). Variability was even higher between individual wethers under the MACD with F1 score ranging

from 52% to 97% (Table 2.4). However, the variability of algorithm performance across rams was reduced greatly under the MACD+.

F1 scores improved when individual optimal thresholds were used rather than the overall optimal threshold as described above, particularly for the MACD algorithm (Table 2.6). The F1 scores under the MACD were above 80% for all males except one. There were minimal increases in the performance of the MACD+ when individual optimal thresholds were applied (Table 2.6).

Table 2.6: The performance of the MACD and MACD+ algorithms in the detection of mounting behaviour of rams and wethers under individual optimal thresholds.

	MACD		MACD+	
	Best Threshold ¹	F1 score (%)	Best Threshold	F1 score (%)
Ram				
A	0.40	96	0.30	97
B	0.40	81	0.30	88
C	0.55	89	0.40	95
D	0.55	88	0.45	87
E	0.45	94	0.45	96
F	0.35	89	0.30	85
Wether				
A	0.35-0.40	95	0.25-0.45	97
B	0.60	85	0.35-0.55	96
C	0.45-0.60	100	0.45-0.50	100
D	0.50-0.55	91	0.35-0.40	89
E	0.50	84	0.20-0.40	82
F	0.55	84	0.30-0.50	95
G	0.55	67	0.15-0.40	100
H	0.20-0.35	91	0.10-0.20	94

¹If multiple thresholds had the equal highest F1 score, they were presented as a range.

2.5 Discussion

The MACD and MACD+ algorithms developed in the present study were highly sensitive and precise in the detection of mounting events. The algorithm's success in a controlled environment in trial 1 demonstrated that harness-attached accelerometers outperformed collar-attached accelerometers. Collars may be less labour-intensive to fit on animals but this is traded off with precision. Studies that have utilised collar-attached sensors to monitor behaviour in sheep have attributed the variability of results to the variability in sensor position (Barwick et al., 2020). This may be true of the present study however, we hypothesise that the looser fitting collars introduced more noise into the data, thereby reducing performance. Reliance on a single axis for the detection of mounting events using the MACD means that sensor position must be accurate and consistent. Harnesses are tighter fitting and more secure than collars, which has likely aided their success although care must be taken to ensure the accelerometer fixed to harnesses is correctly aligned. Checking the static acceleration of acceleration on the z-axis would aid in ensuring that the accelerometer is positioned correctly. The greater motion of the neck compared with the withers is also likely to have contributed to the poorer performance of collar-attached sensors.

Rams did not exhibit agonistic behaviour in trial 1 because they were individually placed in the pen with the ewe, but this was observed in both rams and wethers during trial 2 when animals were mixed with ewes in pens. Other behaviours such as running were also more frequently observed in-paddock. Both behaviours contributed to a decrease in the precision of the MACD when it was applied to data collected from the paddock. This was due to the fact that mounting, headbutting, being headbutted,

and running are characterised by high forces of acceleration compared with state behaviours. Mozo et al. (2019) also reported a precision of 84% in their application of a mount detection algorithm to data from a harness-attached accelerometer. The nature of the false positives encountered was not described by Mozo et al. (2019), however, the eight rams used in that study were joined with ewes in a syndicate, so it can be presumed that agonistic behaviours did occur.

To counteract the decrease in precision from the pen trial to the paddock trial, the MACD+ was created by applying a set of conditions to the MACD. These conditions were based on features from the x- and y-axes, which measured acceleration along the lateral and longitudinal planes of the male. Mounting behaviour is characterised by less acceleration in the lateral plane and greater, more consistent acceleration along the longitudinal plane. This contrasts with running, which is characterised by greater force across the lateral plane and agonistic behaviour, which is characterised by negative forces along the longitudinal plane during recoil or inertia caused by contact with another male. Precision increased when the MACD+ was applied and the proportion of false positives arising from agonistic and running behaviour was greatly reduced. These results demonstrate the need to validate algorithms under independent conditions to those in which these are developed.

Despite the differences in precision between the two algorithms, both were highly sensitive in detecting mounting events when applied to accelerometer data from rams and wethers. A sensitivity of over 90% was observed for rams under the MACD and for both male types under the MACD+. A similar sensitivity was achieved by Mozo et al. (2019), decision tree algorithm detected 94% of all observed services (mounts in which

a ram achieves intromission and ejaculates) and 78% of all mounts. It measured both the angle of the accelerometer with respect to the ground and the xy-axis in different phases of the mount behaviour. It also used time intervals to determine if an event was too brief to be classified as a mount (<1.75 s) and whether the mount was a service or not by assuming a six-minute refractory period. Mounting is characterised by such a force of acceleration compared with state behaviours that it is evident why mounts were detected with such high sensitivity across both studies. Mounts observed in the current study were occasionally briefer than 1.75 seconds, which may explain the decreased sensitivity Mozo et al. (2019) encountered in detecting mounts. The current study did not discern between mounts and services, and it is unknown if similar results could be achieved by assuming the same refractory period.

Androgenised wethers are commonly used in artificial breeding programs and research studies to both tighten the synchrony of oestrus through the male effect and detect when ewes come into oestrus (Crocker et al., 1999, 1982; Maddison et al., 2016). Despite the difference in sizes between wethers and rams, this study demonstrated that there was no significant difference in the peak heights between the two male types. The optimal thresholds for rams and wethers did differ by 0.05 g for both the MACD and MACD+ however, if the wethers assumed the optimal thresholds of the rams, the decrease in F1 score was negligible. This indicates that the same threshold settings could be used for both wethers and rams in a future commercial sensor.

For the purpose of this study, evaluations of the two algorithms were based on the respective thresholds that returned the highest F1 score. As earlier described, the F1 score is the harmonic mean of precision and sensitivity. It is worth noting that

precision and sensitivity may not be valued equally in every scenario. For example, a producer conducting single sire joining may value selecting a threshold that favours sensitivity over precision, because rams rarely engage in agonistic behaviour without other rams present. The trade-off between precision and sensitivity is a dynamic relationship that should be considered at the user endpoint.

The sensitivity and precision of the MACD varied between individual males. While this variation was reduced when the MACD+ was employed, tailoring the optimal threshold to individuals also improved the performance of the MACD. Body size is likely to be the one source of this between-male variation. Although the height and weight of males were not measured in the present study, theoretically the closer in size a male is to a ewe, the more perpendicular the angle at which he mounts her must be, therefore increasing the force of acceleration. A similar conclusion was drawn by Mozo et al. (2019) who hypothesised that the failure of a ram to meet their algorithm feature thresholds was linked to its large size. It is unlikely that this is the only explanation for variation between individuals in the current study as the height of MACD peaks did not differ significantly between rams and the smaller-bodied wethers. Variation between individual sheep in the classification of accelerometer data was also encountered by Barwick et al. (2018) who speculated that differences in accelerometer positioning, conformation and gait contributed to the varying performance of a classification model between sheep. Differences in conformation and the manner in which rams jump are also likely to affect the performance of the MACD. A calibration period in which the average peak acceleration is measured for individual rams is a possibility for future commercial sensors and would enable the MACD to be utilised with improved performance outcomes for individual sheep.

The present study is one of three that have used sensors to monitor mounting behaviour in rams. Alhamada *et al.* (2017a) developed the Alpha-Detector, a mating detection system that uses a combination of a pneumatic sensor and radio-frequency identification (RFID) reader on the ram to identify the ewe through an RFID transponder fixed to the rump. This system reported a sensitivity of 94% and a precision of 100% although gluing transponders to ewes is not a practically feasible solution for the Australian sheep industry due to the extensive nature of sheep production, the time required to perform the process and the potential for damage to the wool. The decision tree algorithm to detect mating behaviour in rams (Mozo *et al.*, 2019) reported lower precision and similar sensitivity compared to the current study but managed to distinguish between mounts and services. Though the libido correlates with serving capacity, making this distinction is important in rams as producers are ultimately interested in the number of successful mounts.

The sampling rate of the accelerometers in this study was higher than that used by Mozo *et al.* (2019), who applied a 12.5 Hz sampling rate. The authors reported changing the battery every four to five days during a 26-day trial while the ActiGraph is reported to have a battery life of 22.5 days when programmed to a rate of 30 Hz. A commercial mounting sensor would need to withstand the entire joining period, which is typically five weeks long. Reducing the sampling rate of the accelerometer would reduce power consumption and extend the battery life. Given that the MACD is a simple smoothing equation, it is likely that there would be minimal losses in accuracy following its integration and translation into a commercial sensor with a lower sampling rate. Indeed, this would require validation. Sampling rates of 10 Hz

and below are typical of sensors in the dairy industry, both those used in research and those that are commercially available (Benaissa et al., 2020, 2019; Finney et al., 2018; Shahriar et al., 2016b). Be that as it may, these sensors measure activity based on state behaviours (including standing, rumination and walking), not event behaviours which are shorter in duration and lower in frequency. Notably, Mozo *et al.* (2019) achieved accurate detection of mating behaviour in rams using a 12.5 Hz sampling rate.

Both the MACD and the MACD+ were sensitive, precise and computationally simple algorithms for detecting mounting behaviour in sheep compared to other studies in the field. Without tailoring the algorithm for individual animals, it is clear that a trade-off exists between algorithm simplicity and precision in this study as the single-feature MACD was less precise than when the additional conditions were added.

Despite the extra conditions of the MACD+, the computational simplicity of both algorithms outstrips those of other studies in the field. This makes the MACD and the MACD+ suitable candidates for commercial sensors that are limited by power capacity and longevity. Future research could investigate the integration of these algorithms onto a commercial on-sheep sensor and explore how other technology such as Bluetooth or RFID could be used to determine the identity of the ewe associated with a mounting event. By combining this information, producers could precisely monitor fertility and the conception date of a lamb, in addition to a ram's sexual performance.

2.6 Acknowledgements

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Chapter 3 – Monitoring the sexual behaviour of rams in real-time through a wireless sensor network

3.1 Abstract

In 2012 it was predicted that precision livestock farming (PLF) would revolutionise extensive agricultural production systems. Since then, there has been exponential growth in on-sheep sensor research but little of this has been translated into commercial solutions for producers. This study presents the first sensor to monitor ram mating activity and sheep behaviour in real-time through a wireless sensor network. An algorithm previously developed by the authors to identify mounting activity in rams (MACD+) was integrated into an on-animal sensor with commercial potential (Smart Tag; Australian Wool Innovation Limited). To validate the performance of the MACD+ following integration into the Smart Tag, the sensor was first tested in a controlled environment in which rams were fitted with sensors ($n = 9$) were introduced a ewe in a pen. This returned a sensitivity of 84% and a precision of 92%. This was equivalent to the previously reported performance of the MACD+, which was 91% sensitive and 94% precise. As minimal losses in performance were observed following the integration of the MACD+ algorithm into the Smart Tag, its performance was further investigated in-paddock, whereby the mounting behaviour of rams ($n=6$) was measured in real-time through a wireless sensor network. Here, the Smart Tag identified mounting events with a precision of 86% and a sensitivity of 88%. This is comparable to the performance of previously developed mounting detection sensors, thus strengthening the potential of the Smart Tag as a commercially suitable PLF technology.

