BIOMARKER FOR ASSESSING PREGNANCY IN SLAUGHTERED EWES AT THE ABATTOIR

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Abstract-This study was conducted by using ovine pregnancy-associated glycoproteins (ovPAG-1) to determine the pregnancy status of ewes at the point of slaughter. This was done by collecting whole blood samples from clinically healthy Dhone Merino ewes (n=60) over a period of one year from a high throughput abattoir. The samples were analysed using TaqMan Reverse Transcription-Polymerase Chain Reaction (RT-PCR mRNA). The results revealed that a total of 55.2% of these ewes within a range of $2.881*10^{-5}$ to $8.977*10^{-7}$ of ovPAG-1 mRNA were pregnant at slaughter. It was also observed that pregnant six-tooth ewes within 56-60kg slaughter weight were mostly converted into mutton during their luteal and first trimester phases. The use of ovPAG-1mRNA however served as a reliable biomarker for detecting the pregnancy status of ewes at slaughter.

Key words: Complementary DNA (cDNA), sheep slaughter, Reverse Transcription-PCR

I.INTRODUCTION

Meat species of both sexes are constantly slaughtered for meat in backyard and commercial slaughterhouses [1]. This practice has not spared the livestock industry of losing viable genetic materials from the productive and pregnant animals with singleton, twins, triplets or multiple foetuses at different gestational stages [2-4] The development of a method to accurately estimate pregnancy status-stage when precise mating dates are not available was thus deemed appropriate for this study.

Hence, the choice of ovine pregnancy-associated glycoproteins (ovPAG-1) which are detectable in the maternal circulation as they create an avenue for producing recombinant protein useful to develop diagnostics for embryonic development and early stage of pregnancy [5,6]. Currently, the extent to which the slaughter of the prized Dhone Merino [7,8] results in losses of genetic materials is not yet documented in South Africa. A dearth of information is therefore noticed regarding the proportion of the indigenous ewes that are converted to mutton at the high throughput abattoir. Against this concern, we aimed at using ovPAG-1 mRNA as a biomarker to determine the pregnancy status of Dhone Merino ewes at the point of slaughter.

II. MATERIALS AND METHODS

Data collection

Data were generated at a high-throughput Halal abattoir in the Eastern Cape Province of South Africa. The live weights of the 60 clinically healthy ewes (having four tooth; six tooth and full mouth) were determined at the lairage using an electronic weighing scale. Their ages were estimated through a general chronological guide using cementum or dentition method [9]. Whole blood samples (5 to 10 ml) were collected from the jugular vein into heparinised vacutainer tubes from the 60 Dohne Merino ewes during exanguination at the abattoir.

RNA Extraction

Total RNA was rapidly extracted from the previously collected blood samples using the Zymo Whole-Blood RNA MiniPrep™ kit for its suitability to extract high quality RNA (*A*260/*A*280 >1.8, *A*260/*A*230 >1.8) in all downstream RNA-based manipulations. A buffer system combined with *Fast-Spin* column technology (according to manufacturer's instructions), was used for the extraction. Probes and primers were designed using the primer express software version 1.0 following manufacturer's instructions (Applied Biosystems, Foster City, CA).

Primer and Probe Design

The sequence of primers and probe for PAG-1 was chosen from the regions of

nucleotide sequence that provided the most variability from the other members of bPAG and bPRP families (GenBank: M73961.1). Fifty nanograms of total RNA was reverse-transcribed into complementary DNA (cDNA) and used for this real-time RT-PCR analysis.

Real-Time PCR Detection

A quantitative detection method, TaqMan real-time RT-PCR was used for analysing mRNA levels in the blood samples. Real-Time RT-PCR detection was done using ABI PRISM 7700 sequence detector and the software version 1.7 (Applied Biosystems). The thermal cycling proceeded with 40 cycles of 95^{o}C for 15 seconds and 60^{o}C for 1 minute. The standard curve was generated by serial dilution of plasmid containing ovPAG-1 mRNA to quantify concentrations of samples.

III.RESULT AND DISCUSSION

The amplicons for the ovPAG-1 were generated with the pregnancy primers amplified within 332-358 (for forward) and 359-419 (for reverse) at 24 bp regions (Table 1). The results showed high amplification efficiency (82.59%) and strong correlation coefficient (R^2=0.997) of the PCR quantitation (qPCR) assay that was ideally optimised (Figure 1). This therefore enhanced the increased throughput and reduced the chance of carryover contamination as earlier reported by Dorak [10]. A single band of mRNA (PCR) product corresponding to 24 base pairs (bp) represents a fraction of the expressed region of ewes recording 55.2% embryonic or foetal losses (Figure1). This is therefore consistent with the one earlier reported by Goossens *et al.* [11], where 60% pregnant goats and sheep in The Gambia lost their foetuses when they were slaughtered for chevon and mutton. It is also similar to the loss of viable genetic materials in pregnant ewes (17.2%) with contemporaneous double ovulations and a single foetus from four mixed breeds-Suffolk cross, Cheviot, Grey face and Mountain in Ireland [12].

