An Investigation into the Use of Salivary Progesterone to Diagnose Pregnancy in Sheep

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INTRODUCTION

The need for a pregnancy diagnosis test

The development of a reliable and accurate pregnancy diagnosis test for use on farm animals for the early diagnosis of pregnancy would enable the prompt rebreeding of non-pregnant animals and prevent the culling of pregnant animals in error.

There are many methods of pregnancy diagnosis currently in use, but most are very expensive, difficult to use in the field and require an experienced operator (Gordon, 2005). This investigation looked at the potential development of a pregnancy diagnosis test for use with sheep which would be easy for a farmer to use and would be accurate even at early stages of pregnancy.

Figure 1 Blackface ewe with lamb

Use of salivary progesterone for pregnancy diagnosis

The concentration of steroid hormones in a female animal are an indicator of reproductive status. Progesterone enters the saliva via passive diffusion from the salivary glands. The level of progesterone present in the saliva of the horse and man can be used to diagnose pregnancy by use of an Enzyme-Linked Immunosorbent Assay [ELISA] (Smith, 2005, Kaufman and Lamster, 2002). Therefore, it was reasonable to suppose that the same might be applicable for sheep.

AIMS AND OBJECTIVES OF THE INVESTIGATION

The specific aims of this investigation were to:

- Determine if salivary progesterone could be used for pregnancy diagnosis in sheep.
- Consider the use of an ELISA for saliva sample analysis and to identify its suitability for use in the field.
- Identify if the salivary progesterone concentration in sheep could be used to monitor the oestrous cycle.

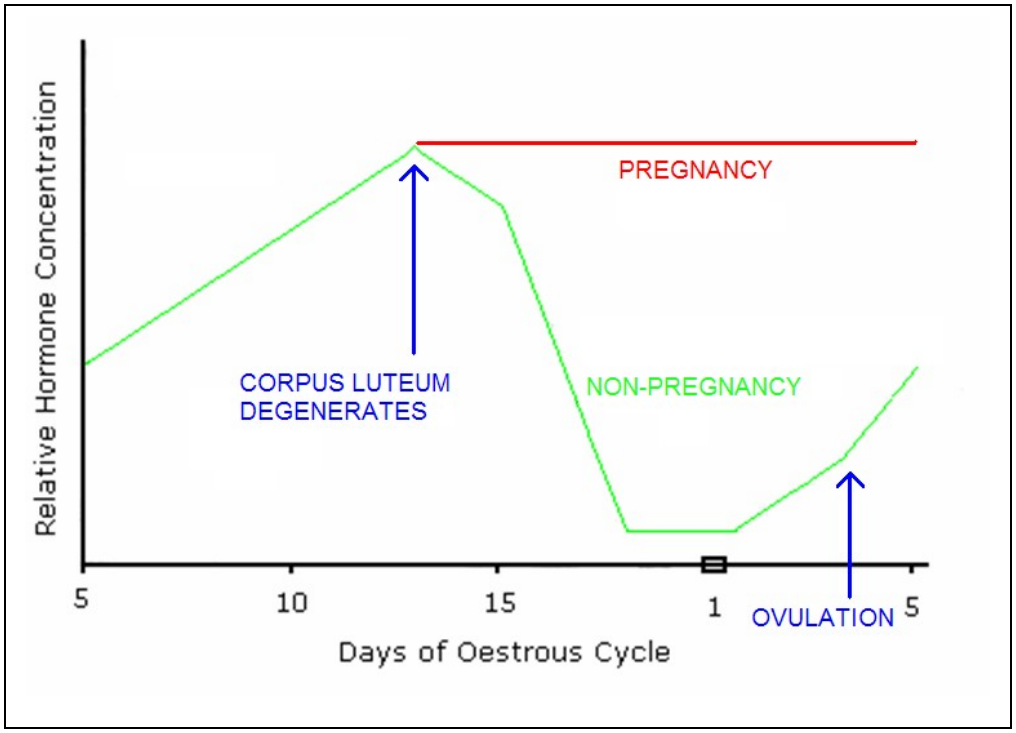

Figure 2: The relative concentration of progesterone during the oestrous cycle.

The ovine oestrous cycle is normally 16-17 days. The level of progesterone rises from the day of ovulation (~day 3 to 4) until approximately day 13 when the level starts to drop (Bearden *et al.,* 2004). The decrease occurs when the corpus luteum starts to degenerate, this is shown as the green 'non-pregnancy' line in Figure 1. If, however, pregnancy does occur then the corpus luteum is maintained and also the level of progesterone, this is shown by the red 'pregnancy' line on Figure 1. The gestation period is 5 months (145-152 days on average). Progesterone drops dramatically on the run up to parturition, thus the highest reading occurs at about 140 days.