3.2 Introduction

The advent of on-animal sensors has given rise to Precision Livestock Farming (PLF), a practice in which technology is used to monitor livestock at the individual level rather than the entire flock or herd. Activity monitors, proximity loggers, Global Navigation Satellite Systems (GNSS) or other such sensors are used to monitor the health, welfare and behaviour of individual animals. Information gleaned from PLF technologies acts as a decision support tool and allows producers to tailor management decisions to the level of individual animals.

The commercial availability of PLF technologies is dependent on the industry they are developed for. On-animal sensors are available to the dairy industry by the hundreds (Stygar et al., 2021). The intensive nature of dairy farming facilitates more frequent contact with animals, existing infrastructure to support PLF technologies, a greater likelihood of connectivity and a propensity to invest more capital per head than extensive animal industries. Extensively farmed livestock are grazed in greater numbers over greater parcels of land, often in more remote areas of the country. On-animal sensors developed for the sheep industry must therefore be more robust, economical and self-sufficient than those available to the dairy industry (Masters, 2021). By contrast, there is currently only one on-animal sensor available to sheep producers (SmartShepherd, 2021).

Although the commercial availability of on-sheep sensors is limited, it is a rapidly growing area of research (Fogarty et al., 2018). Industry bodies, researchers and producers alike are increasingly interested in the ways in which on-animal sensors could increase production efficiency. Reproduction is one key area of focus as the

behaviour and performance of individual sheep during joining have historically been lost to the extensive landscape in which sheep are farmed. Without the ability to monitor the behaviour of individuals and their subsequent performance, sources of reproductive wastage can often go undetected. On the male side alone, more than a quarter of rams are 'low performers' that display little to no interest in ewes. The current method used to predict ram libido, known as a serving capacity test, is predictive at best and involves isolating a ram in a pen with 3-5 ewes for a period of at least 30 minutes (Kilgour, 1985; Kilgour and Whale, 1980). Other than the variability in test scores, the test is highly labour-intensive. An on-animal sensor that can detect mating events between sheep would provide a more accurate indicator of a ram's libido with little labour expense.

It has already been established that on-sheep sensors are able to detect mounting behaviour with high precision and sensitivity. Alhamada et al. (2017a) used a combination of a pneumatic sensor and RFID technology to identify mating events and the identity of the animals they occurred between. Despite its success, the system requires the adhesion of RFID chips to the ewe's rump, rendering the technology infeasible in the Australian sheep industry as it detracts from the wool commodity. Mozo et al. (2019) used harness-attached accelerometers and applied a decision tree algorithm to identify mating behaviour in rams with a high level of precision and accuracy. Despite the success of this algorithm, it is still very much in its infancy and requires manual extraction and manipulation of data.

The authors of the current study developed a computationally simple variation of a moving average convergence-divergence algorithm (MACD+) to detect ram mounting behaviour in accelerometer data (Goldsmith et al., 2022). The MACD+ identified

mounting events with a sensitivity of 91% and a precision of 94%. The simplicity of this algorithm made it a strong candidate for integration into a commercial sensor. The aim of this study is to assess the performance of the MACD+ algorithm following its integration into the prototype of a commercial sensor (Smart Tag; Australian Wool Innovation Limited) and assess the ability of the Smart Tag to seamlessly transfer data through a wireless sensor network.

3.3 Materials and Methods

This study took place at the University of Sydney's Animal Reproduction Unit, Cobbitty, NSW, Australia (Longitude 150° 39' 57" E, Latitude 34° 01' 02" S). All procedures herein were approved by the University of Sydney Animal Ethics Committee, 2018/1309. Two trials were carried out to fulfil the objectives of this study, with the first under controlled conditions to develop the algorithm to detect mounting events and the second in field conditions to evaluate the algorithm.

Smart Tags and the integration of the mounting detection algorithm

The accelerometer that was initially used to develop the mounting detection algorithm had a sampling rate of 30 Hz. Smart Tags (Australian Wool Innovation Ltd; Sydney, Australia) sample at a rate of 10 Hz, thus the algorithm required translation to a less frequent sampling technology. To do this, variables in the algorithm were divided by three. The MACD of the z-axis was calculated by:

$$Acc_N^z = \frac{A_t^z + A_{t-1}^z + \dots + A_{t-N+1}^z}{N}$$

Where Acc is the moving average acceleration over the last N timesteps, A is acceleration in the z-axis as measured on the ram mounted accelerometer and t is most recent time.

$$MACD_{3-30}^z = Acc_3^z - Acc_{30}^z$$

$$MACD = MACD_{3-30}^z$$

Where $MACD$ is the difference in 3 datapoint Acc of the z-axis (short moving average) and 30 datapoint Acc of the z-axis (long moving average).

The equation for the MACD+ was as follows:

1. Maximum of the next 5 $MACD^x < 0.8 g$
2. At least 12 of the next 15 in $Acc_{10}^y > 0$
3. Minimum of next 10 dp in $Acc_{10}^x > -0.5 g$
4. Maximum swing between positive and negative integers in the next 5 $Acc_{10}^y < 3$

The MACD+ algorithm was written to the microcontroller units (MCU) of Smart Tags using C. Two types of Smart Tags were created: a research-grade tag and a commercial-grade tag.

The research-grade tag transmitted data to an iOS device upon Bluetooth connection and enabled the user to flag mounting events in real-time through a button in the iOS application. As mounting events occurred, observers were able to see whether the event had 1) met the threshold of the MACD, and 2) satisfied the four subsequent conditions of the MACD+. After reviewing the data in real-time, it was exported to a comma separated values (csv) file that contained a timestamp, raw data from the x-, y-

and z-axes as well as from each of the variables in the MACD+ algorithm, a binary classifier for each of the MACD+ conditions and the 'mounting' label applied in real-time by the observer.

The commercial-grade Smart Tag transmitted packets containing mounting event data to a server via a wireless network of readers. Packets were only sent if the MACD threshold had been triggered. Received packets contained a timestamp of the mounting event, the tag number the packet was sent from and the binary classifiers detailing which of the MACD+ conditions the event had satisfied. Raw data was not stored or transmitted by the commercial-grade Smart Tag to conserve battery life.

3.3.1 Trial 1: Pen trial with research-grade Smart Tags

Mature merino rams (n=9) were each fitted a Smart Tag (Digibale Pty Ltd; Sydney, Australia) prior to the trial. The Smart Tags were attached posterior to the withers via a harness (Figure 3.1). The accelerometers were positioned such that the x-axis corresponded with the lateral axis of the ram.

A ewe in oestrus was secured in a headstall in an open 2 x 2 m pen. Oestrus was stimulated in the ewe using methods outlined in Chapter 2.3.2. A technician was stationed by her flank with an artificial vagina in order to intercept the ram prior to intromission. Rams were held in a separate pen 10 m away and were introduced to the ewe one at a time. Once the ram was released into the pen with a ewe, they were given three minutes to perform courting and mating behaviours. The technician intercepted all mounts with an artificial vagina. Observers stationed outside the pen filmed the ram's behaviour. This was repeated three times over the course of the day for each

ram, with at least two hours between each session. Following this period, rams were moved to an adjacent pen before another ram was immediately introduced.



Figure 3.1: The Smart Tag (a) fitted posterior to the withers of a ram via a harness.

The number of observed mounting events correctly identified (true positives; TP), the number of non-mounting events that were incorrectly identified as mounting events (false positives; FP), and the number of observed mounting events that were not identified by the Smart Tags (false negatives; FN) were recorded. If two alerts were registered for a singular mounting event, the second peak was labelled as a FP. Sensitivity, precision and F₁ score (the harmonic mean of sensitivity and precision) were calculated and used to determine the optimal attachment point for the accelerometer and select a threshold that maximised both sensitivity and precision. The definitions are as follows:

$$\text{Sensitivity} = \text{TP}/(\text{TP} + \text{FN})$$

$$\text{Precision} = \text{TP}/(\text{TP} + \text{FP})$$

$$\text{F1 score} = 2 * ((\text{Precision} * \text{Recall})/(\text{Precision} + \text{Recall}))$$

3.3.2 Trial 2: Paddock trial with production-grade Smart Tags

This trial involved the observation of rams (n=5) and ewes (n=40) in a small paddock (0.2 ha). Ewes were oestrus-synchronised by the administration of progesterone through a controlled internal drug releasing device (EAZI-BREED CIDR, Zoetis Pty Ltd.; Rhodes, Australia). Twelve days after insertion, CIDRs were removed and ewes were given an im injection of 400IU eCG. Rams were also fitted with the production-grade Smart Tags for the detection of mounting behaviour (Figure 3.1).

A wireless mesh network was installed in the joining paddock to transmit data from the Smart Tag to a server in real-time. Eight solar-powered readers were installed along the boundary fences of the paddock in the line of sight of adjacent readers. A gateway was installed on a telegraph pole in the centre of the paddock at a height of 4 m to transmit data to the server. When a mounting event was recorded, the Smart Tag transmitted a data packet containing the Smart Tag ID and a timestamp of the event to the gateway through the mesh network of readers. The gateway communicated the data packets to the server via Long Range Wireless Area Network where they were subsequently exported for analysis.

The rams were introduced to ewes in a small paddock (0.2 ha) 48 hours post-CIDR removal. The sheep were grazed on pasture and free to express normal behaviour.

Joining interactions were observed in-person and filmed with an iPhone camera for three hours. Video footage was used to validate the packets sent by the production tags to the server. Microsoft Excel was used to create a list of all mounting events from the video footage and the successful events from the server were cross-checked against these. Any Smart Tag identified mounting events that did not correspond with an observed mounting event were deemed to be a false positive and were labelled with the non-mounting behaviour (Table 3.1). Events where the ram was not in the field of view were ignored.

The performance of the Smart Tags was evaluated on sensitivity, precision and F1 score as in Trial 1.

