

# **Accuracy of a Low-Cost, User-Friendly Handheld Device to Measure Pregnancy Status in Sheep and Cattle**

Amelia Gledhill

AFNR4102 Research Project B / Research Paper

2016

Number of words:	5,100 (exclusive of abstract, tables, figures and references)
Number of references:	35
Number of tables:	07
Number of figures:	06

# Table of Contents

<b>ABSTRACT .....</b>	<b>3</b>
<b>1 INTRODUCTION.....</b>	<b>4</b>
<b>2 METHODS .....</b>	<b>8</b>
2.1 <i>DATA COLLECTION</i> .....	8
2.2 <i>PREGNANCY TESTING</i> .....	8
2.2.1 Sheep.....	9
2.2.2 Cattle.....	9
2.3 <i>FAT AND MUSCLE DEPTH</i> .....	10
2.3.1 Cattle.....	10
2.4 <i>DATA PROCESSING AND STATISTICAL ANALYSIS</i> .....	11
<b>3 RESULTS .....</b>	<b>14</b>
3.1 <i>PREGNANCY TESTING</i> .....	14
3.1.1 Sheep.....	14
3.1.2 Cattle.....	15
3.2 <i>FAT AND MUSCLE DEPTH</i> .....	17
3.2.1 Cattle.....	17
<b>4 DISCUSSION .....</b>	<b>22</b>
<b>5 CONCLUSION .....</b>	<b>27</b>
<b>6 ACKNOWLEDGMENTS .....</b>	<b>28</b>
<b>7 REFERENCES.....</b>	<b>29</b>

## ABSTRACT

Fifty-three Merino ewes and one hundred and twenty-five Limousin x Charolais and Angus cows and heifers were scanned for pregnancy status and fat and muscle depths using the Renco Preg-Alert Pro A-mode ultrasound device. Body condition score via visual approach by looking at the animal from behind and from the side was also obtained for cattle. The results from the device were compared to the results of the local veterinarian. The ewes were restrained in the race and the cattle were restrained in a cattle crush. The ewes were scanned during one stage of gestation (121-135 days) and the cattle were scanned during three stages (90-115, 190-210 and 265 days until calving). The overall accuracy of the device for sheep pregnancy status was 81%. The accuracy of the device for pregnancy status of cattle was 76% at days 90-115, 63% at days 190-210 of gestation and 43% from days 265 of gestation to calving. Only cattle were tested for fat and muscle depth and the device showed a mean of 4.92mm for fat depth and 58.89mm for muscle depth, with body condition scores ranging from 1.25 to 3.75 (1-5 scale). Fat and muscle depth results were a lot more difficult to obtain than pregnancy status, with only 72% of cattle recording a fat depth and 37% of cattle recording a muscle depth. The study concluded that the Renco Preg-Alert Pro A-mode ultrasound device is not accurate when detecting pregnancy status in sheep and cattle at all stages of gestation and the fat depths corresponded a lot better with the body condition score of the cow compared to the muscle depths. A veterinarian would be a more reliable option when checking the pregnancy status of sheep and cattle.

# 1 INTRODUCTION

Pregnancy diagnosis is extremely important when running any breeding enterprise on a farm. Testing the animals quickly and effectively for pregnancy supports ideal management of sheep and cattle, expansion of farm profit and efficient use of resources. Knowledge of the pregnancy status of livestock will assist the farmer to cull any non-pregnant animals out of the herd, which will allow the pregnant animals to utilise the pastures. Reproductive and production losses from abortions, stillbirths and the birth of weak lambs and calves can be reduced by separating the flock/herd into pregnant and non-pregnant groups (Wani *et al.* 1998).

There are many benefits in knowing the fat and muscle depth as well as body condition score in sheep and cattle. When breeding livestock, it can assist the farmer to know which individual animals are more likely to conceive and which will have a higher chance of producing twins and/or triplets, and again being able to utilise different pastures for the animals (Young *et al.* 2016). Animals that are carrying twins or triplets would be put in paddocks with better quality pasture and a higher stocking density, compared to animals carrying a single foetus.

A farmer can avoid a heavy price penalty when sending their livestock to market, if they are able to determine which animals will be in the right range and which should be sent back to the paddock to continue feeding and gaining weight if they don't reach market specifications. Animals that meet the weight requirements will get more money than those that are under weight.

Currently sheep are tested for pregnancy status via ultrasonic scanning over the skin of the belly. There is need for early pregnancy diagnosis in sheep, reliable techniques for early diagnosis aids in culling or rebreeding of ewes and offers a valuable tool for managed breeding programs (Ishwar 1995). This is because sheep have seasonal breeding patterns, where loss of a breeding opportunity means loss of a productive year for the ewe (Ganaie *et al.* 2009). If a producer is unable to detect early pregnancy status, there can be economic losses in lamb production.

Various methods have been used to diagnose pregnancy in sheep and cattle. In sheep less practical techniques include the management method (non-return to oestrus), abdominal palpation and laparotomy (a surgical incision into the abdominal cavity) (Ishwar 1995). While more practical techniques include radiography, pregnancy protein assays, hormonal determination and ultrasonography (Ganaie *et al.* 2009). Visual assessments via abdominal palpation can be used and gives an indication of pregnancy, but has a low accuracy. The laparotomy, laparoscopy and vaginal biopsies are accurate methods, however, these methods are unrealistic under farm conditions (Goel and Agrawal 1992; Gordon 1999).

Pregnancy diagnosis is one of the most frequently performed processes undertaken on cattle (Romano *et al.* 2006). The current recommendations from the major beef industry research and development organisations in Australia is for each mated female to be pregnancy tested at least once a year (Meat & Livestock Australia 2004; Gargiulo *et al.* 2012). The most widely used forms of pregnancy testing in cattle are either manual rectal palpation of the reproductive tract (per rectum) or ultrasonographic examination of the reproductive tract. A veterinarian will most commonly provide these services to farmers on a fee-per-cow or time charge basis. Both of these procedures are invasive and present risks to the veterinarian and the pregnancy,

such as an abortion. Extensive training is required to undertake the procedure safely at adequate accuracy and speed for the service to be economically feasible (Gargiulo *et al.* 2012). The accuracy of these methods can be improved with the knowledge of four key pieces of information: the animal's reproductive history, palpation of uterine horns, vaginal examination and progesterone determinations (Hazen *et al.* 2000). Veterinarians using ultrasound can reliably diagnose pregnancy from 30 days gestation, whereas testing using manual palpation can diagnose pregnancy status from 35 days (Gargiulo *et al.* 2012). Both methods can diagnose pregnancies from days 30/35 through to full term (282 days) with sensitivities and specificities exceeding 95% (Badtram *et al.* 1991; Fricke and Lamb 2005; Silva *et al.* 2007).

