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THE INFLUENCE OF SPERMATOZOAL MOTILITY ON CONCEPTION AND PREGANCY STATUS FOLLOWING ARTIFICIAL INSEMINATION IN SHEEP

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SUMMARY

Reproductive efficiency is a key driver of productivity and profitability in many sheep enterprises. Both ewe and ram genetics influence reproductive efficiency, along with environmental influences. However, past research has heavily focused on the influence of the ewe. Reproductive failure from the paternal side has a significant influence, as a single ram may inseminate hundreds of ewes during their lifetime. Hence, the objective of this study was to investigate the influence of ram spermatozoal motility on conception outcomes and foetal scan count following artificial insemination (AI). Spermatozoal motility was found to significantly influence conception outcomes following logistic regression and approached significance for foetal scan count following ordinal regression. Therefore, spermatozoal motility could play a key role in improving reproductive efficiency in sheep.

INTRODUCTION

Host genetics from the ram and ewe both impact factors which influence reproductive efficiency, including conception outcomes and foetal scan count. Comparatively, relatively little emphasis has been placed on investigating the ram's influence on reproductive efficiency despite rams having the potential to inseminate hundreds of ewes annually, and many more over their lifetime, having a substantially larger impact on the flock. The objective of this study was to characterise the influence of spermatozoal motility on both conception outcomes (binary variable) and foetal scan count (ordinal variable).

MATERIALS AND METHODS

Historic AI data was provided by a commercial artificial breeding company which included progressive spermatozoal motility via visual microscopic assessment of ejaculates used in on-farm AI. A retrospective dataset for pregnancy scan results (scanning to identify the number of foetuses) and pedigree was also supplied by Sheep Genetics (Meat & Livestock Australia) for all ewes inseminated. Ejaculates with at least 40% motility were used in AI. Data quality control steps included the removal of AI records where the ejaculate's motility was not assessed, the type of semen (fresh, straw- or pellet-frozen) was not noted, and small number of records for ewe and sire breed AI combinations. A total of 2,608 insemination records from 2,289 ewes across 4 breeds and 90 rams from 5 breeds, spanning 11 AI sites across 5 years were contained in the final dataset.

Statistical analysis. Fixed effects including the AI site (n=11), type of semen used for insemination (n=3), ewe age (n=8), and spermatozoal motility were included in the final model following significance when fit individually in preliminary linear regression via ASReml (version

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4.2) (Gilmour *et al*. 2021). As breed was confounded with AI site, it was not fitted in the final model. Logistic regression was performed using conception outcomes (binary trait) including fixed effects of the AI site and type of semen used for insemination, as well as covariates like spermatozoal motility and ewe age, while the inseminating ram was fit as a random polygenic effect. The full model used for logistic mixed-model regression analysis in ASReml was as follows;

$$Logit(n) = Wb + Za + e$$

where $Logit(n) = ln\frac{N(Y=1)}{1-N(Y=1)}$; $N(Y = 1)$ is the probability of conception success as the outcome Y following on-farm AI; b is the vector of fixed effects; a is the random polygenic effect; W and Z are the incidence matrix relating to the fixed effects and random polygenic effects, respectively. Log odds of conception at each level of spermatozoal motility was estimated using the predict statement. The heritability of conception outcomes was estimated via ASReml.

An ordinal regression was used for foetal scan count (ordinal variable = 0, 1, 2, 3) via ASReml. Fixed effects included AI site and type of semen used for insemination with covariates (spermatozoal motility and age of the ewe) and a random polygenic effect fitted in the final model. A multinominal error distribution was assumed.

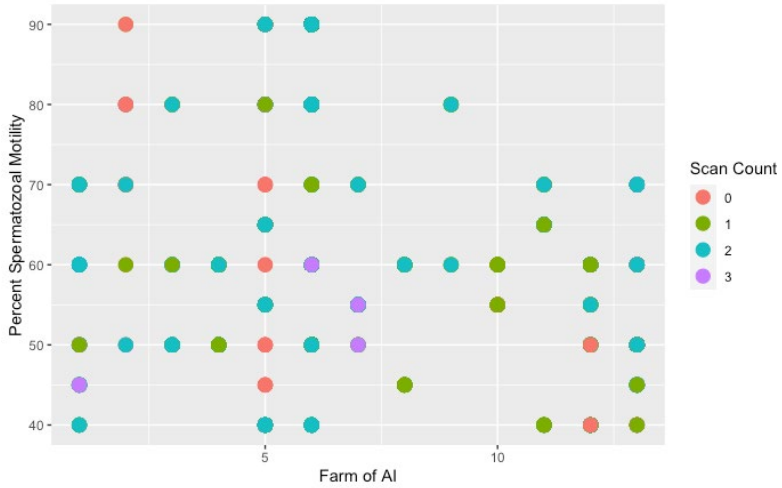

Figure 1. Range in spermatozoal motility for each AI site and the corresponding foetal scan count result following on-farm AI

RESULTS AND DISCUSSION

AI site had a significant influence on conception outcomes (P <0.001) and foetal scan count (Table 1). Spermatozoal motility also significantly influenced conception outcomes (P = 0.024) but not foetal scan count (P = 0.079). The age of the ewe had a significant impact on foetal scan count (P <0.001) but not conception outcome. The type of semen used for insemination was not significant for either conception outcome or foetal scan count. Results from past studies (Spanner *et al*. 2024; David *et al*. 2015; Wierzbowski and Kareta 1993; and Morris *et al*. 2001) align with the results of the present study showing that spermatozoal motility has an influence on conception outcomes.