Table 3.3: Descriptions of observed behaviours in rams

Behaviour	Description
Mounting	Male bends knees and surges forward on his hind legs, connects his brisket with the ewe's rump (this may include intromission and ejaculation) and then dismounts and resumes a standing position
Running	Forward motion at a pace faster than a walk
Head-butting	Forceful head-forward movement of aggression directed at another sheep
Head-butted	Reception of a forceful head-forward movement of aggression from another male
Courting	Licking or pawing at a ewe, often with a bent neck, to gauge receptivity
Walking	Forward motion at a slow pace, where each hoof hits the ground independently
Grazing	Foraging while standing or walking with head lowered to the ground
Lying	Recumbent on the ground
Standing	Sedentary in an upright position supported by all four legs

3.4 Results

3.4.1 Trial 1: Pen trial with research-grade Smart Tags

The Smart Tags identified 58 of the 64 mounting events performed by rams in the pen trial. Overall sensitivity was 84% and precision was 92% (Table 3.2). All but one of the false positives resulted from running behaviour. The other was created by courting behaviour.

Table 3.4: The classification performance of the Smart Tags, including correctly identified mounting events (TP), mounting events that were not identified (FN), other behavioural events that were falsely classified as mounting events (FP), and the sensitivity, precision and F1 score.

Ram	TP	FN	FP	Sensitivity (%)	Precision (%)	F1 score (%)
1	6	1	0	86	100	92
2	5	0	0	100	100	100
3	14	4	1	78	93	85
4	2	0	0	100	100	100
5	14	0	3	100	82	90
6	7	0	1	100	88	93
7	4	0	0	100	100	100
8	3	1	0	75	100	86
9	3	0	0	100	100	100
Total	58	6	5	84	92	88

The average peak height during mounting behaviour was 0.57 ± 0.017 . The mounts that were not identified by the MACD+ all had a peak height of less than $0.4 g$. The average peak height for individual rams ranged from $0.49 \pm 0.017 g$ to $0.70 \pm 0.069 g$ (Figure 3.2). This confirmed the suitability of $0.4 g$ as the threshold for the MACD.

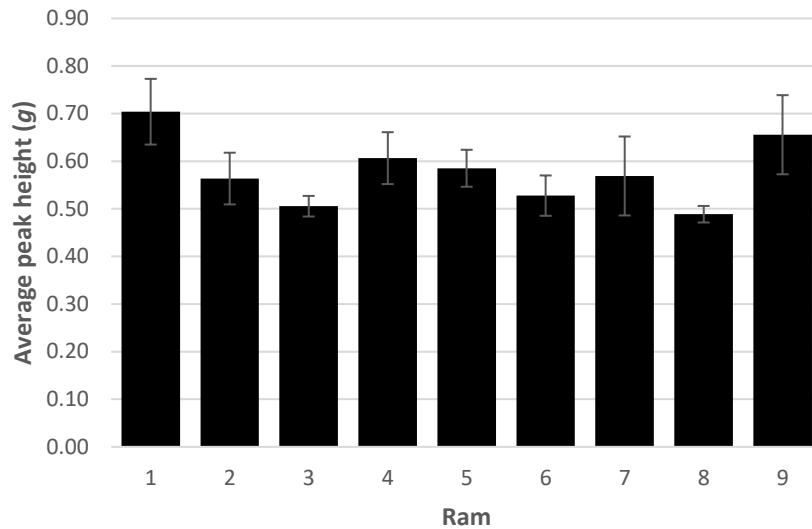


Figure 3.2: The average peak height (g) of the MACD during mounts $\pm$ SEM

3.4.2 Trial 2: Paddock trial with production-grade Smart Tags

The Smart Tags identified 136 of the 154 total mounting events performed in the paddock trial (Table 3.3). This equated to an overall sensitivity of 88%.

Table 3.5: The classification performance of the Smart Tags, including correctly identified mounting events (TP), mounting events that were not identified (FN), other behavioural events that were falsely classified as mounting events (FP), and the sensitivity, precision and F1 score.

Ram	TP	FN	FP	Sensitivity (%)	Precision (%)	F1 score (%)
1	27	3	6	90	82	86
2	24	4	5	86	83	84
3	22	3	7	88	76	81
4	20	3	1	87	95	91
5	43	5	4	90	91	91
Total	136	18	23	88	86	87

A total of 23 false positives were captured by the Smart Tags, giving an overall precision of 86%. Agonistic behaviour led to more than half of these misidentifications, with a total of 13 headbutting events detected by the Smart Tags.

The remaining false positives were caused by running and courting behaviour (each with three events). There were also three occasions when a mounting event was registered twice by the Smart Tag.

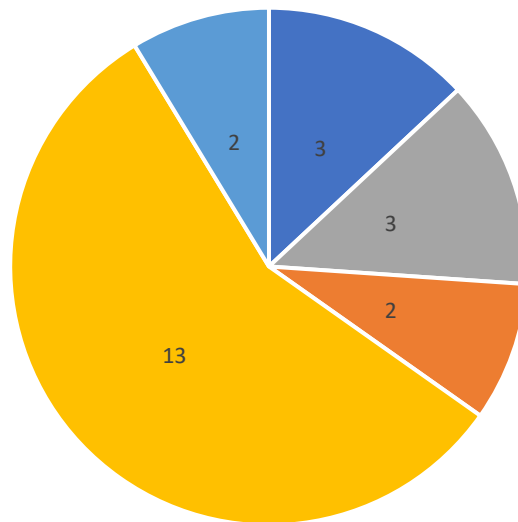


Figure 3.3: The proportion of behaviours falsely classified as mounting events (FP) by the Smart Tags, including headbutting (yellow), courting (light blue), running (dark blue), dismount (grey) and being headbutted by another ram (orange).

3.5 Discussion

This is the first study that has used sensors to remotely monitor the behaviour of rams in real-time. Previous studies that have employed sensors to measure mating behaviour in rams have required a manual extraction or manipulation of data. It is also one of the few studies that have used an integrated wireless system to map sheep behaviour.

The performance of the MACD+ algorithm was retained following the transfer from the research-grade accelerometers in our previous study to the Smart Tags (Goldsmith et al., 2022). The MACD+ was 88% sensitive in the current study, only three percentage points lower than the sensitivity of the algorithm when applied to data collected from research-grade accelerometers. There was a slight loss in precision, from 94% in the previous study to 86% in the current study. We hypothesise this slight decrease in performance resulted from the lower sampling frequency of the Smart Tags (10 Hz) compared to the accelerometers used in the previous study (30 Hz). Walton et al. (2018) reported a similar decrease in precision after reducing the sampling rate of accelerometers from 32 Hz to 8 Hz. A decrease in sensitivity was also reported but this didn't reflect the findings in the current study. This is likely owed to the high-intensity peaks produced by mounting events in accelerometer data, which maintained integrity despite the reduced sampling frequency (Figure 3.2).

Approximately two-thirds of the false positives in this study arose from agonistic behaviours (Figure 3.3), including headbutting and being headbutted by another ram. This trend was also observed in our previous study, where agonistic behaviours comprised one-third of the false positives. It should be noted that in both this study

and in our previous study, rams were stocked at a much higher density than they would be during joining on a commercial property. Validating the technology under commercial conditions may alter the primary source of false positives, however further research is required.

The performance of the Smart Tags is comparable with other studies that have used sensors to remotely monitor ram reproductive behaviour. Mozo et al. (2018) proposed a manual decision tree tool to identify mounting behaviour in data retrieved from harness-mounted accelerometers. The MACD+ algorithm surpassed the performance of this decision tree, both in the Smart Tags and when it was applied to raw accelerometer data. Mozo et al. (2018) reported a global sensitivity of 78% compared to the 88% sensitivity of the Smart Tags and the 91% sensitivity of the MACD+ algorithm in our previous study (Goldsmith et al., 2022). The precision of the Smart Tags and the algorithm developed by Mozo et al. (2018) were similar at 86% and 85%, respectively. While the nature of the false positives encountered by Mozo et al. (2018) was not disclosed, their algorithm featured a high pass filter, so it is probable that these misclassifications arose out of high-activity behaviours such as running or headbutting. It is worth noting that rams in this study were joined to ewes at 1:20, a similar ratio to the current study. This suggests that agonistic behaviour would have been frequent.

The single other ram mating behaviour sensor study featured a novel mating detection system called the Alpha-Detector (Alhamada et al., 2017a). This system consisted of a pneumatic sensor and a RFID reader, which were fitted to the chests of rams via a harness, and a RFID chip which was adhered to the rumps of ewes. While the Alpha-

Detector was highly accurate at identifying mating behaviour, with a sensitivity of 94% and a precision of 100%, a system that requires the gluing of transponders to fleece would be difficult to promote in the Australian sheep industry. Price penalties for damaged wool would likely deter producers from adopting the technology.

The MACD+ also performed well across individual rams with precision ranging between 76% and 96% and sensitivity ranging between 86% and 90%. The Smart Tags outperformed the algorithm developed by Mozo et al. (2018) in this regard, where sensitivity ranged from 54% to 90% and precision ranged from 79% to 100% for individual rams. Minimising variation in classification accuracy between animals is often overlooked in favour of reporting global performance, however it is extremely important if a PLF technology is to function as a decision support tool for individuals.

Beyond the accuracy of the tag, it will also be important to establish a threshold to identify low-performing rams. This has not been explored in this thesis nor in current literature though it will be a key element of a commercial on-sheep sensor, should it be developed. The lower bounds of precision and accuracy must be taken into account when developing this threshold. Taking the MACD+ in this study as an example, we would assume that at least 86% of mounting events are captured and that 76% of events identified by the sensor are mounting events. It is difficult put a figure to this threshold without further research to ascertain the number of lambs a ram is expected to produce in a single syndicate joining and across its lifetime.

On-sheep sensor research has undergone exponential growth since the early 2010s, however much of this research employs research-grade sensors (Fogarty et al., 2018). Data must be removed manually from these types of sensors and, in many cases,

manipulated from its raw form before it is able to be interpreted. This is true for both the sensors used by Mozo et al. (2018) and Alhamada et al. (2017). This research is integral to the development of PLF technologies, however, it is curious that so few of these studies have been translated into sensors fit for commercial purpose.

Beyond economic and technological constraints, a lack of interoperability in PLF technologies has been cited as a significant barrier to the adoption by farmers. Bahlo et al. (2019) were critical of 'single-purpose' technologies in their discussion of on-animal sensors and highlighted that multiple data flows and interfaces hinder the progression of PLF technologies. Masters (2021) agreed and noted that an overwhelming proportion of on-sheep sensor research was conducted with a single sensor and purpose. While the current study has indeed developed a technology for the singular purpose of monitoring libido in rams, the Smart Tag has scope for development into a flock-wide monitoring system. The Smart Tags are simultaneously being developed to monitor disease, predation and grazing behaviour (Australian Wool Innovation, 2021). This would offer producers a central interface to monitor not only the reproductive performance of their rams but the welfare and productivity of their flocks.