Ultrasonic techniques are used to examine subsurface structures in living tissues with an A-scan, B-mode ultrasonography or Doppler methods (Ishwar 1995). There are moving tissues, which are reflected by the ultrasound and has a high degree of safety to the technician and the animal. A number of previous studies have revealed that trans-abdominal real time B-mode ultrasonography and Doppler ultrasound techniques remained at a constant 100% accuracy until lambing (Karen *et al.* 2006; Ganaie *et al.* 2009).

The present study was performed to compare the accuracy of a handheld ultrasonic device to current veterinary practices for the diagnosis of pregnancy status in sheep and cattle and fat and muscle depths at various stages of gestation of cattle. The main aims of this study are:

- To determine the accuracy of a low-cost handheld ultrasound device to measure:
  - Pregnancy diagnosis in sheep and cattle; and
  - Fat and muscle depth in cattle

- To assess the relationship between body muscle, fat and body condition score in cattle during gestation

## **2 METHODS**

### *2.1 DATA COLLECTION*

The present study used data collected from university teaching facilities: John Pye Farm, Greendale and the Sheep Reproductive Unit, Cobbitty, New South Wales. The two facilities are located south-west of Sydney with a temperate climate and an average annual rainfall of 790 mm. This project had the approval of the University of Sydney Animal Ethics Committee (AEC).

The data was collected using a handheld ultrasound device called the Renco Preg-Alert Pro. The device is designed and made in the USA and costs approximately AU\$2,500. It is a microprocessor-based instrument, using a low power A-mode ultrasound for the detection of pregnancy in mammals and the measurement of fat and muscle tissue thickness. An A-mode ultrasound is a pulse of ultrasound sent out, the echo processed and a determination made on the basis of the time it took to go and return. When a visual display is used, the presentation is a series of spikes rather than a picture (Renco Corporation 2009). The device diagnoses pregnancy by detecting the echoes from the interface between amniotic fluid and the far side of the intrauterine wall and a distinct line will appear on the digital screen. The device also measures back fat and loin muscle thickness in millimetres. The testing process is completely non-invasive, which minimises the chance of infection and disease transmission.

### *2.2 PREGNANCY TESTING*

For the purpose of this study veterinarians were routinely called to complete pregnancy testing with their chosen methods and within 10 days the animals were re-tested using the



Renco Preg-Alert device. The veterinarian and handheld ultrasound testing will not be completed on the same day to avoid holding the animals in the yards for long periods and to avoid a bias effect on the results. The overall results were compared to whether or not the ewe or cow had lambed or calved.

### **2.2.1 Sheep**

A total 53 Merino ewes were analysed within the study and scanned for pregnancy status using the Renco Preg-Alert Pro. The ewes were inseminated on April 1<sup>st</sup> 2016 through laparoscopic artificial insemination. The device was set to pregnancy scanning mode and the sheep pregnancy screen was set, with sensitivity 3 being chosen. The Renco sensitivity scale is from 1 (least) through 4 (greatest). The ewes were restrained in the race and the probe was covered in ultrasound gel before being inserted through the rails of the race to the area just lateral to the right mammary gland, making sure it was a wool and dirt free area. It is really important to have strong contact between the probe and the skin, as air bubbles can have an effect on outcome of results. The probe was moved slowly over the belly for 30 seconds using a rotating-rocking motion, to ensure all air bubbles are removed and pregnancy was indicated by a series of spikes on the screen of the device. The ewes were scanned at 121-135 days gestation. It was assumed the ewe was not pregnant if no spikes appeared.

### **2.2.2 Cattle**

A total of 125 Limousin x Charolais and Angus cows and heifers were analysed within the study and scanned for pregnancy status using the Renco Preg-Alert Pro. The cattle were inseminated on October 15<sup>th</sup> 2015 through live cover. The device was set to pregnancy scanning mode, with the cow pregnancy screen set and sensitivity 3 chosen. The cattle were restrained in a crush and the right side access grilles opened. The cattle had a small section of

hair shaved off halfway between the hook bone and the long ribs, as the device requires skin contact. The conductive medium used was cooking oil and this was sprayed heavily over the shaved section with the probe moving slowly over it, for 30 seconds, using a rotating-rocking motion, ensuring strong contact with pregnancy indicated by a series of spikes on the screen of the device. The cattle were scanned during three stages of gestation: 90-115 days, 190-210 days and 265-calving. It was assumed the cow was not pregnant if no spikes appeared.

## **2.3 FAT AND MUSCLE DEPTH**

The fat and muscle depths of the cattle were taken at what is commonly known as the ‘P12’ site, which is located over the last rib and 6.5 cm off the backbone and the ‘P8’ site, which is located on the rump. The P8 site is defined as the point at the junction of a line centred on the crest of the third sacral vertebra and a line parallel to the backbone and the P12 rib site is located between the 12<sup>th</sup> and 13<sup>th</sup> ribs (Upton *et al.* 2005).

Fat and muscle depth in sheep was not recorded in this study as there were no sheep producers willing to let us shave a section of wool off the sheep to allow the ultrasound probe contact with skin.

### **2.3.1 Cattle**

The 125 cows and heifers that were tested for pregnancy also had their fat and muscle depths tested at the same time. Two small sections of hair were shaved from the right hand side of the animal at the ‘P12’ and ‘P8’ sites and the two sections were heavily sprayed with cooking oil. The option key on the device was pressed to change the screen to fat and the probe was pressed against the skin to obtain a measurement, it was held against the animal for 30

seconds. The option key was pressed again, which changes the device to the loin screen and the probe was pressed against the skin to obtain a measurement, it was held against the animal for 30 seconds. A number will appear on the screen corresponding to the fat or muscle depth in millimetres (mm).

Body condition score was measured via visual approach by looking at the animal from behind and from the side. Condition scoring was based on a scale of 1-5, with 1 having a skeletal body outline, 3 having a body outline almost smooth and 5 having a body outline bulging due to fat (Queensland Government 2015).

Liveweight was measured with a walk over weighing system. After the cattle were tested in the crush, they were released and walked over the scales before returning to the yards. The system was set up to the data was sent straight to a computer.

