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Comparing pregnancy diagnosis through blood sample and through transrectal ultrasound scanning in dairy cattle

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1. Executive Summary

Pregnancy specific protein-B (PSPB) is a chemical that is produced by a pregnant ruminant, cow, sheep, goat, camelid, bison and buffalo to mention a few, that is a reliable predictor of pregnancy in these animals.

Four dairy farmers in Carmarthenshire, with a total herd size of about 1,700 animals, have been involved in the project to investigate whether PSPB can be used as an early indicator of pregnancy in dairy cows, within 30 and 120 days post-service. Although suggesting relevant infertility treatments was outside the scope of this project, early recognition of infertility in a dairy herd led to timely and appropriate treatments in consultation with the farm's vet.

Studies in Idaho in the US have indicated that the PSPB concentration in blood serum is more reliable for testing purposes than those in milk, during the 30 to 120 day post-service period. At the start of the project the cows were split at random into two groups, pregnancy diagnosis by ultrasound scanning and pregnancy diagnosis by blood sample. The actual calving dates of the animals were used as the benchmark for comparison with the results of both methods.

Each method returned three results, 'positive' for when a pregnancy was detected, 'negative' for when the animal was either not pregnant or that it was too early to detect a pregnancy, and finally 're-check' for when a suspected embryonic death or foetal reabsorption was taking place.

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PSPB was found to be 94% accurate in sensitivity and ultrasound was 95% accurate in sensitivity. The difference between these two results was not statistically significant. Also, PSPB was 87% accurate in specificity and ultrasound was 86% accurate in specificity. The difference between these two results was also not statistically significant.

2. Abbreviations & Key Words

ELISA - Enzyme-linked immunosorbent assay
MHz – Megahertz
OD – Optical density
PSPB – Pregnancy specific protein B
TMR – Total mixed ration
UltraS – Transrectal ultrasound scanning

3. Introduction

The purpose of this project was to investigate any differences between two methods of pregnancy diagnosis in cattle, transrectal ultrasonic examination and PSPB by blood sample.

The aim is to establish a pregnancy rate for dairy cows from 28-30 days post-service and beyond, by testing for PSPB in a blood sample. Allowing an early detection of pregnancy enables us to recognise infertility problems early in a dairy herd. Implementing an early diagnosis protocol into the breeding routine could improve reproductive efficiencies greatly. Investigating the above will provide a comparison between ultrasound scanning and PSPB in blood and help us ascertain which method is best to be used on farm. It was not within the scope of this study to identify the best treatment regimens for bovine infertility.

4. Participants

This project started in July 2020 and ended in June 2021. During that period regular visits were undertaken, depending on the breeding pattern of each participating herd, in order to carry out pregnancy diagnosis of the breeding adult cattle.

The four participant farms were:

a. *Robin Thomas of Dolau Ltd, Dolau Gleision, Llandeilo*

This is a 500 dairy cow herd, of British Friesians and Holstein cows. They are calving all year round. All animals are put to beef bulls and replacement calved heifers are purchased throughout the year.

Cows are milked twice daily through a 24:48 swingover herringbone high-line parlour. The average daily milk yield, expressed as the arithmetic mean is 29 litres per cow per day. The 305-day milk yield is 8,652 litres.

During the winter housing, milking cows are kept in cubicles, with mats and sawdust as bedding. Cows graze grass in the summer and fed forage during the winter. Concentrate feed is added in the parlour feeders.

b. Rheinallt Harries of Llwynmendy Uchaf Farm, Bethlehem

This is a 180 dairy cow herd, of Friesian and Jersey crosses, early spring calving between February and March, rearing own replacements.

Cows are milked twice daily through a 24:48 swingover herringbone high-line parlour. The average daily milk yield at peak, expressed as the arithmetic mean is 26 litres per cow per day. The 305-day milk yield is 7,641 litres.

During the winter housing, milking cows are kept in cubicles, with mats and sawdust as bedding. Cows graze grass in the summer and fed forage during the winter. Concentrate feed is added in the parlour feeders.

c. Janet Watkins of Pantglas Farm, Llanfynydd

This is a 380 dairy cow herd, of Holstein cows. They are calving all year round, rearing own replacements.

Cows are milked twice daily through a 24:48 swingover herringbone high-line parlour. The average daily milk yield, expressed as the arithmetic mean is 39 litres per cow per day. The 305-day milk yield is 10,652 litres.