The Smart Tag has real scope for implementation in the Australian sheep industry and beyond. This is the first sensor developed to monitor the libido of rams in real-time. It is a highly accurate alternative to outdated methods of estimating ram reproductive performance with strong commercial potential. The ability to monitor the reproductive performance of rams on-farm would allow producers to eliminate unproductive rams from the flock and boost the reproductive efficiency of future

generations. Further studies should therefore investigate the relationship between siring ability and the libido of rams during joining.

3.6 Acknowledgements

We gratefully acknowledge Australian Wool Innovation Limited and the McCaughey Memorial Institute for their support and funding of this research. We would also like to thank Ms Trinity White for her assistance in the field.

Chapter 4 – The application of on-animal accelerometers (Smart Tags®) to investigate the link between ram libido and siring ability

4.1 Abstract

The ability of rams to sire offspring is predicated on three factors: fertility, the physical ability to mate and the sexual motivation to do so. The singular importance of each of these traits to siring ability has been investigated but their interaction with one another remains unclear. Further to this, previous studies have used serving capacity tests as a measure of libido, but the validity of these tests is contentious. The Smart Tag is a recently developed 'on-animal' sensor that measures the libido of rams during joining by identifying mounting behaviour. The aim of this study was to investigate how fertility and libido, as measured using Smart Tags, interact to determine a ram's siring ability. The semen quality (wave motion, motility, volume, concentration, and morphology), testosterone levels, and scrotal circumference of 19 rams were measured prior to joining (with testosterone and scrotal circumference also measured post-joining). Rams were fitted with Smart Tags and subsequently joined to ewes (n=960) for 35 days. Parentage testing was conducted on the lamb crop to determine the number of lambs per sire. Libido, as measured by the number of mounts per sire, was positively correlated with the number of lambs sired ($R = 0.405$; $p < 0.05$). Pre-joining scrotal circumference, the change in scrotal circumference over joining and semen volume were also significant predictors of siring ability ($p < 0.05$) however due to low correlation values and lack of variation within the ram cohort, these findings were not biologically relevant. The relationship between libido and siring ability was

strengthened by the rams with the highest and lowest levels of sexual activity siring the highest (n=102) and one of the lowest (n=11) number of lambs, respectively.

Previous studies have reported that approximately 30% of rams are 'low performers' that have little to no sexual interest in ewes. In the present study, only one ram was definitively classed as a low performer with an average of 3 ± 0.5 mounts per day. The remaining rams performed at least 27 mounts per day. This may suggest that the prevalence of low performers has been overestimated in previous literature, where serving capacity tests were used to determine sexual motivation. Whether or not this is the case, the Smart Tag demonstrates value in identifying low-performing rams. The elimination of low-performing rams from breeding flocks will increase pregnancy rates, tighten lambing windows and decrease the likelihood of poor sexual performance in further generations.

4.2 Introduction

Reproductive efficiency is key to the profitability and productivity of the Australian sheep industry. It hastens the rate of genetic gain and determines the level of output for a producer in a given year (Henry et al., 2012). Sheep are polygynous breeders, meaning that rams are the main route through which valuable genetics are passed. Rams influence the genetic profile of 40-60 lambs per year, while ewes are limited to one to two (Meat & Livestock Australia, 2021). For this reason, producers tend to invest more in individual rams than ewes.

Australian Sheep Breeding Values (ASBV) estimate the genetic component of a ram's performance. This includes carcase and wool quality, growth, reproduction and disease resistance (Brown et al., 2007). The national catalogue of ASBVs allows producers to purchase rams or semen that will accelerate their breeding objectives. The ability of a ram to distribute these genetics during natural joining, also known as their siring ability, is predicated on three key traits: fertility, the physical ability to mate and the sexual desire to mate. The singular importance of each of these traits in relation to the number of lambs sired has been studied but their relationship to one another remains unclear.

A breeding soundness evaluation (BSE) can be conducted prior to joining to assess a ram's physical condition and potential fertility. To pass a BSE, rams must be free from disease (such as ulcerative posthitis and brucellosis), have progressive sperm motility of 50% or greater and normal sperm morphology of over 30%, a scrotal circumference of greater than 30 cm (Van Metre et al., 2012). The BSE does not consider a ram's libido, even though it is estimated that almost 30% of rams exhibit little to no sexual interest in ewes (Fitzgerald and Perkins, 1991; Hulet et al., 1964). These rams, called 'low performers', sire fewer lambs than high-performing rams and extend the lambing window, meaning that producers must recruit surplus rams to the breeding flock to compensate (Fitzgerald and Perkins, 1991; Hulet et al., 1964). The process of identifying low performers involves extensive behavioural monitoring, which is no mean feat in extensive livestock production.

Serving capacity tests are the current industry method used to measure ram libido but are not widely undertaken due to their high labour cost. Serving capacity tests involve

monitoring the mating behaviour of rams in a pen with a small number of ewes for a period of at least twenty minutes but up to one hour (Kilgour, 1985; Mattner et al., 1971). In addition to the labour intensity, several studies have failed to link serving capacity score with siring ability. A number of factors have been attributed to the variability of serving capacity tests, including an unfamiliar testing environment, the proximity of other rams and the sexual attractiveness of ewes (Kilgour and Whale, 1980; Lindsay et al., 1976; Tilbrook and Lindsay, 1987).

Measuring the sexual performance of rams during joining is evidently the gold standard. Recent research has demonstrated that on-animal sensors are highly effective at detecting the mating behaviour of rams (Alhamada et al., 2017a; Mozo et al., 2019). Rather than performing a predictive test, sensors identify the mating events of rams with a sensitivity and precision upwards of 85%. Until recently, these sensors were in their infancy.

The authors of the current study developed the first commercially viable sensor to detect ram mating behaviour in the Australian sheep industry. The Smart Tag identifies mounting events in real-time and transmits data to the operator through a wireless sensor network. The advanced autonomy of this on-sheep sensor is unrivalled in the precision livestock farming (PLF) space. The Smart Tag is a compelling candidate for adoption on-farm and has scope to replace serving capacity tests as a method for determining the libido of rams.

The aim of this study was to describe the cumulative effect of libido, semen quality, testosterone levels and scrotal circumference on the ability of rams to produce lambs. Additionally, we aimed to investigate whether semen quality or other physiological

factors were correlated with libido. This is the first study that has used on-animal sensors to measure libido in this context.

4.3 Materials and methods

This study was carried out in accordance with the Australian *Animal Research Act 1985* No. 123 and in adherence to the Australian code for the care and use of animals for scientific purposes 8th edition (2013). Experimental procedures were conducted with approval from the University of Sydney Animal Ethics Committee (approval 2021/1865).

All animals used in this trial were housed at Arthursleigh Farm, Marulan, NSW, Australia (34°33'36.12" S, 150°3'37.7" E). Mature Merino rams ($n=19$; aged 3-4 years) and ewes ($n=960$) were maintained as a single mob in a 30 ha paddock with ad libitum access to improved pasture and water. Rams and ewes were joined for a total of 35 days throughout April and May at a joining rate of 2%.

Rams were randomly selected for trial by the Arthursleigh farm manager. Rams were excluded from the study if low body condition score (BCS; 1-2), testicular lesions or disease (Flystrike) were observed, conditional to veterinary inspection.

4.3.1 Semen collection

One ejaculate per ram was collected via electroejaculation two weeks prior to joining by a licensed veterinarian. Rams were sedated with an im injection of xylazine (7.5 mg, Troy Laboratories Australia Pty Ltd) ten minutes prior to electroejaculation. Rams

were then restrained in a recumbent position and the prepucial area was cleaned. An Electro Ejaculator stimulator (Minitube Pty Ltd; Victoria, Australia), with a rectal probe 16cm long, 2.54cm in diameter, containing three electrodes, was used. The rectal probe was lubricated and placed into the rectum above the prostate gland. Voltage was applied in 2-second pulses, with incremental increases in voltage over the course of three minutes to approximately 8 V or until an ejaculate was produced and collected into a warmed sterile tulip glass. Ejaculates were immediately assessed.

4.3.2 Assessment of ram semen parameters

Ejaculate volume and concentration

Ejaculates were transferred to a 15 mL tube and volume was determined using a pipette. Concentration was measured using a cell counter (NucleoCounter® SP-100™; Chemometec, New South Wales, Australia). A 50 µL aliquot of raw semen was diluted with 40 mL of Reagent S100 based upon the anticipated sperm concentration in a 15 mL centrifuge tube. Concentration was recorded as millions of spermatozoa per millilitre of semen.

Wave motion and motility

Ejaculate wave motion was determined at 100 X magnification as per industry protocol and given a score from 0 (no motion) to 5 (numerous rapid waves). Semen was then diluted to a concentration of 100×10^6 sperm/mL with Salomon's Sheath Fluid (tris-citric acid, fructose, 0.3% bovine serum albumin; Evans et al., 1987). Motility was

determined subjectively by assessing the proportion of motile sperm in a sample observed under 200 X magnification.

Sperm morphology

Morphology slides were prepared by further diluting the 100×10^6 sperm/mL sample 1:1 with a 3% NaCl solution. The number of morphological abnormalities was subjectively determined per 200 cells for each ejaculate digitally (Infinity Analyse Software) at 400 X magnification (Olympus CX41 Microscope) as per industry standard (Evans et al., 1987).

Scrotal circumference

Scrotal circumference was measured after ram sedation and prior to semen collection by placing the testes measuring tape (ReliaBull; Minitube, Australia) at the widest circumference. Scrotal circumference was also measured post-joining.

Testosterone

Approximately 5-10 mL of peripheral blood was collected from the jugular vein of each ram following the administration of xylazine just prior to electroejaculation. Samples were centrifuged in vacutainers at $3500 \times g$ for 20 minutes to separate plasma from blood cells. The separated plasma was transferred into clean 15 mL tubes and stored at -20°C until analysis. A second blood sample was also collected post-joining.

Testosterone was quantified in duplicate using ImmuChem™ Double Antibody Testosterone 125 radioimmunoassay kit (MP Biomedicals, Ohio, United States) as per the manufacturer's instructions. The assay sensitivity was 0.01ng/mL. The intra-assay CV was 10.1% and inter-assay CV was 12.2%.

4.3.3 Fitting of Smart Tags and automated detection of mounting activity

Smart Tags (AWI Pty Ltd, New South Wales, Australia) were attached posterior to the withers of rams via a standard mating harness (Figure 4.1). They were developed and validated for the detection of mounting activity in rams. They were powered by two AA batteries.



Figure 4.1: The Smart Tag (a) fitted posterior to the withers of a ram via a harness

A wireless mesh network was installed in the joining paddock to transmit data from the Smart Tag to a server in real-time. Thirty solar-powered readers were installed along the boundary fences of the paddock in the line of sight of adjacent readers. Three readers were also placed in the centre of the paddock using a star picket when there were no readers in the line of sight. A gateway was installed on a telegraph pole in the centre of the paddock at a height of 4 m to transmit data to the server. When a mounting event was recorded, the Smart Tag transmitted a data packet containing the Smart Tag ID and a timestamp of the event to the gateway through the mesh network of readers. The gateway communicated the data packets to the server via Long Range Wireless Area Network where they were subsequently exported for analysis.