## 2.4 DATA PROCESSING AND STATISTICAL ANALYSIS

Pregnancy status data was analysed using Microsoft Excel. Sensitivity and specificity were calculated based on the results obtained by the device, the veterinarian and whether the animal had lambed or calved. The indices determined were:

- *Sensitivity* (Se %): Accuracy in detecting pregnant sheep and cattle
  - $TP/(TP + FN) \times 100$
  
- *Specificity* (Sp %): Accuracy in detecting non-pregnant sheep and cattle
  - $TN/(TN + FP) \times 100$
  
- *Accuracy* (%): Number of correct diagnoses made, of the total diagnoses made

- $(TP + TN)/(TP + FP + FN + TN) \times 100$
- *Precision (%)*: How close estimates from different samples are to each other
  - $TP/(TP + FP) \times 100$
- *Negative Predictive Value (%)*: Proportions of negative results that are true negative
  - $TN/(TN + FN) \times 100$
- *Matthews Correlation Coefficient*: Correlation coefficient between the observed and predicted binary classification.
  - $(TP \times TN) - (FP \times FN) / \sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}$

A true positive (TP) is where the pregnant animal is correctly identified as pregnant, true negative (TN) where the non-pregnant animal is correctly identified as non-pregnant, false positive (FP) where the non-pregnant animal is incorrectly identified as pregnant and false negative (FN) where the pregnant animal is incorrectly identified as non-pregnant.

Matthews Correlation Coefficient is a value between -1 and 1. A coefficient of 1 is perfect prediction, 0 is no better than random prediction and -1 is total disagreement between prediction and observation.

Fat and muscle depth and body condition score data in cattle was analysed using Microsoft Excel and a minimum, maximum, standard deviation and mean were calculated. A scatter graph was created of all the fat and muscle depths recorded against all body condition scores. Column graphs were then created to show the average fat and muscle depth against the body

condition score, removing all the outliers. Outliers were body condition scores that only had one or two fat or muscle depth measurements.

Statistical analysis for pregnancy status was conducted using Genstat<sup>®</sup> 14<sup>th</sup> Edn, and significant effects were assessed with a significance level of  $P = 0.05$  (Genstat 2011). A logistic regression analysis was created to determine the significance of the relationship between the device and the veterinarian.

Fat and muscle depth and body condition score statistical analysis was conducted using Statistical Analysis Software (SAS) and significant effects were assessed with a significance level of  $P = 0.05$ . A linear regression model was created to determine the significance of the relationship between fat and muscle depth and body condition score.

## 3 RESULTS

### 3.1 PREGNANCY TESTING

The proportion of pregnant and non-pregnant sheep and cattle were found to be 64% and 36% and 69% and 31% respectively.

#### 3.1.1 Sheep

The veterinarian had higher overall results in all tests completed. The veterinarian had a sensitivity of 100%, while the device only had 82%, however specificity was similar. Accuracy was also higher with 94% in the veterinarian and 81% in the device (Table 1). The Matthews Correlation Coefficient for the device and veterinarian (0.60 and 0.88) were both close to 1, which is considered to be a perfect prediction.

**Table 1. Comparison of the accuracy for the device and the veterinarian at 121-135 days gestation in sheep**

	121-135 Days Gestation					
	Se (%)	Sp (%)	Accuracy (%)	Precision (%)	NVP (%)	MMC
<b>Device</b>	82	79	81	88	71	0.60
<b>Veterinarian</b>	100	84	94	92	100	0.88

**Se: sensitivity, Sp: specificity, NVP: negative predictive value and MCC: Matthews correlation coefficient**

The true negatives were very similar between the device and the veterinarian (Table 2 and 3), the veterinarian had all true positives and no false negatives, while the device had six false negatives.

**Table 2. True negative, false positive, false negative and true positive outcomes of the device compared to lambing outcome**

DEVICE	LAMBED	
	NO	YES
NO	15	6
YES	4	28
<b>TOTAL</b>	19	34

**Table 3. True negative, false positive, false negative and true positive outcomes of the veterinarian compared to lambing outcome**

VETERINARIAN	LAMBED	
	NO	YES
NO	16	0
YES	3	34
<b>TOTAL</b>	19	34

The odds of the veterinarian correctly selecting whether the ewe is pregnant is four times that of the device ( $P < 0.05$ ).

### 3.1.2 Cattle

The veterinarian overall had much higher accuracy in detecting pregnancy status than the device. The device had the highest specificity, accuracy and precision in the 90-115 days of gestation, while sensitivity and the negative predictive value was the highest in the 190-210 days of gestation. During the 265-calving stage the sensitivity, specificity, accuracy, negative predictive value and Matthews Correlation Coefficient were the lowest, with accuracy only reaching 46% (Table 4).

The results from the veterinarian were 75% or higher in every category tested and overall had 95% accuracy and 97% precision. The device however, had overall results 74% or lower and had an overall accuracy of only 62%.

**Table 4. Comparison of the accuracy for the device and the veterinarian at the three stages of gestation tested and the overall outcome in cattle**

Stage of Gestation (Days)	Device						Veterinarian					
	Se (%)	Sp (%)	Accuracy (%)	Precision (%)	NVP (%)	MMC	Se (%)	Sp (%)	Accuracy (%)	Precision (%)	NVP (%)	MMC
<b>90-115</b>	81	60	76	86	50	0.38	90	90	90	97	75	0.76
<b>190-210</b>	84	33	63	64	60	0.20	100	100	100	100	100	1
<b>265-Calving</b>	43	55	46	72	26	-0.02	100	82	95	94	100	0.88
<b>Overall</b>	69	46	62	74	38	0.14	97	92	95	97	92	0.89

In all stages of gestation the veterinarian had more true positives compared to the device (Table 5 and 6). The false positives and false negatives were considerably higher in the

device, especially in 265-calving (17), compared to the veterinarian (0) and overall (27) compared to (3).

The overall odds of successful test in 190-210 days gestation compared to 90-115 days gestation are 0.8856 in favour of failure and successful test in 265 days gestation to calving compared to 90-115 days gestation are 0.4275 in favour of failure. This indicates that overall pregnancy gets more difficult to detect in the later stages of gestation.

**Table 5. True negative, false positive, false negative and true positive outcomes of the device compared to calving outcome at the three stages of gestation tested and the overall outcome**

DEVICE	CALVED							
	90-115 Days		190-210 Days		265-Calving		Overall	
	NO	YES	NO	YES	NO	YES	NO	YES
NO	6	6	6	4	6	17	18	27
YES	4	25	12	21	5	13	21	59
<b>TOTAL</b>	10	31	18	25	11	30	39	86

**Table 6. True negative, false positive, false negative and true positive outcomes of the veterinarian compared to calving outcome at the three stages of gestation tested and the overall outcome**

VETERINARIAN	CALVED							
	90-115 Days		190-210 Days		265-Calving		Overall	
	NO	YES	NO	YES	NO	YES	NO	YES
NO	9	3	18	0	9	0	36	3
YES	1	28	0	25	2	30	3	83
<b>TOTAL</b>	10	31	18	25	11	30	39	86

The odds of the veterinarian correctly selecting whether the cow is pregnant is 13 times that of the device ( $P < 0.05$ ).



### 3.2 FAT AND MUSCLE DEPTH

There was only a small number of cattle tested for fat and muscle depth, with only 72% of cattle recording a fat depth and 37% of cattle recording a muscle depth.