Cattle are housed all year round and are kept in cubicles, with deep sand as bedding. Their diet consists of TMR, while concentrate feed is added in the parlour feeders.

d. John Davies of Plasbach Farm, Ffairfach

This is a 180 dairy cow herd of Holstein cows. They calve all year round, rearing own replacements.

Cows are milked twice daily through a 20:20 doubled-up low-line parlour. The average daily milk yield, expressed as the arithmetic mean is 32 litres per cow per day. The 305-day milk yield is 9,197 litres.

During the winter housing, milking cows are kept in cubicles, with mat and straw as bedding. Their diet consists of TMR, while concentrate feed is added in the parlour feeders.

All farm references and herd results for the remainder of the report will be randomised and anonymised into Farm A, Farm B, Farm C and Farm D.

5. Materials & Methods

a. Variables

PSPB. This is an explanatory variable and is based on the results of the PSPB ELISA test. The resulting values were 1 for when the test returns a pregnant result and 0 for all other results, namely open and recheck.

UltraS. This is an explanatory variable and is based on the results of the ultrasound scan test. The resulting values were 1 for when the test returns a pregnant result and 0 for all other results, namely open and recheck.

The Truth. This is an outcome variable and is based on the date that the subject actually gave birth. Once the project finished, further data were collected as to the date that each enrolled cow gave birth. From the date of actual birth, the actual days pregnant were extrapolated and compared to the result that the test in each randomised group returned. The resulting values were 1 for when The Truth and the randomly allocated test agreed and 0 for when The Truth and the randomly allocated test did not agree.

Sensitivity: This represents the percentage of tested animals that were actually pregnant. It was calculated for each test by dividing the total number of animals each test identified as positive against the total number of animals The Truth returned as positive.

Specificity: This represents the percentage of tested animals that were actually not pregnant. It was calculated for each test by dividing the total number of animals each test identified as not positive against the total number of animals The Truth returned as not positive.

b. Enrolment

All animals from each of the participating farms, that had calved at least once were eligible for enrolment. If an enrolled animal from a participating farm was already presented for a test, only the first record was retained and any subsequent ones were discarded. Enrolled animals that were removed from each herd before the conclusion of the trial, were excluded from the analysis. Animals that were marked for culling and were not intended for breeding, were also excluded.

Animals were enrolled on a monthly basis and their timing of enrolment during the study depended upon their insemination records in the previous months of enrolment. 30 days post-service was the earliest date selected for enrolment. Lists were drawn up by the lead vet and sent to the participating farmers in advance of the pregnancy diagnosis visits. Each farm was visited on a monthly basis to examine the enrolled animals. The seasonality of the pregnancy diagnosis visits, was based on the calving pattern of each herd. If a participating herd was breeding all year round, the project visits took place throughout the duration of the study. On the other hand, if the participating herd was breeding seasonally, the project visits would only take place for part of the duration of the study.

c. Randomisation

All eligible animals were randomised into two cohort groups, one for blood PSBP and a second for UltraS. Animals in each cohort were examined differently during the veterinary visits, but they were not farmed or bred separately. Each participating farmer carried on with their usual breeding programme, as recommending infertility treatments was outside the scope of this project. The randomisation method used was the RANDBETWEEN function of Microsoft Excel © program, Microsoft Corporation.

d. Data collection

During each pregnancy diagnosis visit further information was collected. Firstly, each of the four participating farms had its own unique identifier, that was logged on the recording sheet of each visit. Each presented cow was identified by its freeze brand number or management tag. In cases of ambiguity, the official UK animal ear tag number was recorded also. Finally, there was a record of the date of visit.

For those animals that were enrolled on the PSPB cohort group, a blood sample was collected by the lead veterinary surgeon. The preferred method of collection was by paracentesis of the coccygeal vein. The blood sample was placed in a 7 millilitre glass sample tube, under vacuum with no anticoagulant or preservative. Once collected, each tube was shaken gently three times. Each blood sample tube had a unique identifier, that was also recorded during each visit.

As soon as possible after each visit, all blood samples were returned to the Mendip Vets BioPD laboratory in Llandeilo and were refrigerated until they underwent PSPB laboratory analysis. Laboratory testing was carried out twice a week. The laboratory analysis was based on the BioPRYN© reagents, that were devised by BioTracking LLC. The testing process consisted of three parts, preparation, immunoassay stages and interpretation. The preparation involved the centrifuging of samples at 3,200 revolutions per minute for three minutes. Preparation allowed for the blood serum to be separated and be extracted from the clotted blood sample. The immunoassay stages were ones of direct ELISA on 96-well polystyrene plates, where positions A1 & B1 were occupied by a known positive sample and positions C1 & D1 were occupied by a known negative sample. During this immunoassay procedure, any PSPB concentration in the serum sample was detected, enhanced, isolated and marked with a coloured die. The plate was read by an ELISA reader and the results deciphered by the BioPRYN computer software that was connected to the ELISA reader.