4.3.4 Parentage testing

DNA samples were taken from rams post-joining and from lambs at weaning. A tissue sampling unit (Allflex Australia Pty Ltd; Capalba, QLD) was used to collect a notch of tissue from the ear. Parentage was determined by Neogen Australasia Pty Ltd using a 160 single nucleotide polymorphism (SNP) chip (GeneSeek® Genomic Profiler™; Illumina, Inc., San Diego, US).

4.3.5 Statistical analysis

All statistical analyses were conducted using the RStudio software package (R Core Team 2021) and a significance level of $P < 0.05$. Results are expressed as means ($\pm$ SEM). The number of days used in statistical analyses was reduced to $n=27$ after tag

fitting days (days 1 and 2) and days where greater than two rams had missing data, were removed.

Analysis was conducted using a generalised linear mixed model. Continuous predictor variables were centred due to the discrepancy between units of measurement. A negative binomial distribution was used to model variables due to overdispersion of the response variate. All predictors were included in the full model and were assessed on their variance inflation ratio (VIF). Any predictors with a VIF greater than five were removed from the model. Once a model free from collinearity was achieved, model selection was conducted using stepwise selection based on the AIC. Variables were considered significant if $p < 0.05$.

4.4 Results

4.4.1 Descriptive statistics

Table 4.1 displays all descriptive statistics of the predictor variables measured, showing means ($\pm$ SEM) of scrotal circumference, ejaculate volume, sperm concentration, wave motion, subjective motility, normal morphology, testosterone concentration and number of mounts.

Table 4.1: Descriptive statistics of variables measured from mature Merino rams ($n=19$) describing the mean, standard error of the mean and the range of the data.

Variable	Mean $\pm$ SEM	Range
Scrotal Circumference		
Pre-joining (cm)	37 $\pm$ 0.9	30.1 – 43.5

<i>Post-joining (cm)</i>	34 ± 0.5	29.8 – 37.4
<i>Percent change (%)</i>	-7 ± 1.7	-27.9 – -9.6
Semen characteristics		
<i>Volume (μl)</i>	1537 ± 98.1	450 - 2300
<i>Concentration ($\times 10^6$ sperm/ml)</i>	2284 ± 323.1	581.4 - 5873
<i>Wave motion</i>	4 ± 0.1	2.5 - 5
<i>Subjective motility (%)</i>	79 ± 2.1	60 - 90
<i>Normal morphology (%)</i>	72 ± 1.8	58.5 - 83.5
Testosterone concentration		
<i>Pre-joining (ng/ml)</i>	1.16 ± 0.304	0.05 – 3.73
<i>Post-joining (ng/ml)</i>	0.05 ± 0.013	<0.01 – 0.2
<i>Percent change (%)</i>	-69 ± 0.16	-100.0 – 160.0
<i>Number of mounts (per day)</i>	55 ± 7.4	1 - 196

4.4.2 Libido

Over the course of the trial, rams exhibited significant differences in the number of mounts per day ($P < 0.05$) (Table 4.2). A peak of mounting frequencies was observed in the first 16 days of joining, which significantly tapered off in the second half of the joining period ($P < 0.05$; Figure 4.2). The mean number of mounts performed by rams per day was 55 ± 2.0 . Ram 5 was deemed to have the lowest libido, performing an average of 3 ± 0.5 mounts per day. This was exceptionally lower than the individual with the second lowest performance (Ram 20), who recorded an average of 27 ± 5.3 mounts per day.

Table 4.2: The average number of mounts performed per day by individual rams and the number of lambs they sired.

Ram	Mounts/day ($\pm$ SEM)	Number of offspring
3	47 $\pm$ 9.0	55
4	82 $\pm$ 15.7	9
5	3 $\pm$ 0.5	11
6	71 $\pm$ 13.6	57
7	107 $\pm$ 20.7	7
8	37 $\pm$ 7.1	48
9	76 $\pm$ 14.6	65
11	122 $\pm$ 23.5	102
12	37 $\pm$ 7.1	56
13	56 $\pm$ 10.8	44
14	28 $\pm$ 5.4	28
16	75 $\pm$ 14.4	75
17	30 $\pm$ 5.8	38
18	49 $\pm$ 9.5	21
19	42 $\pm$ 8.1	10
20	27 $\pm$ 5.3	73
21	47 $\pm$ 9.0	19

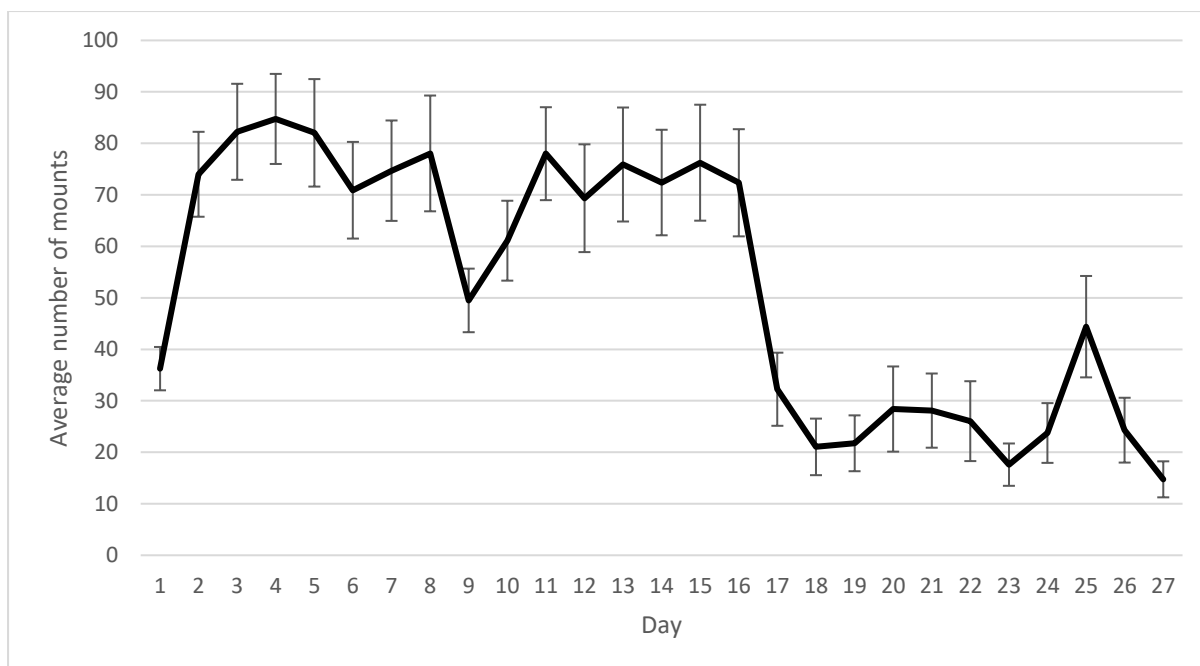


Figure 4.2: The average number of mounts performed by all rams across each day of the trial. Error bars represent the SEM.

4.4.3 Semen quality

The mean semen volume of ejaculates collected from rams was 1537 ± 98.1 μ L. Mean sperm concentration was $2284 \pm 323.1 \times 10^6$ sperm/mL. The average wave motion score was 4.3 ± 0.16 and mean subjective motility was $79.21 \pm 1.96\%$, ranging from 60-90%.

The mean proportion of morphologically normal sperm in rams was $71.8 \pm 1.70\%$, with a range from 58.5 – 83.5% (Table 4.1). Morphological abnormalities observed included abnormal acrosome, broken/bent tail, amorphous head, proximal droplet, dag defect, distal reflex, asymmetry, coiled tail, pyriform and detached heads.

4.4.4 Ram scrotal circumference

Mean scrotal circumference (SC) decreased significantly from pre-joining (37.3 ± 1.59 cm) to post-joining (34.1 ± 0.09 cm) ($P < 0.001$).

4.4.5 Testosterone

Mean serum testosterone levels (T) also decreased significantly from pre-joining (1.1 ± 0.28 ng/mL, with individuals ranging from 0.05 to 3.73 ng/mL) to post-joining (0.05 ± 0.01 ng/mL, <0.01 to 0.20 ng/mL) ($P < 0.0001$).

4.4.6 Siring ability

A total of 718 lambs were sired by 17 rams (Table 4.1). The number of lambs sired by an individual ram ranged from 7 to 102, with a mean of 42.2 ± 6.72 . The highest-performing ram sired 14.6 times more lambs than the lowest-performing ram.

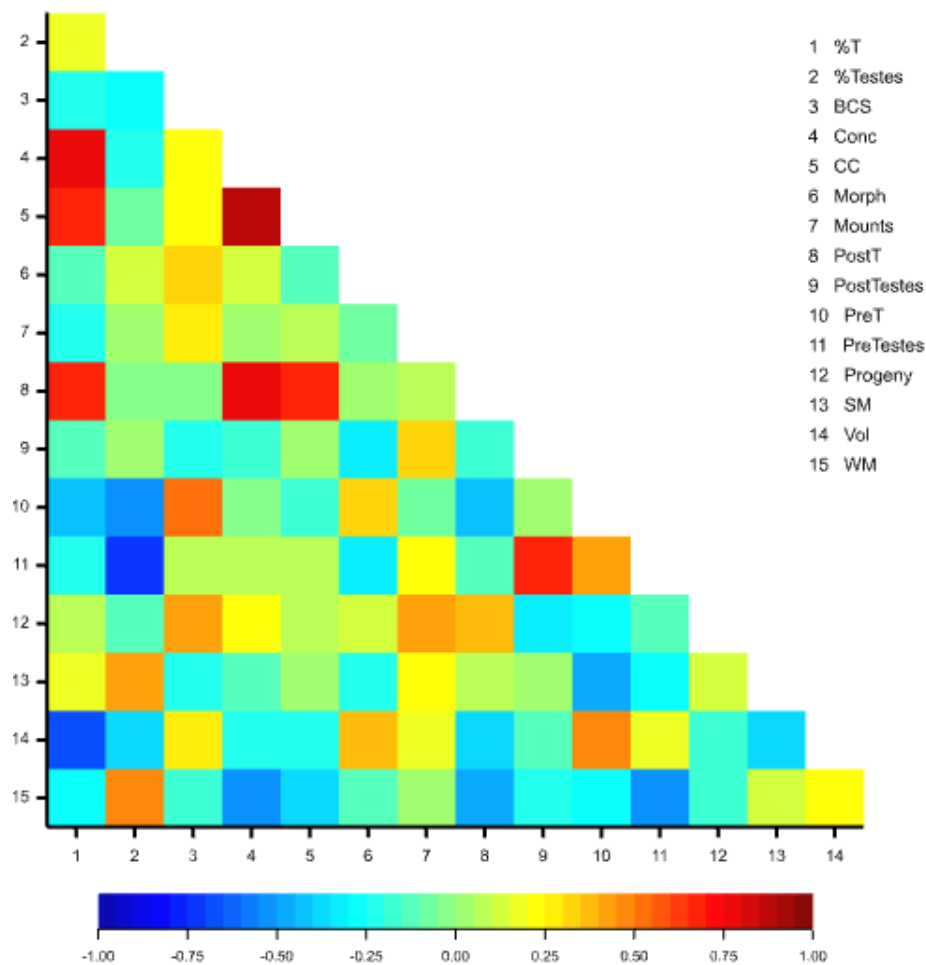


Figure 4.3: A correlation matrix for the variables included in the study where blue indicates a strong negative correlation; red indicates a strong positive correlation; and 1 = percent change in testosterone, 2 = percent change in scrotal circumference, 3 = body condition score, 4 = sperm concentration, 5 = colour and consistency, 6 = percent normal sperm, 7 = average number of mounts per day, 8 = post-joining testosterone, 9 = post-joining scrotal circumference, 10 = pre-joining testosterone, 11 = pre-joining scrotal circumference, 12 = number of lambs sired, 13 = subjective motility, 14 = semen volume, and 15 = wave motion.