#### 3.2.1 Cattle

There was a very large difference between the minimum and maximum muscle depth (70mm), compared to a difference of 5mm in the fat depth. Results only worked for the device 90 and 46 times out of a total of 125 for fat and muscle depths respectively. Some of the cattle had a very poor body condition score of 1.50 and the average was not much higher at 2.67. The average liveweight of the cattle was 546kg, with the lightest heifer weighing 315kg and the heaviest cow weighing 772kg (Table 7).

**Table 7. Minimum, maximum, standard deviation and mean of muscle and fat depths, body condition score (BCS) and liveweight in cattle. n = number of cattle tested**

	Minimum	Maximum	SD	Mean	n (observed)	n (total)
<b>Fat (mm)</b>	4	9	1.43	4.92	90	125
<b>Muscle (mm)</b>	36	106	15.06	58.89	46	125
<b>BCS (1-5)</b>	1.50	3.50	0.41	2.67	125	125
<b>Liveweight (kg)</b>	315	772	104.44	546.06	107	125

The majority of cattle tested had body condition scores between 2.25 and 3 and fat depths ranging from 4-8mm. There were four outliers of body condition scores 1.50 and 3.50 with fat depths of 4 and 7mm and 4 and 9mm respectively (Figure 1).

There were very similar results for the muscle depths with most cattle tested having a body condition score of 2.25-3.00 and muscle depths ranging from 50-90mm. Outliers in the data came from cattle with body condition score 1.50 and 3.50 (Figure 2).

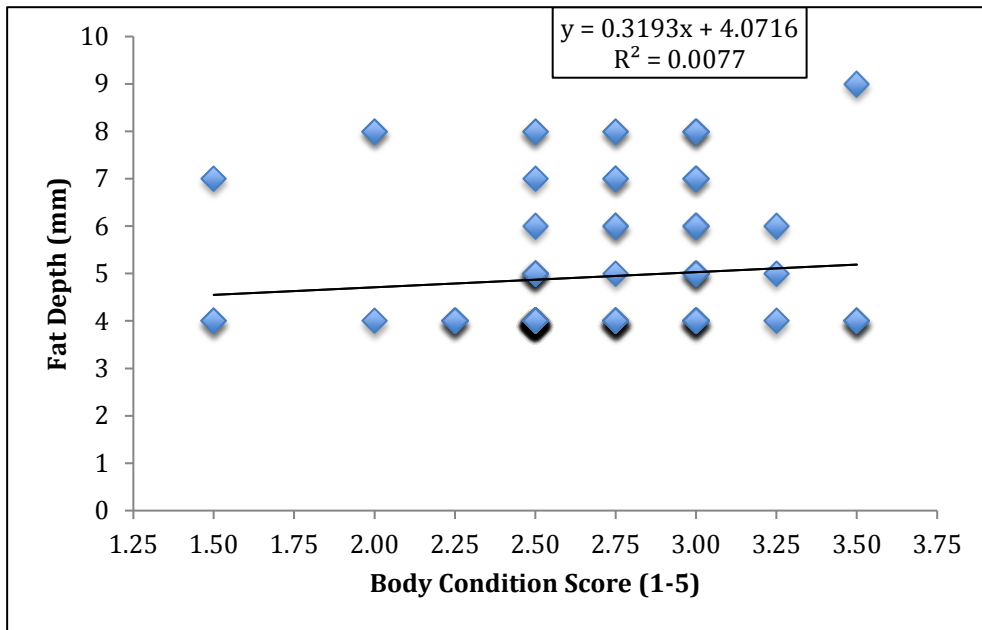


Figure 1. Variation of fat depth compared to the body condition score of the cattle tested. Data is expressed as the total number of fat depths tested on cattle with different body condition scores (n = 90).

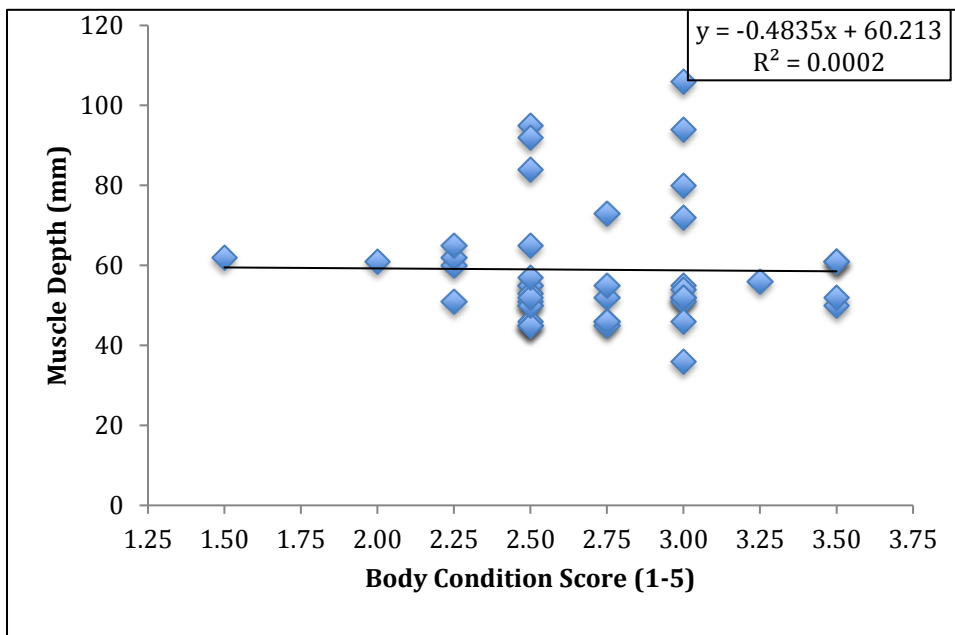
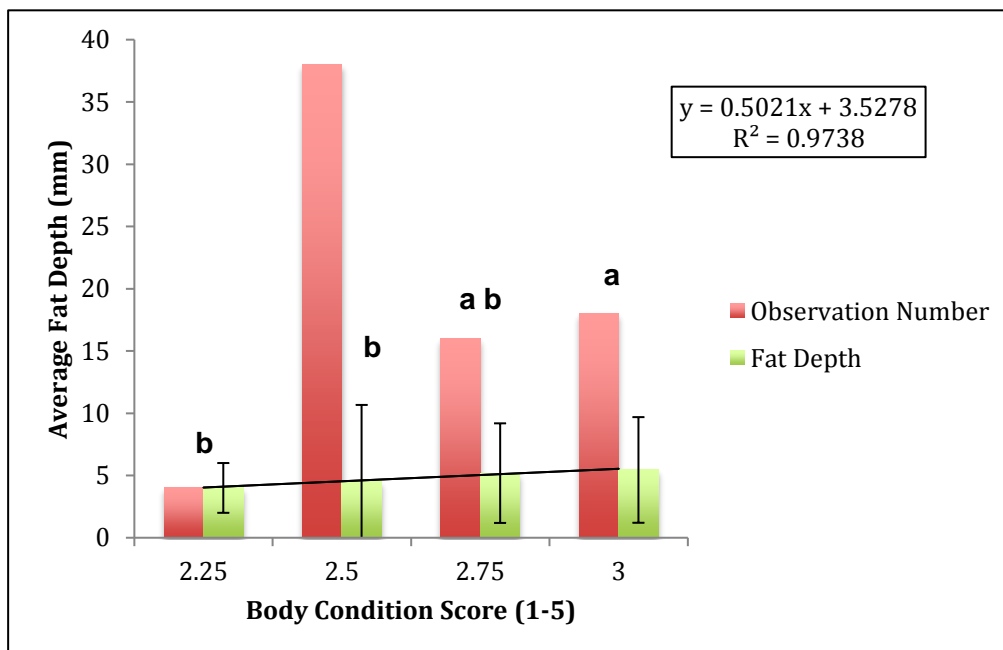


