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BRIEF COMMUNICATIONS: Development of a Measurement of Anogenital Distance in Sheep

TR Boyce[a], LP Carlyon[a], KR Petrovski[a], BRC Alves[a], LJ Flanagan[a], & M Caetano[a*]

[a] School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy Campus, Mudla Wirra Road, South Australia 5371

[*]Corresponding author Email: mariana.caetano@.adelaide.edu.au[*]

Abstract

Fertility is a fundamental phenotypic trait used for achieving optimal production efficiency and, no easily adoptable or precise tools are currently available. In this study, anogenital distances (AGD; anus to clitoris - AC and anus to fourchette - AF) were measured twice from 80 Merino ewes and 80 ewe hoggets to predict fertility. Fertility was measured by evaluating the pregnancy scanning results. Measurements of ADG were performed by using digital callipers and high-resolution images. Overall means of measures were greater for the AF in pregnant ewes compared to dry ewes and no association was found for the AC distance except being shorter for hoggets with callipers. The relationship found between one of the AGD, the AF, and pregnancy status demonstrates the potential to use this trait as a cost-effective method for estimation of fertility that can be easily adopted by producers, particularly in hoggets.

Keywords: breeding, clitoris, imaging, pregnancy status, sheep

Introduction

Anogenital distance (AGD) has been defined as the distance from the centre of the anus to the base of the clitoris and is used as a method of identifying those with potential infertility in humans, pigs, cattle, and rodents (Shourabi et al. 2022, Carrelli et al. 2021, Seyfang et al. 2018, Wainstock et al. 2017, Szenczi et al. 2014). However, to our knowledge, there is no current literature on sheep. The aim of the current study was to estimate the capacity of measurements of anogenital distance (AGD; n=2 types) to predict fertility using pregnancy status of Merino ewes and ewe hoggets.

This study was conducted to evaluate the capacity of the first (AC) and second (AF) anogenital measurements to predict fertility using the pregnancy status of Merino ewes and ewe hoggets. The first objective was to determine whether the AGD measurements varied between pregnancy status. The second objective was to compare the different methods (callipers or image analysis) of measuring the AGD. The third objective was to estimate the repeatability of the same AGD measurement when taken twice per sheep.

Material and methods

Animal handling and measurements

The sheep utilised in this study were from a commercial farm located approximately 15 km from Cummins in rural South Australia (Latitude: -34° 14' 60.00" S Longitude: 135° 42' 59.99" E), under approval by the Animal Ethics Committee (S-2023-001). Eighty ewe hoggets and eighty ewes were randomly selected during pregnancy scanning (65 days after the end of joining) with age, body condition score (BCS) (scale 1 – 5), and pregnancy status recorded (0 or 1) until equal numbers of pregnant and dry per age category were reached. All females were naturally served. All measurements were made during pregnancy scanning. Percentages of pregnant ewes and hoggets were at 150% and 154% respectively.

Experimental design

The AGD measurements included the distance from the anus to the clitoris (AC) and the distance between the centre of the anus to the fourchette (AF). Digital callipers (CS-P8"S; Mitutoyo; Takatsu-ku, Kawasaki, Kanagawa, Japan) with 0.01mm resolution were used to measure each AGD (in mm), twice for each sheep by a single observer. High-resolution images were captured using a prototype device and used to measure AGD with an image analysis software (CMEIAS-IT 1.28; Michigan, America). The calliper and image measurements were mirrored for creating duplicate data.

Statistical analysis

Data analysis was carried out using SAS 9.4 software (Statistical Analysis Software, Cary Inc, USA). The effect of the method used to measure AGD (n = 2; callipers or image analysis) and age of animals (n = 2; ewe hoggets and ewes) on the AGD measurements (AC and AF) including both measurements per sheep was estimated using mixed model in PROC MIXED. BCS had no effect on AGD measurements. The level of significance was set at $P<0.05$. Correlations between measurements of the AGDs between the first and second measures were estimated in PROC CORR.

Results

Means of AF measurements were longer in all pregnant females with callipers ($P = 0.05$) and for ewe hoggets only with image analysis ($P = 0.0001$). However, means of AC measurements were shorter in ewe hoggets with the calliper only ($P = 0.05$; Fig 1) There was a very high correlation between the first and second measurements performed by assessor, being $r=0.98$ ($P<0.0001$) for image or high correlation, being r=0.70 (P<0.0001) for callipers respectively (Table 1).