4.4.7 Predictors of siring ability and correlations between variables

The final predictive model included mounts, pre-joining scrotal circumference, sperm concentration, semen volume, sperm morphology and the change in scrotal circumference from pre-joining to post-joining (Table 4.3). Of these, libido, semen volume, pre-joining scrotal circumference and the change in scrotal circumference

from pre-joining to post-joining were significant predictors of the number of lambs sired ($p < 0.05$).

Table 4.3: Estimated effects from a negative binomial to predict the number of lambs sired

Co-efficient	Estimate	S.E.	P-value
Intercept	2.989	0.3155	<0.001
Average number of mounts per day	0.0169	0.005143	<0.05
Pre-joining scrotal circumference	-0.1478	0.0643	<0.05
Sperm concentration	-1.7E-05	0.000115	0.146
Semen volume	-0.00133	0.000508	<0.01
Sperm morphology	0.0409	0.02447	0.094
Change in scrotal circumference	-11.8	3.763	<0.01

Libido

Libido was positively correlated with the number of lambs sired ($r = 0.405$, $P < 0.05$; Figures 4.3 & 4.4). The ram that performed the highest number of mounts per day (121 ± 23.5) sired the greatest number of offspring (102). Conversely, the lowest-performing ram (3 ± 0.5 mounts per day) sired only 11 lambs. The R^2 value was low, indicating a high level of variability around the regression line.

To further interrogate the effect of libido on the number of lambs sired, a second analysis excluding outliers (rams 5 and 11) was conducted. This revealed that the average number of mounts per day was not a significant predictor of lambs sired. The significance of all other predictors remained unchanged.

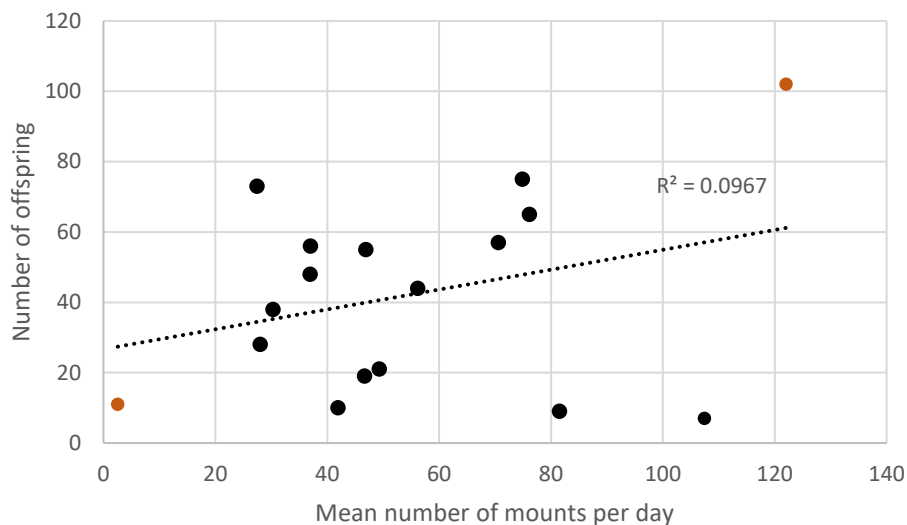


Figure 4.4: The interaction between the mean number of mounts performed by a ram per day and the number of lambs sired. The rams with the lowest and highest average number of mounts per day are presented in orange.

Scrotal circumference

A ram's pre-joining scrotal circumference was negatively correlated with siring ability ($r = -0.289$; $P < 0.05$; Figures 4.3 & 4.5), however the R^2 value was low (0.0877), indicating high variability around the regression line. The change in a ram's scrotal circumference from pre-joining to post-joining was also a significant predictor of siring ability (Figure 4.6). A greater percentage reduction in a ram's scrotal circumference from pre-joining to post-joining was weakly correlated to a greater number of lambs produced ($r = -0.115$). Again, the R^2 value was low (0.0201). There was also a moderate positive correlation between pre-joining scrotal circumference and pre-joining testosterone level ($r = 0.444$; Figure 4.3).

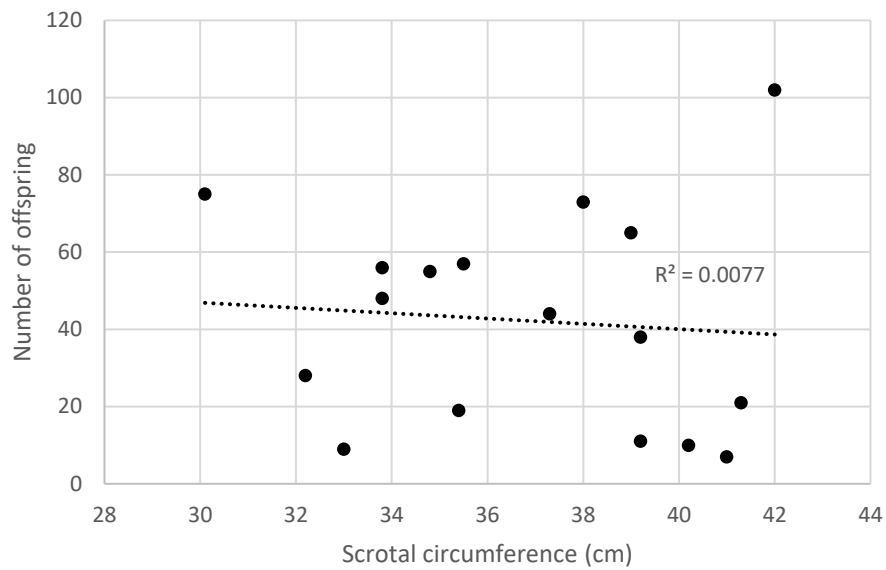


Figure 4.5: The interaction between the scrotal circumference of a ram prior to joining and the number of lambs sired.

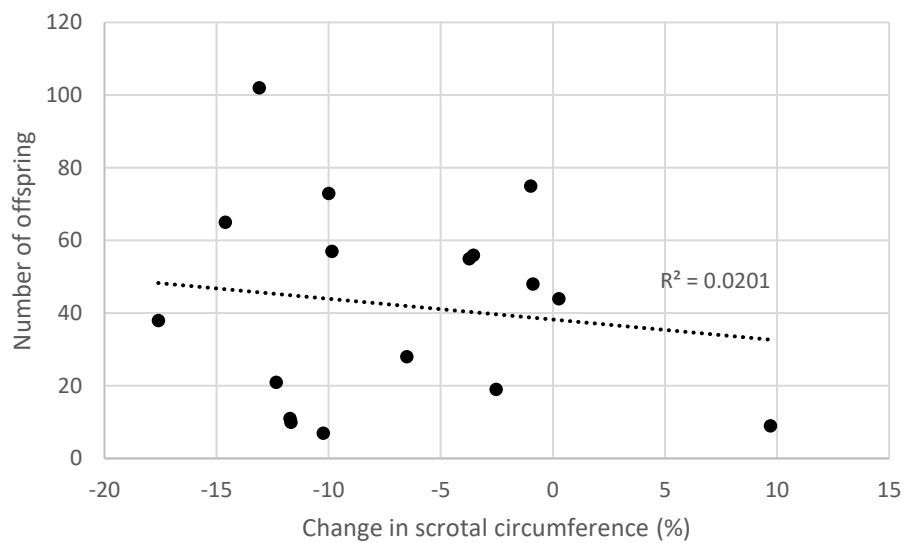


Figure 4.6: The interaction between the change in scrotal circumference of a ram from pre-joining to post-joining and the number of lambs sired.

Semen volume

Semen volume was negatively correlated to siring ability ($r = -0.186$; $P < 0.05$; Figures 4.3 & 4.7), though this appeared to be a weak relationship. Again, the R^2 value was low (0.0205). There was also a moderate positive correlation with pre-joining testosterone level ($r = 0.499$) and a strong negative correlation with the change in testosterone from pre-joining to post-joining ($r = -0.685$).

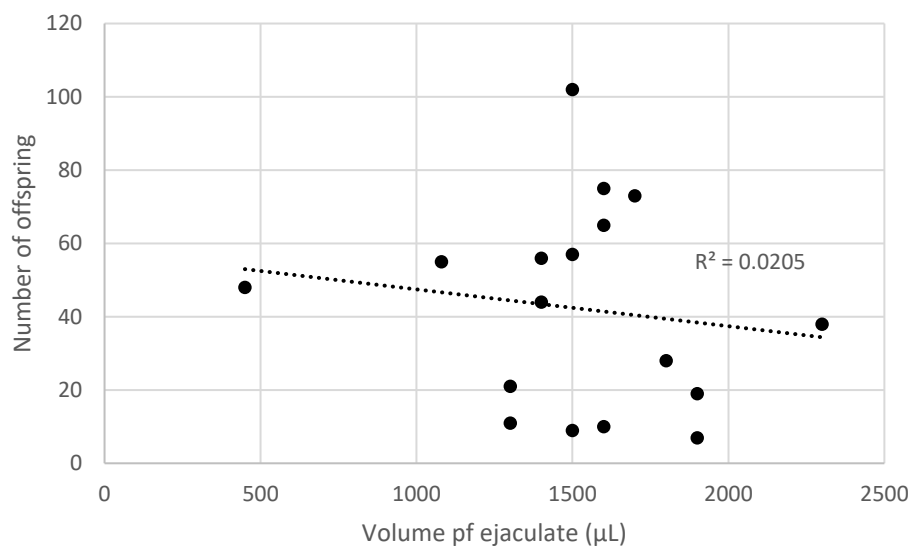


Figure 4.7: The interaction between ejaculate volume and the number of lambs sired by a ram.