The interpretation of the test was based on reflectometry of high intensity light that was shone onto and reflected from the wells of the plate, then compared to the known positive and negative samples. Depending on the amount of coloured die that each well carried, an OD value was assigned to them by the computer software. Each OD result was associated with a likelihood of a positive or with a likelihood of a not positive result. The PSPB OD result for each cow was recorded and entered in the statistical analysis.

For those animals that were enrolled on the UltraS cohort group, transrectal ultrasound scanning took place. The probe used was a 4.0 MHz curved linear with 23 centimetre penetration and a resulting image that was fanned at 90 degrees. The scanner model was XTC© by ReproScan Technologies LLC. A number of parameters were recorded after each examination, such as presence of pregnancy, stage of pregnancy in days, presence of multiple foetuses and whenever possible the gender determination of the foetus.

All data from this project were analysed with Stata© MP 16.1, Stata Corp LLC.

6. Results

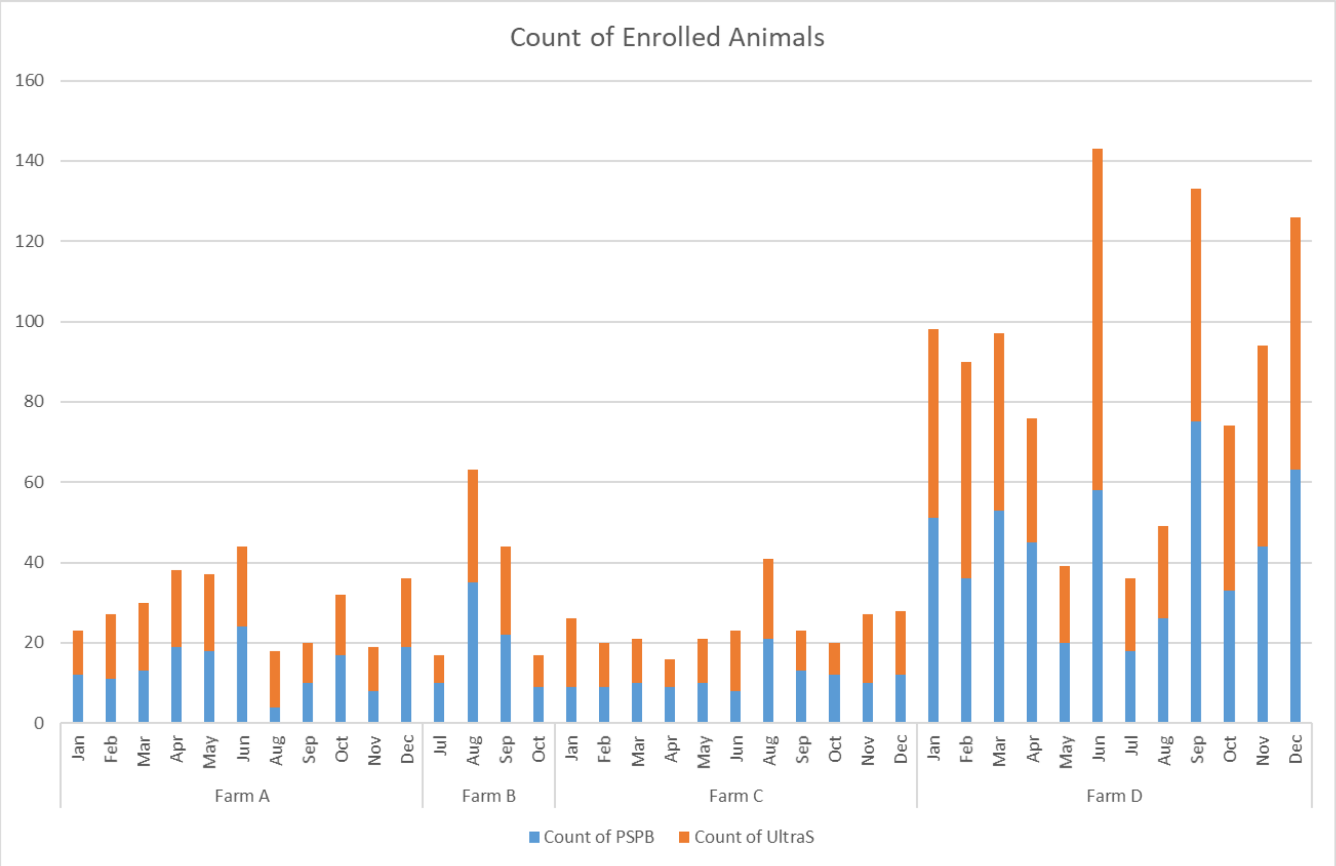
The total number of animals enrolled per participating farm and the randomised allocation of the pregnancy diagnosis cohort group is shown in the following table (Table 1):