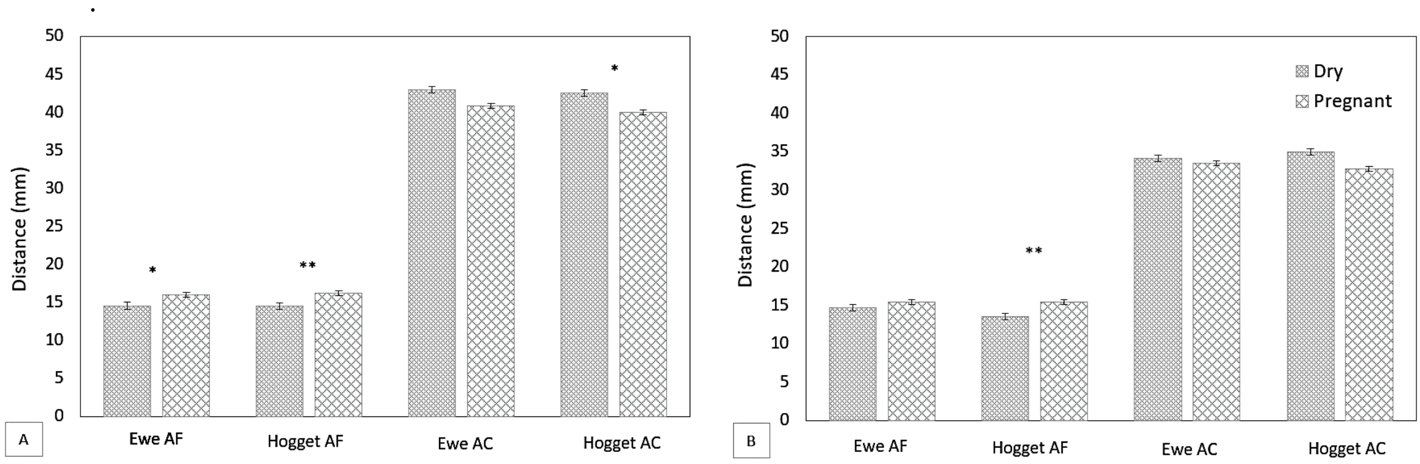

Figure 1: Anogenital measurements (AC = distance from the anus to the clitoris; AF = distance from the anus to the fourchette) between dry and pregnant Merino sheep using callipers (A) and image analysis (B). * P<0.05; ** P<0.0001 between pregnancy groups per age (ewes or hoggets only).

Table 1: Summary table of the AGD (AC = distance from the anus to the clitoris; AF = distance from the anus to the fourchette) values obtained between first and second measurements in mm.

AGD	Measurements	Category	Calliper	Image
			Mean ± SEM	Mean ± SEM
AF	1st	ewe	14.92 ± 1.47	15.21 ± 1.47
AF	1st	hogget	15.36 ± 1.62	14.3 ± 1.61
AC	1st	ewe	37.32 ± 1.45	30.27 ± 1.4
AC	1st	hogget	38.12 ± 1.435	38.12 ± 0.97
AF	2nd	ewe	15.09 ± 1.47	15.27 ± 1.47
AF	2nd	hogget	14.90 ± 1.61	14.34 ± 1.61
AC	2nd	ewe	37.69 ± 1.45	30.48 ± 1.4
AC	2nd	hogget	38.88 ± 1.14	38.71 ± 0.96

Discussion

The comparisons between ewe hoggets and the ewes indicated that in developing females only one of the AGD measurement, the AF, could be used to estimate the fertility (Fig 1), as there was a difference between pregnant and non-pregnant females and methods used. Similar results have been reported for the AGD measurement of the anus to the clitoris in cattle and other species (Carrelli et al. 2021). Thus, the methodology used in this study could be used in a large majority of mammalian species.

An interesting finding from the current study is how the measurements relate to the estimated fertility of the ewe hoggets. The AC distance had minimal difference in pregnant and non-pregnant sheep being shorter only in pregnant hoggets on calliper assessment. In contrast, pregnant hoggets had a longer AF length. This indicated that AGDs used in this study were probably independent. In other studies, the shorter AGD from the anus to the clitoris correlated with better fertility in nulliparous heifers (Rajesh et al. 2022 and Carrelli et al. 2021). Contrary, in Murciano-Granadina goats, aged between one to four years, females with a longer distance between the anus to the clitoris had better reproductive performance (Shourabi et al. 2022).

Based on the results of this study, callipers can be used for AF measurement in all females whilst AC in ewe hoggets only. The image analysis showed an AF measurement being correlated with fertility, but only in ewe hoggets.

The intra-observer correlation between the first and second anogenital measurements of the same AGD was very high (r=0.98) for the image analysis and high (*r*=0.70) for callipers. This indicated that there is no need for repeated measuring. Notably, calliper to image analysis measurement in ewes had higher variability than in hoggets. Means of calliper measurement were much higher than image analysis in ewes (Table 1). This may have been a result of higher stretch on calliper measurements in ewes and may have skewed the data. As image analysis is based on data capture at one point of time without stretching, seem that is more precise.

Our study had a limited number of observations. Further evaluation of the AGD measurements to estimate fertility with the aim to ensure applicability to external populations is required with a larger sample size in a commercial setting. Furthermore, with the accumulation of data, the use of either a ranking or categorising system can be used to predict fertility. The extra-genetic and genetic factors will need to be evaluated for their effects on fertility and correlations to AGDs. In commercial settings, measuring AGDs to identify fertile ewes is difficult because of changes in anatomical configuration with aging and parturition. Additionally, the fertility of the ewes is already known, and retrospective prediction is fruitless. Therefore, the measurements should be taken earlier in life. Results of this study suggest that this was possible in hoggets after their first joining.

Using the AGDs to estimate the fertility in sheep can allow for the earlier selection of breeding flock. However, there are limitations to using this method in commercial settings. Although minor, additional handling is required that slows down the usual farming practices. Additionally, individual records are required. This may not be feasible for commercial farms. Indeed, it can be feasible for stud farms with smaller flock sizes. Sheep stud farms are more involved with the improvement of their flock and will be more likely to take advantage of using the AGD to estimate the ewe fertility. If they do implement this methodology, it may potentially have a flow-on effect on the commercial farms.

This research showed that the AGD measurement, the AF distance was related to fertility. Based on the higher repeatability of intra-observer measurements, the image analysis method for measuring AF was more accurate and can allow for potential artificial intelligence. Overall, the AGDs measured in this preliminary study had some evidence to be used as predictors for Merino ewe hoggets and, less likely, ewes' fertility.

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