4.5 Discussion

This study is the first to explore the relationship between the libido of a ram during joining and siring ability. We found that the average number of mounts was positively correlated with the number of offspring sired. This relationship was affected by the sires at the two extremes of libido, with the highest performer siring the greatest number of lambs and the poorest performer siring one of the lowest numbers of lambs. Between these two extremes, the relationship between libido and siring ability is less clear. Other than the ram with the poorest libido, all rams performed more than

27 mounts per day on average. Given that rams can be joined to ewes at a rate of 1:100 over a five-week joining period (Fowler, 1982), this level of sexual activity indicates that all but one ram were satisfactory, if not high, performers.

Previous studies have shown that 29.6% and 28.6% of rams are low performers which have little to no sexual interest in ewes (Fitzgerald and Perkins, 1991; Hulet et al., 1964). Until the present study, the sexual performance of rams has only been measured through predictive serving capacity tests. It is unclear what specific thresholds were used by Hulet et al. (1964) and Fitzgerald and Perkins (1991) to class rams as low performers. Mattner et al. (1971) previously defined low performers as rams that performed less than four ejaculations within a single 30-minute serving capacity test. No revisions to these thresholds have been made since the study by Mattner et al. (1971). High-performing rams were defined as those that performed an average of five to six services. Although no standardised definition of a low-performing ram currently exists, especially not for in-paddock libido, the ram that averaged the lowest number of mounts in the present study (3 ± 0.5 mounts per day) is unquestionably a low performer.

There are three explanations for the strong proportion of high performers in the current study. It may be that high performers were overrepresented in the population of rams selected. A more likely theory, however, is the overestimation of low performers in previous literature. The use of pen tests to determine libido may not be a true indication of the sexual performance of rams during joining. Kilgour and Whale (1980) demonstrated that rams initially deemed to be low performers increased their performance in pen tests with repetition and longer periods of testing. This hypothesis

is supported by data from Mozo et al. (2019), who reported a relatively low level of variation in the libido of seven rams. Further trials with the Smart Tag should be conducted on a broader cohort of rams with a greater range of known libido to investigate this hypothesis.

The sole low-performing ram in this study only sired 11 lambs. This result suggests that a threshold of mating activity must be reached before a ram can sire an acceptable number of offspring. Further research with a greater incidence of low-performing rams is required to strengthen this result. Should future investigations demonstrate the same relationship between libido and siring ability, the Smart Tag will be a valuable tool for producers who wish to identify and eliminate low-libido rams from their breeding flock.

At the other end of the spectrum, whereby the highest performing ram sired the greatest number of lambs, the relationship between libido and siring ability is more obscure. It was hypothesised that in a fertile ram, the number of lambs sired would increase with the number of ewes mated though this was not met by results. For example, the ram with the second and third highest number of mounts in this study sired a low number of lambs. Clearly, there are other factors at play that were not revealed by investigations in this study, as rams with high libido and low siring ability had acceptable semen quality, testes size and circulating testosterone.

Previous studies have shown that some rams are highly selective and prefer to mate with a select few ewes frequently rather than spreading their efforts over the wider flock (Price et al., 1996; Tilbrook et al., 1987; Tilbrook and Lindsay, 1987). A sensor that could register the identity of the ewe being mated with would unveil whether this

factored into poor siring ability. This was a hallmark of the technology developed by Alhamada *et al.* (2016), whereby the identity of joined ewes was registered by an RFID scanner attached to the harness the ram wore. The paired RFID transponders were glued to the ewes' rump. While this technology was accurate and allowed for the identification of individual mating pairs, adhering RFID chips to the wool of ewes is not commercially feasible in an industry that relies on wool as a product.

In addition to sexual motivation, siring ability is determined by fertility and physical condition. The model developed in the current study found that scrotal circumference prior to joining, the change in scrotal circumference over joining and semen volume were negatively correlated with the number of lambs sired. The biological relevance of these results is dubious despite their statistical significance. The r-squared value for each of these predictors was low with little evidence of correlation when graphically presented, particularly in pre-joining scrotal circumference and semen volume.

The importance of scrotal circumference to siring ability is unclear, both in the current study and surrounding literature. We found that scrotal circumference had a weak negative correlation with the number of lambs sired. Caution must be exercised when interpreting this result as there was not a large amount of variation in scrotal circumference between rams. The Breeding Soundness Evaluation deems that a scrotal circumference greater than or equal to 33 cm is satisfactory. All but one ram in this study satisfied this criterion. Bennett *et al.* (2021) found a similar result in bulls, where a 1.0 cm increase in scrotal circumference was associated with a decrease of 1.56 calves. The authors hypothesised the true relationship between scrotal circumference and siring ability could not be extricated as only bulls that had scrotal circumferences

greater than breeding soundness standards were used. Similarly inconclusive results with negative or weak positive correlations between scrotal circumference and siring ability have been found in both rams and bulls (Makarechian and Farid, 1985; Mickelsen et al., 1981; van Eenennaam et al., 2014).

As mentioned above, only one ram in this study had a scrotal circumference of less than 33 cm. Despite failing the BSE as per the criteria used by Van Metre et al. (2012), this ram produced 75 lambs. While this is a small insight into the imperfect nature of BSEs, this finding was reflected by Alexander (2021) who demonstrated no link between BSE score and siring ability. Mickelsen et al. (1981) also demonstrated that scrotal circumference did not affect conception rate. Despite this, it has been shown that there is a high correlation between scrotal circumference and the number of sperm in the testes (Lino, 1972). This was not evident in the current study (Figure 4.3), however this may be a reflection of the method used to collect semen as electroejaculation reaps poorer quality ejaculates (Mattner and Voglmayr, 1962).

Ejaculate volume was negatively correlated with siring ability in the present study but as with scrotal circumference, there was little variation in ejaculate volume. Cameron et al. (1987) found that maximum fertility could be achieved during natural mating with as few as 140×10^6 spermatozoa per ejaculate. The rams in the current study were therefore capable of achieving maximum fertility on the basis of the volume and concentration of the ejaculate. It is thus reasonable to assume there was no biological relevance of semen volume on siring ability in this study despite its statistical significance. As with the low instance of rams with poor libido, it is possible that

studying rams with a greater range of ejaculate traits would uncover a stronger relationship between semen quality, scrotal circumference and siring ability.

Of all the parameters measured in this study, only libido had a consequential effect on siring ability. While this was only apparent in one ram, it demonstrates the value that Smart Tags and similar on-animal sensors could present to the Australian sheep industry. Research has shown that sexual performance is moderately heritable (Kilgour, 1985; Snowden et al., 2002). Bench et al. (2001) demonstrated that the male offspring of high-performing rams performed significantly more ejaculations and mounts without ejaculation in serving capacity tests than ram lambs of low-performing rams. The sexual performance of ewe lambs born from low-performing rams was also affected, with the first oestrus occurring on average eight days later than ewe lambs born from high-performing rams. Additionally, low-performing rams decrease scanning rates and extend the lambing (Hulet et al., 1964; Perkins et al., 1992). Although the true rate of low-performing rams in a flock has been questioned by this study, there is no doubt that the Smart Tag would aid producers in removing these rams from their breeding flock.

Siring ability remains a complex phenomenon, one that cannot be distilled into a formula of reproductive parameters. While this study could not tease out the nuances of the relationship between fertility, physical condition and libido, and its effect on siring ability, it did identify a low-performing ram that could not widely disseminate his genes. The removal of low-performing rams from the breeding flock has been shown to increase pregnancy rates, tighten lambing windows and improve the reproductive efficiency of the next generation. The Smart Tag has a demonstrable

ability to identify low-performing rams, thus making it a valuable tool for producers looking to increase productivity.

4.6 Acknowledgements

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Chapter 5 – General Discussion

Monitoring the behaviour of extensively farmed sheep is notoriously difficult. This is particularly true for reproductive behaviours, as sheep are joined for a period of five weeks with little intervention or observation. Measures of performance are often based on the entire flock rather than individual sheep and are reported as an overall pregnancy or lambing rate. This is concerning as it has been reported that almost a third of rams are ‘low-performers’ with little to no sexual interest in ewes (Fitzgerald and Perkins, 1991; Hulet et al., 1964).

Until recently, the sole way to measure the libido of rams was through a serving capacity test. The validity of serving capacity tests has been called into question over the years with some studies demonstrating that serving capacity test scores are not indicative of in-paddock performance (Allison, 1978; Chaill et al., 1975; Kilgour and Whale, 1980; Kilgour, 1985). This has been attributed to the unfamiliarity of the testing environment, the proximity of human and ram audiences and the attractiveness of the individual ewes to the ram being tested (Lindsay et al., 1976; Tilbrook and Lindsay, 1987).

Parentage testing remains a reliable measure of serving capacity and as its affordability increases, its accessibility to growers increases too. Libido is just one component of a ram’s serving capacity. It is affected by a ram’s physical condition but does not give any indication of their fertility (Brook et al., 1966; Sargison, 2009; Van Metre et al., 2012). That said, libido is a moderately heritable trait (Kilgour, 1985; Snowden et al., 2002) and despite results from this thesis, rams with low libido have been reported as

ubiquitous (Fitzgerald and Perkins, 1991; Hulet et al., 1964). Identifying the libido of rams should be a consideration prior to purchasing or breeding.

The advent of on-animal sensors has allowed for the remote monitoring of individual animals in their natural environment without the need for in-person observation (Brown et al., 2013). Prior to this thesis, two studies utilised on-sheep sensors to measure reproductive behaviour in rams. Alhamada et al. (2017a) used a combination of a pneumatic sensor and RFID technology to detect ram mounting behaviour and the individual ewes that were joined by the ram. Meanwhile, Mozo et al. (2019) used accelerometers to create a unique signature of mating behaviour in rams. Each of these studies reported high levels of sensitivity and precision though they have not been translated into farm-ready technologies. This is a common theme for on-sheep sensor research as there are a unique set of barriers to developing PLF technologies for extensively farmed livestock, many of which aren't faced by intensive animal industries.