Table 1. Number of animals enrolled on project and allocation to treatment groups

Participating Farm	**PSPB Tests**	**UltraS Tests**	**Total**
Farm A	155	169	324
Farm B	76	65	141
Farm C	123	143	266
Farm D	522	533	1055
Total	**876**	**910**	**1786**

The total number of animals enrolled per month and the randomised allocation of the pregnancy diagnosis cohort group is shown in the following graph (Figure 1).

Figure 1. Enrolment of animals per month and allocation to treatments



The following table shows the calculation of sensitivity and specificity for PSPB, as well as the calculation of sensitivity and specificity for UltraS, when each of the diagnostic techniques are compared to The Truth (Table 2):

Table 2. Calculation of sensitivity and specificity for the treatment groups

	PSPB - Pregnant	**PSPB - Non-Pregnant**	**UltraS - Pregnant**	**UltraS - Non-Pregnant**
The Truth - Pregnant	412	26	433	22
Sensitivity	*94%*		*95%*	
The Truth - Non-Pregnant	57	381	64	391
Specificity		*87%*		*86%*

We conclude based on Table 2, that PSPB was found to be 94% accurate in sensitivity and ultrasound was 95% accurate in sensitivity. The difference between these two results was not statistically significant. Based on the same table, we also conclude that PSPB was 87% accurate in specificity and ultrasound was 86% accurate in specificity. The difference between these two results was also not statistically significant.

7. Discussion

Putting aside costs for carrying out either method of pregnancy diagnosis, there are advantages and disadvantages with either technique. Ultrasound scanning is a relatively quick method to get a reliable result. It also allows us to identify multiple pregnancies (twins, triplets and so on), the stage of pregnancy (certain equipment will allow accurate ageing within 7-10 days) and depending on the image and stage of pregnancy, gender determine the foetus. The need for the latter examination has diminished with the introduction of sexed semen, but is still relevant in suckler herds that use natural service or for any herd that uses artificial insemination with conventional semen.

The disadvantage of this method is operator experience, as there is evidence that the scanning result improves with training and with years of practice. Another disadvantage of this method is that very often the selected animals for the ultrasonographic examination have to have their daily routines disrupted, as they are kept in a pen before or after milking waiting to be examined.

PSPB allows for a trained farmer, to collect the samples at any time that suits their and their cows' schedule therefore the disruption to the animal's routine is minimal. There are a number of disadvantages of this technique, the collection of a blood sample falls under the remit of the Veterinary Surgeon's Act. Therefore, the person who harvests those samples has to be trained by their own vet in the procedure. Secondly, although PSPB predicts relatively accurately a pregnancy result, it cannot ascertain the length of the pregnancy, twinning rate or be used for gender determination. This test works best with a known service date, otherwise the result may be meaningless without knowing whether the animal will calve in one or 255 days from the date of the PSPB result.

PSPB diagnosis can be implemented as part of the weekly management task, similar to foot trimming, drying cows off and be carried out on specific days of the week. Once the PSPB results are returned from the laboratory, farm records can be updated about the pregnant animals and for those that were tested not pregnant can be presented to the vet. In turn, the vet can ascertain why they are unable to conceive and administer appropriate treatments.

Such a protocol provides an additional pregnancy diagnosis tool which is inexpensive and pro-active resulting in better pregnancy rates, but also allows the herdsperson to work closely with the farm's veterinary surgeon. The ultimate result is one of "win-win" for all parties involved, the veterinary surgeon, the farmer and the cow. The animal's behaviour and productivity are inhibited minimally, the farm enterprise can save money, and the veterinary surgeon can continue their involvement and oversight of the herd.

8. Conclusions

This project worked with four diverse dairy farming systems and concluded that pregnancy diagnosis through a blood sample is a viable alternative to conventional methods, which in turn results to positive change around herd performance. The effects are multi-faceted, the vets benefit, the cows benefit, so does the farming enterprise.

9. Conflict of Interest

The author offers laboratory services on pregnancy testing to ruminant clients in the UK.

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