Therefore, as the level of progesterone present in a pregnant sheep is maintained throughout gestation it should be possible to determine from the level of progesterone present in a sample whether an animal is pregnant or not.

METHODS

Sample collection
The saliva samples for this investigation were collected from ewes which were part of an embryo transfer program. Their cycles were synchronised by use of a sponge impregnated with artificial progesterone which prevents the ewe from returning to oestrus and ovulating. Once the sponge was removed the ewe would show signs of oestrus within 24-48 hours if it was not pregnant.

Samples were collected at different stages of pregnancy (day 14, day 50 and day 140). Three sheep were sampled at day 14, six sheep at day 50 and six sheep for day 140. Two samples were taken from each sheep on each occasion and mixed together. Saliva samples were taken using commonly available cotton buds. The samples were then prepared and analysed using a competitive ELISA plate, this resulted in the progesterone concentration being obtained for each sample.

Use of the ELISA
An ELISA can be used to measure and detect a range of materials in biological samples. They use enzymes that are attached to one of the reactants to allow quantification through colour development. A monoclonal antibody ELISA kit which was originally designed for measuring progesterone concentration in bovine milk was used for this investigation. It has already been shown that it can be used successfully to measure the concentration of progesterone in saliva (Smith, 2005).

Once the ELISA had been run a standard curve was produced automatically by the microtitre plate reader software program. This allowed the progesterone concentration of the unknown samples to be read off from the standard curve. Some cotton buds were also dipped in water or substrate buffer to determine if there was any 'background' level of progesterone present. These were analysed using the same method as the other samples.

RESULTS

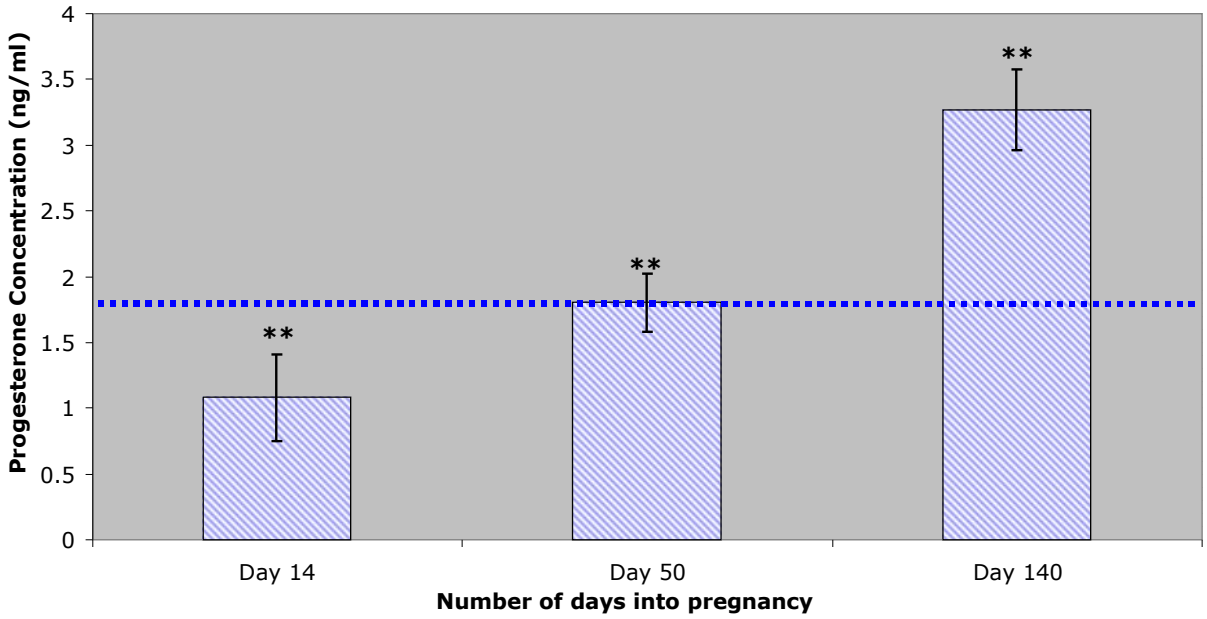

Figure 3: Comparison of the progesterone concentration at different stages of pregnancy. The blue line is the background concentration from the cotton buds **p<0.001).