The heritability for conception outcomes following on-farm AI was 0.27 (±0.06). This was higher than previous heritability estimates for ewe conception outcomes 0.06 (Peñagaricano *et al*. 2012), 0.05 - 0.23 (Bunter *et al.* 2016; Bunter *et al.* 2021). Differences in the number of animals inseminated and time period may have contributed to the higher heritability in this study. The breed of both the ewes and rams was confounded with the site of AI, hence, the heritability of the present

study could have potentially overestimated the additive genetic variance, leading to the higher heritability estimate. As such, it would be beneficial to conduct further research on a larger dataset to evaluate the influence of both breed and AI site on conception outcomes following on-farm AI.

Table 2. Fitted effects and their significance for logistic and ordinal regression with conception outcomes and foetal scan count, respectively

Fixed effect	Effect type	P-value
Conception outcome (Logistic regression)		
AI site	Fixed (n = 11)	<0.001
Spermatozoal motility	Covariate	0.024
Age of ewe	Covariate	0.477
Type of semen used for insemination	Fixed (n = 3)	0.419
Foetal scan count (Ordinal regression)		
AI site	Fixed (n = 11)	<0.001
Spermatozoal motility	Covariate	0.079
Age of ewe	Covariate	<0.001
Type of semen used for insemination	Fixed (n = 3)	0.407

As spermatozoal motility increased, so too did the log odds of a positive conception outcome following on-farm AI (Table 2). While further research and validation is required using a larger dataset, these results indicate the potential to improve AI conception outcomes by using sperm with higher percent motility. A recent study by Spanner *et al*. (2024) reported spermatozoal motility assessed via CASA influences the odds of pregnancy following on-farm AI, amongst other parameters like spermatozoal morphology and ewe uterine tone. However, Spanner *et al*. (2024) only used Merino sheep, hence, further validation on a larger dataset include multiple breeds is required. It is important to note that ejaculates with less than 40% motility were not used for AI, due to industry practice of discarding low motility ejaculates (Van Metre *et al*. 2012).

Table 2. Log odds ratio for each level of spermatozoal following logistic regression for conception outcomes

Spermatozoal motility (%)	Lod odds (±SE)
40	1.534 (±0.557)
45	1.719 (±0.497)
50	1.903 (±0.442)
55	2.087 (±0.397)
60	2.272 (±0.365)
65	2.456 (±0.348)
70	2.640 (±0.349)
80	3.009 (±0.403)
90	3.378 (±0.504)

Spermatozoal motility has been reported to significantly influence lambing percentages (David *et al*. 2015; Wierzbowski and Kareta 1993) and *in vitro* fertilisation (Morris *et al*. 2001). However, these studies used a different phenotype to determine reproductive efficiency (i.e. lambing rate, *in vitro* fertilisation (IVF), semen assessment (i.e. gross motility) and method of insemination (i.e. cervical insemination, IVF). The retrospective data available for the present study was limited to foetal scanning data, hence litter size was not able to be directly assessed. Furthermore, other factors, like parity and ewe nutrition (Kelly *et al*. 1992) as well as the weather conditions at lambing (Masters *et al*. 2023) have a large influence on conception, fecundity and lamb survival. It is likely these

factors have a cumulatively larger impact on overall reproductive efficiency than spermatozoal motility.

The present study did not assess the influence of spermatozoal morphological abnormalities on conception outcomes, as this data was not available. While morphological abnormalities are not routinely assessed as part of industry protocols, the inability to account for the potential influence of morphological abnormalities on conception outcomes is a limitation of the present study. Porcine studies have shown that morphological abnormalities in spermatozoa can affect reproductive efficiency, whereas spermatozoal motility has not been shown to have an impact (McPherson *et al*. 2014). Spanner *et al*. (2024) suggest spermatozoal morphology also has a significant influence on pregnancy in sheep following on-farm AI.

CONCLUSION

Ram spermatozoal motility potentially influences conception outcomes, along with the AI site. Further, the farm of AI and age of the ewe significantly influence foetal scan count. While further research is recommended to determine the exact extent of impact that ram semen has on reproductive efficiency, including investigations across multiple breeds as well as cross breeds at the same AI site, performing AI with a higher spermatozoal motility may ultimately lead to increased reproductive efficiency.

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