Figure 2. Variation of muscle depth compared to the body condition score of the cattle tested. Data is expressed as the total number of muscle depths tested on cattle with different body condition scores (n = 46)

The average fat depth showed an increase in fat depth as the body condition scores increased. Most cattle had a body condition score of 2.5, which resulted in more fat depth observation numbers (38), compared to body condition score 2.25, where only 4 fat depth observations were recorded (Figure 3).

The average muscle depth showed an increase in muscle depth in body condition scores 2.25 and 2.5, to then decrease at 2.75 and increase again at body condition score 3. Observation numbers were highest in body condition scores 2.5 and 3, which also have the highest average muscle count, 58 and 62mm respectively (Figure 4).

If any two means do not share a common letter they are statistically different ( $P < 0.05$ ).



**Figure 3. Changes in fat depth compared to the body condition score of the cattle tested. Data is expressed as average fat depth tested on cattle with different body condition scores. Only scores from 2.25-3 were used (n = 76). Vertical bars show standard error of the mean.**

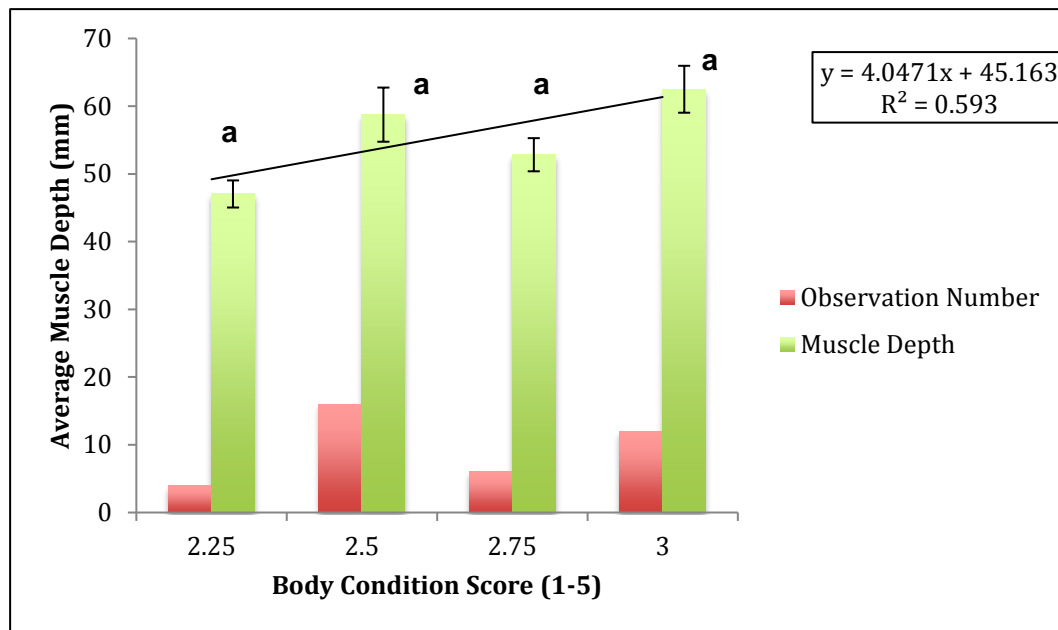


Figure 4. Changes in muscle depth compared to the body condition score of the cattle tested. Data is expressed as an average muscle depth tested on cattle with different body condition scores. Only scores from 2.25-3 were used (n = 38). Vertical bars show standard error of the mean.

At the individual animal level, there was a significant relationship between fat depth and body condition score ( $P = 0.0030$ ). Body condition score increased with fat depth (Figure 5). The relationship between muscle depth and body condition score was not significant ( $P = 0.6869$ ) (Figure 6).