This thesis presents the first wearable on-animal sensor to measure not only the libido of rams but the mating behaviour of extensively farmed sheep in real-time. A computationally efficient algorithm to detect mounting behaviour in rams and wethers was developed in Chapter 2. The performance of this algorithm (MACD+) was on par with that of other mount-detection technologies in the literature. Both the performance and computational simplicity of the MACD+ made it a suitable candidate for integration into a wireless sensor network in Chapter 3. There was little decrease in the performance of the MACD+ between its development in Chapter 2 and its integration into the Smart Tag in Chapter 3. Following the promising results in

Chapter 3, the Smart Tag was implemented as a decision support tool in Chapter 4 to investigate the effect of libido on siring ability. Semen quality, scrotal circumference and circulating testosterone were also measured to investigate their role in siring ability. The results from this study indicated that libido was positively correlated with siring ability, although further investigation of this relationship is recommended. The performance of the MACD+ developed in Chapter 2 was equivalent to that of other mounting detection sensors in the field (Alhamada et al., 2017a; Mozo et al., 2019). The MACD+ was able to identify mounting behaviour with 94% precision and 91% sensitivity. This was compared to a precision of 84% and 78% in the study by Mozo et. al. (2019), and a precision of 100% and sensitivity of 85% for the sensor developed by Alhamada et al. (2017). The performance of the MACD+ decreased slightly following its integration into the Smart Tag in Chapter 3, though this loss in accuracy was minimal.

The computational simplicity of the MACD+ algorithm was an important consideration in the progression of the thesis, aiding its integration into a wireless sensor network in Chapter 3 and application as a decision support tool in Chapter 4. Much of on-sheep sensor research employs complex machine learning algorithms to identify behaviour (Fogarty et al., 2018). While these algorithms are effective, particularly when identifying a suite of behaviours, they are extremely power-consumptive (García et al., 2020). Power availability is an overlooked limitation of PLF technologies developed for extensive livestock industries and is rarely discussed in on-sheep sensor research. To withstand the long deployment periods demanded by extensive livestock farming, sensors must either have an extended battery life or have

self-sufficient energy sources (ie solar power), and battery consumption needs to be minimised. This limits the transmission of large amounts of data in real-time (Bailey et al., 2021).

The process of developing algorithms to detect event behaviours, such as mounting, is a double-edged sword. Machine learning models are inherently biased towards ubiquitous behaviours (ie state behaviours). Researchers are thus forced to develop novel algorithms to amplify signals of targeted event behaviours, which are characterised by their short duration. This is evident not only in this thesis through the development of the MACD+, but in the studies by Mozo et al. (2019) and Alhamada et al. (2017), both of which employed entirely novel, non-machine learning methods to detect ram mating behaviour. The upside is that these algorithms are far less complex and power consumptive than those employed to identify a suite of behaviours, allowing data processing to occur on the sensor. This facilitated the integration of the MACD+ into a wireless sensor network in Chapter 3.

Further to its ability to detect mounting behaviour, the Smart Tag is also the first sensor to monitor the behaviour of extensively farmed sheep in real-time. Mounting events were identified as they occurred in Chapter 3 and despite the transition from an accelerometer sampling at 30 Hz to one sampling at 10 Hz, there was minimal loss in accuracy. As previously discussed, the availability of PLF technology for extensive livestock producers is limited. There is only one wearable sensor on the market for the Australian sheep industry and this features a store-on-board memory (SmartShepherd, 2021). This is contrasted with the wealth of real-time PLF technology available to the dairy industry (Table 1.2).

When the Smart Tag was applied as a decision support tool in Chapter 4, it was found that the number of mounts performed by a ram was a significant predictor of the number of lambs sired. The strength of the correlation between siring ability and libido was moderate to weak. It was bolstered by the ram with the lowest number of mounts siring a substantially small crop of lambs while the rams with the highest number of mounts produced the greatest number of lambs. This correlation likely would have been stronger had there been greater variation between the sexual performance of rams.

Previous studies have reported that almost 30% of rams are low performers with little to no sexual interest in ewes. This contrasted with our findings where only one ram of the breeding cohort was deemed to have low libido. This ram performed an average of five mounts per day, whereas the rest of the cohort performed at least 27 mounts per day. This was supported by results from Chapters 2 and 3, which indicated a high level of libido across the board. Notably, this study is the first to investigate the relationship between in-paddock libido and siring ability, whereas the studies by Fitzgerald and Perkins (1991) and Hulet et al. (1964a) used serving capacity tests to measure the sexual motivation of rams. As outlined in Chapter 1, the validity of serving capacity test scores is contentious, with results affected by the unfamiliar testing environment, ewe attractivity and both ram and human audiences. Based on the results from Chapter 4 and the supporting evidence in Chapters 2 and 3, we hypothesise that serving capacity tests have historically overestimated the incidence of low performers. Though this thesis has questioned the incidence of low libido in rams, we have uncovered a definitive link between libido and the number of lambs they produce. The

Smart Tag has value as a decision support tool through the identification of rams with low libido. Removing low-performing rams from joining syndicates has been shown to increase pregnancy rates and tighten lambing windows (Hulet et al., 1964; Price et al., 1996; Stellflug et al., 2006). Further to that, libido is a heritable trait, so the Smart Tag would also aid in improving the reproductive efficiency of future generations (Bench et al., 2001; Kilgour, 1985; Snowden et al., 2002). The economic cost of low libido rams to production has not been explored in this thesis or in the literature. It is recommended that this is investigated further by first determining the true proportion of low-performing rams during joining and then generating a cost-benefit analysis for the adoption of the Smart Tags. Further research is also required to determine a threshold for identifying rams with poor sexual performance. This has been determined for traditional serving capacity tests, but not for in-paddock libido.

The number of rams recruited for this thesis, and in particular Chapter 4, was constrained by the desire to replicate industry-standard joining rates, simulating joining as it would occur on a commercial farm. The recommended joining rate in extensive sheep industries is between 1-2 rams per 100 ewes. This was adhered to in Chapter 4 but increased beyond this in Chapters 2 and 3 to collect sufficient data during the development and integration phase of the sensor. We have presented the hypothesis that the incidence of rams with low libido has been overestimated by serving capacity tests, however, it cannot be ruled out that the high sexual performance of the rams in this thesis was an anomaly. This highlights the need for further investigations into the relationship between siring ability and the sexual performance of rams during joining.

Studying a broader representation of the Australian sheep flock would also clarify the role that semen quality, scrotal circumference and other fertility markers play in siring ability of rams. Other than the lack of libido observed in one ram, we were not able to conclusively point to factors that influenced the number of offspring a ram produced. Semen volume and scrotal circumference were negatively, but weakly, correlated with siring ability. We concluded that these results were not biologically relevant as, overall, the studied rams had acceptable semen quality, scrotal circumferences and testosterone levels as per BSE criteria. This made it difficult to elucidate the relationship between breeding soundness and siring ability. This frustration has been echoed by authors that presented similarly counterintuitive results when variation in scrotal circumference was not substantial (Bennett et al., 2021; Mickelsen et al., 1981; Van Eenennaam et al., 2014). A re-investigation of the factors that contribute to siring ability should make efforts to recruit rams with a wide range of purported breeding soundness. The addition of advanced semen testing to the protocol may also uncover markers of infertility and their interaction with libido and BSE parameters.

Despite the leaps made by this research, the road to the adoption of the Smart Tag is one that stretches beyond the life of this thesis. Extensive animal industries face a unique set of barriers to the adoption of PLF technologies. These include poor network coverage, geographical signal interferences, hostile weather conditions, limited power supply and the scale of the landscape (Masters, 2021). This is in addition to the barriers common to both intensive and extensive livestock production systems, such as the cost-benefit of PLF technology and the technological literacy of farmers (Marshall et al., 2021). The Smart Tag prevailed over several of these barriers, including

signal interference (overcome by installing multiple readers in line-of-sight), hostile weather conditions and limitations on power. While internet access in rural Australia is improving, it is doing so at a sluggish pace (Park et al., 2019). Admittedly, the research locations in this thesis had adequate internet access. The application of wireless sensor networks to areas with poor network coverage, such as the rangelands, must be vetted if this technology is developed for commercial use.

A lack of interoperability is another commonly cited barrier to adoption. Bahlo et al. (2019) were critical of on-animal sensors developed for a single purpose, citing numerous and complex data streams as a hindrance to the progression of PLF. While the Smart Tag has been developed with a singular focus on ram mating behaviour in this thesis, there is scope to broaden its applications. The Smart Tag was originally devised by the Australian Wool Innovation as a whole-flock monitoring tool. This technology is simultaneously being developed to monitor sheep welfare, health and disease, and pasture use. Early research indicates that the technology can be used to alert producers to predation events, as sheep increase their distance travelled per day and alter their activity budget in the presence of wild dogs. It was also shown that sheep burdened with Barber's pole worm (worm egg counts between 250-1000 eggs per gram of faeces) grazed less and stood more than control sheep (Australian Wool Innovation, 2022). The capability of the technology to monitor the health, welfare and productivity of a flock, in addition to the sexual behaviour of rams, would improve the interoperability of the Smart Tag and increase the overall return on investment.

Further to that, it has been shown that lambing date by monitoring ram-ewe interactions during joining (Paganoni et al., 2020; Trotter et al., 2022). Bluetooth-

based sensors measured the number of times a ewe was found in proximity to a ram and days with the highest rates of interactions were assumed to occur during oestrus. The Smart Tags used in this thesis are equipped with Bluetooth-based technology to transmit data through a wireless sensor network. It is therefore feasible that oestrus detection could be added to the functionality of the technology. Combining this with the mounting detection function detailed in this thesis, the Smart Tag has scope to map the reproduction life cycle of sheep, from joining to lambing. This would provide a decision support tool for breeding flock selection, allow producers to perform early fetal aging and differentially manage ewes accordingly.

The identification of individual mating pairs, be that through proximity or pneumatic sensors (Alhamada et al., 2017a; Paganoni et al., 2020), would strengthen the ability of the Smart Tag to predict the siring ability of rams. Such a capability would provide more information on the breadth of a ram's libido by detailing the number of unique ewes that are mated. This is important as low-performing rams tend to have a higher incidence of repeated matings, while high-performing rams mate with a greater number of ewes (Price et al., 1996). Should the integration of this technology be possible, future research should repeat the methodology detailed in Chapter 4. Including the unique number of ewes that a ram has mated along with an assessment of semen quality may provide a stronger predictive model for siring ability.

The Smart Tag is a compelling candidate for integration into commercial sheep farms. Its potential benefit to reproductive efficiency is marked. The ability to measure and manage the libido of rams has been shown to increase pregnancy rates, tighten lambing windows and improve the reproductive efficiency of future generations.

Broadening the capability of the Smart Tag to monitor oestrus as well as sheep health and performance outside of joining would bolster its commerciality and increase its threshold for adoption. Beyond the practical benefits of the Smart Tag, this thesis has made significant contributions to the field of Precision Livestock Management through the first integration of an accelerometer-based wireless system into an extensive livestock system. This is a significant step for the Australian sheep industry and extensive livestock systems worldwide.

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