There was an apparent average background progesterone concentration of 1.80ng/ml from each cotton bud.
The samples from the pregnant ewes at day 14, 50 and 140 indicated a rising concentration of progesterone throughout pregnancy. There was a significant difference between the progesterone concentration at day 14 compared to day 140 and day 50 compared to day 140. However, both the samples for day 14 and day 50 are below the background progesterone level.

DISCUSSION

This aim of this study was to determine if measurements of salivary progesterone could be used for pregnancy diagnosis in sheep. The investigation found that progesterone could be measured in the saliva of pregnant sheep using a simple sampling technique and an ELISA, but only at day 140. As far as we are aware this is the first time this information has been reported. Unfortunately, this method is insufficiently sensitive to allow monitoring of the oestrous cycle and hence is not a reliable indicator of early pregnancy.

Background level of progesterone
One of the most important issues highlighted in the results from this investigation was the high background level of progesterone present on the cotton buds. This is not necessarily a problem as it can be taken into account when analysing the samples. However, the background level needs to be fairly constant.

Smith (2005) measured the water uptake for a number of Johnson's cotton buds and demonstrated considerable variation. This raises two further issues of variable background measurements between individual buds and variable sample size. Commercial cotton buds are not manufactured with scientific experiments in mind, consequently, there is no requirement for the tips to be a consistent size. In future investigation a more uniform 'swab' or 'tip' would be needed for accurate sample collection.

Furthermore, 'cotton based absorbent materials' can result in a high reading of progesterone (as well as other steroid hormones) (Shirtcliffe *et al.,* 2001). Whilst this does not appear to have been an issue in other investigations, sheep have a much lower concentration of progesterone that horses and humans. Therefore the results for sheep would be adversely affected by even a small amount of interfering substances from the cotton.

Progesterone in sheep saliva
There is a large body of previous work which has shown that hormones (including progesterone) can be measured in saliva samples from various species, including humans, pigs and horses (Smith, 2005; Gordon, 2005; Kaufman and Lamster, 2002). However, there does not appear to be any evidence of hormones being successfully measured in saliva samples from sheep, or indeed any ruminants. This raises the issue that sheep may not readily transport progesterone into their saliva.

Table 1.1 is a comparison of the ratio of saliva flow to body weight for six different species. It can be seen from this that the ruminants produce a much larger volume of saliva in relation to their body weight than the mono-gastric animals. This may have a diluting effect of the progesterone present as the larger the volume of saliva the smaller the relative concentration of progesterone there will be.

Species	Approximate body weight (kg)	Saliva flow (l/day)	Saliva flow: Body weight (l/day/kg)
Sheep	50	10	0.2
Cow	700	140	0.2
Camel	500	90	0.18
Horse	300	12	0.04
Man	100	0.7	0.007
Dog	20	0.5	0.025

Table 1.1: Comparison of saliva flow: body weight for ruminants and monogastric animals.

The salivary glands metabolise progesterone before it is assayed
Urine and faeces could be collected in large quantities from individual sheep, but progesterone is not easy to measure from these sources because it is secreted as various metabolites. This could also be the case in sheep saliva; progesterone may not be secreted directly, but metabolised first. As the ELISA is specific only to progesterone it would not be able to detect any metabolites which may be present. To determine if this is the case it would be necessary to collect a large amount of saliva from a sheep and carry out either mass spectrometry or high liquid pressure chromatography to produce a profile of the steroids present in saliva.

SUMMARY AND FURTHER WORK

There is a clear practical advantage to collecting saliva rather than blood for hormone analysis. This study has demonstrated the potential for the measurement of progesterone in saliva but the methods need to be examined and refined.

There are two issues that need to be addressed immediately. The sampling method has to be improved to avoid background contamination and variable sample size. Various alternatives appear possible with a simple suction device such as a Pasteur pipette as an obvious candidate. Furthermore, the low concentration of progesterone in the sample may be addressed by repeated sampling on each test day with the pooling of samples.

It would be interesting to determine the precise concentration of progesterone and any metabolites in saliva using more sensitive methods such as HPLC. Similarly a comparison with other ruminants and mono-gastric animals would clarify basic issues in terms of the applicability of the method to domesticated animals.

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AUTHOR PROFILE:

Catherine is 21 years old and graduated in 2007 from the School of Biosciences with an upper second class BSc (Hons) degree in Animal Science. Catherine was interested in many areas of animal science and studied a wide range of modules during her time at Nottingham. She was particularly interested in the application of science in the animal industry.