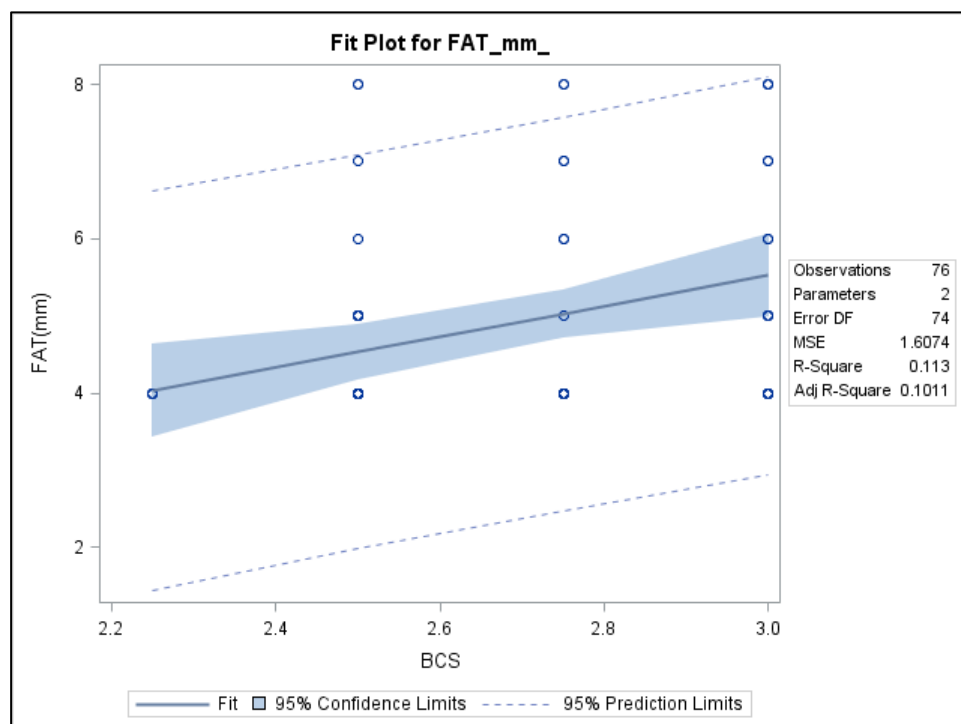
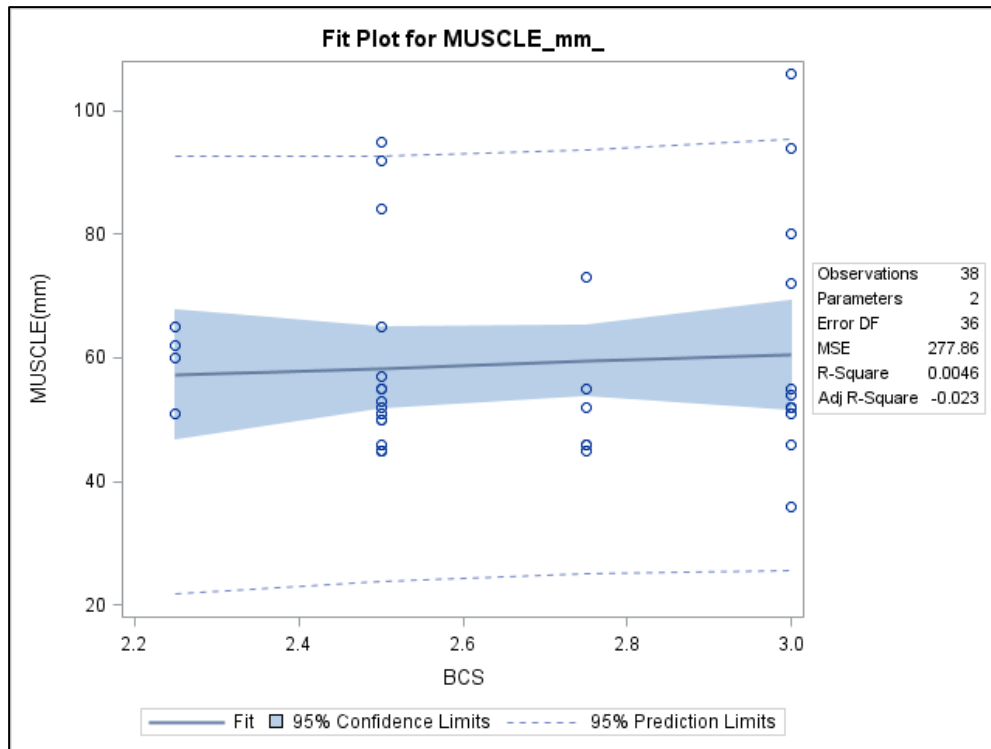


Figure 5. Statistical relationship between fat depth (mm) and body condition score of the cattle tested. Only cattle with body condition scores from 2.2 to 3.0 were included.



**Figure 6. Statistical relationship between muscle depth (mm) and body condition score of the cattle tested. Only cattle with body condition scores from 2.2 to 3.0 were included.**

## 4 DISCUSSION

It was found that the veterinarian was more accurate than the device when detecting pregnancy status in sheep and cattle during all stages of gestation. In sheep the veterinarian had an accuracy of 94% overall compared to 81% accuracy of the device and in cattle the veterinarian had an accuracy of 95% overall compared to 62% for the device. The sheep were easier to test for pregnancy status over the cattle, as they already have a wool free area over the belly that was easy to find and run the probe over. The sheep testing needed more replicates from different stages of gestation, testing from only one stage could be accounting for the higher accuracy percentage compared to the cattle. The device does not show whether the ewe or cow is carrying a single foetus, twins or triplets, it simply shows whether they are pregnant or not.

A study conducted by Ganaie *et al.* (2009) used the Renco Preg-Alert (the model released before the Preg-Alert Pro) to detect pregnancy status during a range of gestation stages in sheep. At 121-135 days, the sensitivity, specificity and accuracy calculated was 79.5%, 100% and 82% respectively. Ganaie *et al.* (2009) had an overall sensitivity, specificity and accuracy of 78.4%, 87.5% and 79.5% respectively. The overall sensitivity, specificity and accuracy results from the current study were 82%, 79% and 81% respectively. These results are quite similar to Ganaie *et al.* (2009), but their study would be more accurate as they had nine stages of gestation that they tested at.

The Preg-Alert device was also used in another study conducted by Ganaie *et al.* (2010), where the study found an accuracy of 56% in days 31-45 gestation, 94% from days 91-105 and 82% from days 136 up until lambing. As the A-mode diagnosis was made on the basis of

detecting the occurrence of fluid in the uterus and sounds related with pregnancy, the possibilities of performing errors because of a filled urinary bladder are reduced (Ganaie *et al.* 2009). It was concluded that B-mode ultrasound is the most accurate, safest, fastest and most economical method of pregnancy diagnosis at the farm level, whereas, the A-mode method can be used under field conditions for pregnancy diagnosis in sheep (Ganaie *et al.* 2009).

The lower sensitivity of the device could be caused by the presence of small amounts of fluid present between the foetus and the uterine wall. The false positive diagnoses may have been attributed to an echo produced by the fluid filled urinary bladder (Ganaie *et al.* 2009). A study conducted by Madel (1983) also had an overall accuracy of 81% for an A-mode ultrasound, but again this study had more replications. Other studies had overall accuracies of 95.8% (Trapp and Slyter 1983), 94% (Watt *et al.* 1984) and 90% (Haibel 1990). Differences in accuracy percentages may be attributed to the changes in breed type, experience of the person testing or a difference in time spent scanning each ewe (Ganaie *et al.* 2009).

In cattle the accuracy of the device continually decreased during each stage of gestation, with a final accuracy of 46% just before calving. Some of the cows tested during this stage had already calved, yet the device was still displaying a positive pregnancy status. One main cause of this problem was from various fluids still inside the uterus (Savc *et al.* 2016). In cattle, fluids can stay inside the uterus 7-30 days after calving (Horan 2016). According to Renco Corporation (2009), pregnancy can be determined by the amniotic fluid and the far side of the intrauterine wall, which is most likely the cause of the false pregnancy readings of the device during the final stage of gestation tested.

The device requires strong skin contact with the probe, which resulted in small sections of hair having to be shaved off the cattle. There is a very high chance that a cow could have been pregnant, but the device did not detect this because not enough hair had been shaved off to provide the necessary skin contact. Shaving off hair became impractical, time consuming and invasive to some cattle. According to Renco Corporation (2009) the number one problem in using their ultrasound instruments is lack of good skin contact and the ultrasound will not pass through air bubbles.