Table 1 Primers and probes used for Real-Time (RT-PCR), including data on the sequence, length, melting temperature, molecular weight and Guanine-Cytosine (GC) content

Name	Accession Number	Sequence	Position
OvPAG-1	M73962	Forward: 5´-CAACCAAGAGACCCTGTAGTAAAC-3´	332-358
		Reverse:5´-AAGTAGATTCTGAAGGTGTCATTG-3´	359-419
Probe[a]		FAM-AAACATCACCAGTCTTCCACCTTCCGGTTT-MGBNFQ	
		Forward primer (OligoAnalyzer 3.1)	
Lenght (bp)	Melting temperature (^{o}C)	Guanine-Cytosine (GC) content (%)	Molecular weight (g/mole)
24	55.1	45.8	7323.8
		Reserve primer (OligoAnalyzer 3.1)	
Lenght (bp)	Melting temperature (^{o}C)	Guanine-Cytosine content (%)	Molecular weight (g/mole)
24	52.7	37.5	7446.9
		TaqMan Probe (OligoAnalyzer 3.1)	
Lenght (bp)	Melting temperature (^{o}C)	Guanine-Cytosine content (%)	Molecular weight (g/mole)
30	63.2	46.7	9036.9

[a] = FAM refers to the reporter and MGBNFQ refers to the quencher ; bp=Base pairs

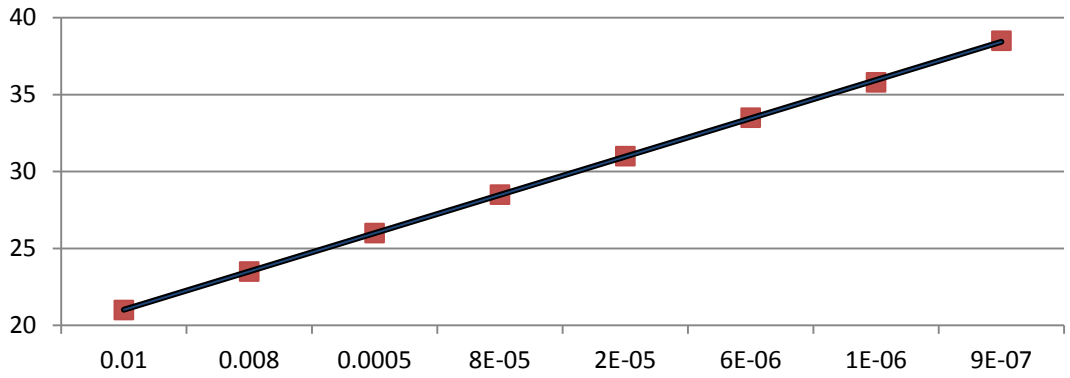

Figure 1 Standard RT-PCR curve for the whole blood from ewes (Standard curve (R^2) = 0.997 and the efficiency=82.59%)

Table 2 showed that more than 50% of the ewes were pregnant at slaughter. At an average age of 30 months, most six-tooth Dhone Merino ewes (51.71%) were in their luteal phase and first trimester respectively (Figure 1). The pattern of results in this study could be compared with the earlier ones where most of the consigned were biochemically pregnant in their *corpus luteual* phase at slaughter [13,14]. A combination of factors such as age, carcass yield and preferred meat quality traits [15-18], could have informed the slaughter of those ewes within the chosen age bracket.

Table 2 Pregnant ewes determined by Reverse-Transcriptase Polymerised chain reaction (RT-PCR)

Sample description	Quantity of mRNA detected	Cycle Threshold (CT)	Percentage of pregnant ewes (%)
Ovine whole blood	0 to 8.977*-^{07}	33.7495 to 57.1343	55.20

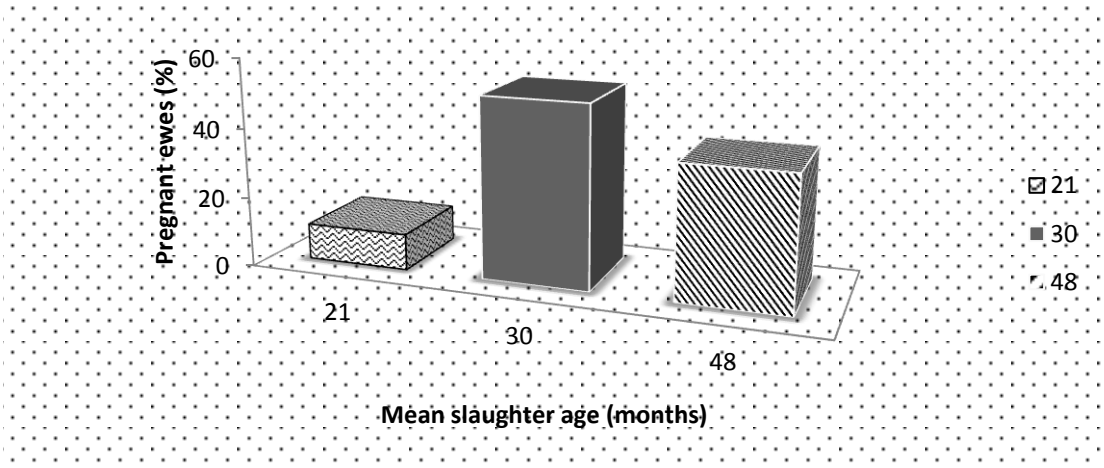

Figure 2 Mean slaughter age and proportion of pregnant Dohne Merino ewes at slaughter

IV.CONCLUSION

This study has revealed that more than 50% of the ewes were pregnant at point of slaughter. This is an indication of losses of viable genetic materials from six-tooth ewes (at 30 month old) that could have enhanced flock restocking and meat production sustainability in the study area.

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