Pregnancy testing in cattle via manual rectal palpation of the reproductive tract is often very invasive, which presents risk to the veterinarian and the pregnancy such as abortion (Gargiulo *et al.* 2012). A study conducted by Franco *et al.* (1987) found that between days 42 and 46 of gestation, the foetal death due to pregnancy diagnosis via manual palpation was estimated to be 11.8%. The device used in the study does not present any risks to the veterinarian or the pregnancy of the cow. It does not require any invasion of the reproductive tract, therefore minimises the chance of infection and disease transmission (Renco Corporation 2009).

Currently, B-mode and Doppler ultrasonography are more commonly used than A-mode (Medan and El-Aty 2010). B-Mode provides the chance to improve the techniques of evaluation of ovarian function and diagnoses of pregnancy in beef cattle (Beal *et al.* 1992). In a study conducted by Quintela *et al.* (2012) showed the overall sensitivity, specificity and accuracy of B-mode pregnancy diagnosis to be 99.95%, 87.31% and 95.29% respectively. These results are a lot higher than the Renco Preg-Alert Pro A-mode ultrasound device. Gargiulo *et al.* (2012) highlighted that a veterinarian using an ultrasound device (B-mode) can reliably identify pregnancy from 30 days gestation and from 35 using manual palpation. The



two techniques can diagnose pregnancy from the days given above through to the full term (282 days) with sensitivities and specificities exceeding 95%.

In the first stage of gestation (90-115 days) the specificity, accuracy, precision and Matthews Correlation Coefficient were the highest compared to the other stages of gestation tested. Although, when comparing these results to the veterinarian, who had results of 75% or higher, it would not be economically feasible for any breeding producer to purchase this device to detect pregnancy status in their cattle. During the final stage of gestation (265-calving) the device gave an accuracy of only 46% and had a negative Matthews Correlation Coefficient. The odds of successful detection of pregnancy status are in favour of the veterinarian by 13 to 1.

It was a lot more difficult to obtain fat and muscle depth results from the device. The device was only able to record 90 fat depths and 46 muscle depths out of 125 cattle. This may be due to not enough hair being shaved off the appropriate recording sites or not enough experience with handling the device and that some of the cattle were in very low body conditions and the device only measures fat depth of 4mm or higher. Some of the results did not match up to the body condition score of the cow. A cow with a body condition score of 1.50 had the device recording 7mm of fat depth and body condition score of 3.50 recording 4mm of fat depth. A cow with a higher body condition score should have a higher fat depth compared to a cow with a lower body condition score. Very similar results occurred when measuring muscle depths. A cow with body condition score 1.50 had a muscle depth of 62mm and another cow with body condition score 3.50 had a muscle depth of 61mm. Although, there was a significant relationship between fat depth and body condition score and no significant

relationship between muscle depth and body condition score. Average fat and muscle depth results are more accurate than the overall fat and muscle depth results.

Ultrasound methods (A-mode and B-mode) of measuring fat thickness in cattle are increasing in popularity, but the subjective (visual) assessment is still the cheapest and most common method used (McKiernan and Sundstrom 2006). A-mode ultrasound is only capable of measuring fat and muscle depth in live animals (Perkins *et al.* n.d.). A study by McLaren *et al.* (1991) scanned a total of 18 cows using an A-mode ultrasound device and obtained an average of 6.7mm fat depth. Another study had an average fat depth of 6.3mm with a standard deviation of 1.9 (Shepard *et al.* 1996). This study had over 1,500 cattle tested, which could contribute to an overall more accurate result for back fat depth compared to the current study.

According to Domecq *et al.* (1995), if the body condition score is significantly associated with ultrasound measurements, the body condition score method can be expected to be as valid as ultrasound measurements to quantify the amount of subcutaneous fat carried by a cow. Their results found a significant relationship between subcutaneous fat and body condition score ( $P < 0.05$ ). Fat scanning can be a very useful instrument for making informed decisions on which cattle are ready to sell (based on P8 EU and MSA market specifications). It will give the producer the ability to objectively measure whether an animal has met the P8 fat depth requirements (Kerr 2014).

The data from this study is significant. There is very little research in using A-mode ultrasound devices to detect pregnancy status in sheep and especially in cattle. This study will be of great use to producers thinking of purchasing this device for their breeding enterprise.

## 5 CONCLUSION

The results of this study suggest that the Renco Preg-Alert Pro A-mode ultrasound device is not accurate when detecting pregnancy status in sheep and cattle at all stages of gestation. A veterinarian would be a more reliable option when checking the pregnant status of sheep and cattle. The device was definitely less invasive and minimised the chance of infection and disease transmission. It was a timely process to shave sections of hair off each cow and with some producers having large breeding herds it just becomes impractical to do it. The device was very simple to use and the results were easy to read off the digital screen.

Although, it was a lot more difficult to obtain the fat and muscle depths from the device, muscle depth was a lot harder to get results for than fat depth. The skin contact issue could have caused this problem, so this device should be trialled on cattle that are less hairy and see whether the results are more easily obtained. The fat depths corresponded a lot better with the body condition score of the cow compared to the muscle depths.

Further pregnancy and fat and muscle depth testing are required at different stages of gestation for sheep and cattle, which will result in more reliable accuracy results overall.

## 6 ACKNOWLEDGMENTS

I would like to express my gratitude to my supervisor, Luciano Gonzalez, whose knowledge, understanding and patience enabled me to write this paper. He went above and beyond to make sure I was safe around the livestock and thoroughly understand every aspect of the project, especially with the data analysis.

A special thanks goes to Farm Manager Paul Lipscombe, who made sure the cattle were in the yards and ready to scan.

I also would like to thank the Australian Wool Education Trust for their Undergraduate Project Scholarship. The scholarship significantly helped with the expenses associated with the project.

I must also acknowledge my Nan and Pop, Jan and John McGill, who provided me with a car and paid for the countless road tolls and petrol tank refills to get me to and from Camden (from Forestville this is a 200km round trip!). Without their support I would not have been able to take on a livestock based research project.

Finally, I would like to thank my family and friends for the support they provided throughout my entire degree.

## 7 REFERENCES

- Badtram GA, Gaines JD, Thomas CB, Bosu WTK (1991) Factors influencing the accuracy of early pregnancy detection in cattle by real-time ultrasound scanning of the uterus. *The Riogenology* **35**, 1153-1167
- Beal WE, Perry RC, Corah LR (1992) The use of ultrasound in monitoring reproductive physiology of beef cattle. *Journal of Animal Science* **70**, 924-929
- Domecq JJ, Skidmore AL, Lloyd JW, Kaneene JB (1995) Validation of body condition scores with ultrasound measurements of subcutaneous fat of dairy cows. *Journal of Dairy Science* **78**, 2308-2313
- Franco OJ, Drost M, Thatcher MJ, Shille VM, Thatcher WW (1987) Fetal Survival in the Cow after Pregnancy Diagnosis by Palpation Per Rectum. *Theriogenology* **27**, 631-644
- Fricke PM, Lamb GC (2005) Potential Applications and Pitfalls of Reproductive Ultrasonography in Bovine Practice. *Veterinary Clinics of North America: Food Animal Practice* **21**, 419-436
- Ganaie BA, Khan MZ, Islam R, Makhdoomi DM, Qureshi S, Wani GM (2009) Evaluation of different techniques for pregnancy diagnosis in sheep. *Small Ruminant Research* **85**, 135-141
- Ganaie BA, Khan MZ, Islam R, Ganaie HA (2010) Ultrasonic techniques for pregnancy diagnosis in sheep. *Indian Veterinary Journal* **87**, 987-990

Gargiulo GD, Shephard RW, Tapson J, McEwan AL, Bifulco P, Cesarelli M, Jin C, Al-Ani

A, Wang N, Schaik AV (2012) Pregnancy detection and monitoring in cattle via combined foetus electrocardiogram and phonocardiogram signal processing. *BMC Veterinary Research* **8**, 1-10

Genstat (2011) 'Genstat for Windows. Version 14.2.0.6297.' 14<sup>th</sup> edn (Lawes Agricultural Trust, Rothamsted Experimental Station: UK)

Goel AK, Agrawal KP (1992) A review of pregnancy diagnosis techniques in sheep and goats. *Small Ruminant Research* **3**, 255-264

Gordon I (1999) Pregnancy testing in sheep. In: Controlled Reproduction in Sheep and Goats. Gordon I. (ed.). *CABI International*, 241-259

Haibel GK (1990) Use of ultrasonography in the reproductive management of sheep and goat-herds. *Food Animal Production* **6**, 597-613

Hazen CH, Pieterse M, Scenczi O, Drost M (2000) Relative Accuracy of the Identification of Ovarian Structures in the Cow by Ultrasonography and Palpation Per Rectum. *The Veterinary Journal* **159**, 161-170

Horan A (2016) The cow post calving (Involution) – <http://moocall.com/blogs/calving/the-cow-post-calving-Involution> [Accessed 30 September 2016]

Ishwar AK (1995) Pregnancy diagnosis in sheep and goats: a review. *Small Ruminant Research* **17**, 37-44

Karen A, Amiri BE, Beckers JF, Sulon J, Taverne MA, Szenci O (2006) Comparison of accuracy of transabdominal ultrasonography, progesterone and pregnancy-associated

- glycoproteins tests for discrimination between single and multiple pregnancy in sheep. *Theriogenology* **66**, 314-322
- Kerr P (2014) Investigating and improving market compliance issues in beef markets in central Queensland. Department of Agriculture, Fisheries and Forestry, *Meat and Livestock Australia*, 1-52
- Madel AJ (1983) Detection of pregnancy in ewe lambs by A-mode ultrasound. *Veterinary Record* **112**, 11-12
- McKiernan B, Sundstrom B (2006) Primefact 282: Visual and manual assessment of fatness in cattle. *NSW Department of Primary Industries, Orange*
- McLaren DG, Novakofski J, Parrett DF, Lo LL, Singh SD, Neumann KR, McKeith FK (1991) A study of operator effects on ultrasonic measures of fat depth and longissimus muscle area in cattle, sheep and pigs. *Journal of Animal Science* **69**, 54-66
- Meat & Livestock Australia: More Beef from Pastures: The producer's manual. Book More Beef from Pastures: The producer's manual. City: Meat & Livestock Australia; 2004
- Medan MS, El-Aty AMA (2010) Advances in ultrasonography and its applications in domestic ruminants and other farm animals reproduction. *Journal of Advanced Research* **1**, 123-128
- Perkins T, Meadows A, Hays B (n.d.) Study Guide for the Ultrasonic Evaluation of Beef Cattle for Carcass Merit. *Ultrasound Guidelines Council Study Guide Sub-Committee*, 1-22

- Queensland Government (2015) Animal Health and Disease Investigation: Animal Body Condition Scoring. Available from URL: <https://www.daf.qld.gov.au/> [Accessed 23 September 2016]
- Quintela LA, Barrio M, Pena AI, Becerra JJ, Cainzos J, Herradon PG, Diaz C (2012) Use of Ultrasound in the Reproductive Management of Dairy Cattle. *Reproduction of Domestic Animals* **47**, 34-44
- Renco Corporation (2009) – [http://www.rencocorp.com/pregalert\\_pro.htm](http://www.rencocorp.com/pregalert_pro.htm) [Accessed 27 April 2016]
- Romano JE, Thompson JA, Forrest DW, Westhusin ME, Tomaszewski MA, Kraemer DC (2006) Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. *Theriogenology* **66**, 1034-1041
- Savc M, Kenny DA, Beltman ME (2016) The effect of parturition induction treatment on interval to calving, calving ease, postpartum uterine health and resumption of ovarian cyclicity in beef heifers. *Theriogenology* **85**, 1415-1420
- Shepard HH, Green RD, Golden BL, Hamlin KE, Perkins TL, Diles JB (1996) Genetic parameter estimates of live animal ultrasonic measures of retail yield indicators in yearling breeding cattle. *Journal of Animal Science* **74**, 761-768
- Silva E, Sterry RA, Kolb D, Mathialagan N, McGrath MF, Ballam JM, Fricke PM (2007) Accuracy of a Pregnancy-Associated Glycoprotein ELISA to Determine Pregnancy Status of Lactating Dairy Cows Twenty-Seven Days After Timed Artificial Insemination. *Journal of Dairy Science* **90**, 4612-4622



Trapp MJ, Slyter AL (1983) Pregnancy diagnosis in the ewe. *Journal of Animal Science* **57**,

1-5

Upton W, Ball A, Wolcott M, Luff A, Hammond K, Graser H (2005) Real time ultrasound scanning applications in livestock assessment. *Animal Genetics and Breeding Unit, University of New England*, 1-73

Wani NA, Wani GM, Mufti AM, Khan MZ (1998) Ultrasonic pregnancy diagnosis in gaddi goats. *Small Ruminant Research* **29**, 239-240

Watt BR, Anderson GA, Campell IP (1984) A comparison of six methods used for detecting pregnancy in sheep. *Australian Veterinary Journal* **61**, 377-382

Young JM, Behrendt R, Curnow M, Oldham CM, Thompson AN (2016) Economic value of pregnancy scanning and optimum nutritional management of dry, single- and twin bearing Merino ewes. *Animal Production Science* **56**, 669-678