

# Ultrasonography versus milk hormonal test for bovine pregnancy evaluation

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It has never been more important to know at the earliest possible time the gestational state of our dairy cows. A positive diagnosis of pregnancy is very good news, but it is determining the negative which has the major initial benefit. The non-pregnant cow needs to be rebred as soon as possible so that the time she is empty is kept to a minimum, reducing the calving interval and producing more milk.

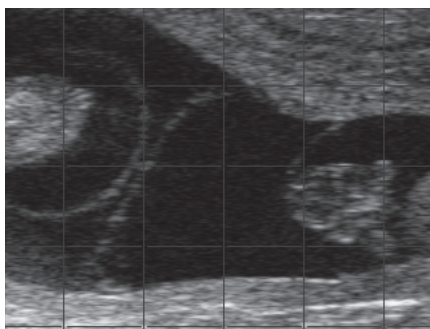
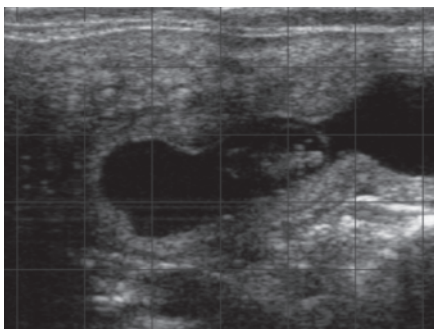
For many years manual palpation was the only method available to diagnose pregnancy, and it was performed very successfully by experienced veterinarians with very good accuracy from 35 days post service.

The development of the first hormone assay test, detecting the hormone progesterone, allowed pregnancy testing to be evaluated via the milk. However, this had major shortcomings, including false positive results, requiring sequential sampling for accuracy.

Manual palpation changed with the introduction of the ultrasound machine, allowing earlier and more accurate diagnosis to be performed. Hormonal testing reached a new level when the less accurate progesterone test was replaced by testing for a pregnancy-specific hormone.

The requirement to establish pregnancy status for the efficiency of herd production

**Fig. 1. A 28 day pregnancy. The earliest gestational stage recommended for 100% accuracy.**



**Fig. 2. Twin pregnancy. The two embryos are close together in this photo, but this is not always the case.**

is unquestionable, with the quest to develop the best and most efficient method of diagnosis a challenge for today's farmer.

## Ultrasonography

Ultrasound diagnosis of pregnancy is performed by using a portable ultrasound machine operating within the frequencies of 3.5-9.0 MHz. There are a number of machines with different ultrasound frequencies in use. The sound waves are conducted through either a linear or sector array of crystals to produce the picture.

The quality and style of the picture produced does influence the interpretational information and the detail visualised and therefore influences the accuracy of the diagnosis.

There are a number of different machines on the market; for the purposes of this article I am assuming diagnosis is being made using one of the machines with a quality image and conducted by an experienced operator.

An accurate ultrasound diagnosis is possible as early as day 28 after insemination. The first visible changes appear during the 13-17 day period but diagnosis cannot be determined at this point. By day 21 after insemination the foetal heartbeat can be visualised confirming a viable pregnancy but it is still too inaccurate to diagnose this early.

Trans-rectal ultrasonography is routinely performed from the 28-day gestation

point, where accuracy is 98-100% in the hands of the experienced operator (Fig. 1).

Ultrasonographic diagnosis has the added advantage of providing additional information on ovarian structures, identification of twins (Fig. 2), determining foetal viability (Fig. 3), accurate gestational ageing (Fig. 4), and determining the foetal sex (Fig. 5). The instant result from the ultrasound diagnosis and the ability to perform a thorough examination of the reproductive tract to establish if any problems are present allows the negative cow to be treated without delay.

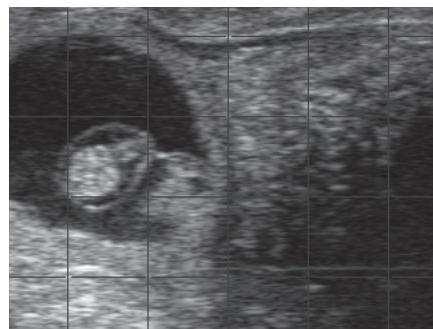
## Hormonal testing

A pregnancy-specific molecule which can be used to determine pregnancy in the bovine has attracted extensive research. The ultimate goal is an in-line milk test for the molecule which gives an instant result which is then fed to the computerised data system. Many different molecules have been and are being investigated. These include the hormones progesterone, oestrone sulphate, Early Conception Factor (ECF) and Pregnancy Associated Glycoprotein (PAG).

PAG has proved to be the most promising so far, to the extent there is a milk test for PAG commercially available. PAG is produced from the trophoblastic cells in the bovine placentomes. PAG hormone requires sending the sample to the

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**Fig. 3. Twin embryos. One is healthy, the other has died. Ultrasonography can determine the foetal viability.**



*Images are taken by Easi-Scan, BCF Technology Ltd.*

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laboratory which takes 1-3 days before results are known.

Its accuracy was determined in an experiment conducted by Paul Fricke, a professor of Dairy Science at Wisconsin, USA. Based on PAG profiles in milk samples collected weekly, the best time to conduct a first pregnancy diagnosis is around 32-39 days after timed artificial insemination (TAI) when milk PAGs are at an early peak in pregnant cows. They were not able to determine the earliest day when milk PAG testing is accurate, so they advised following the manufacturers recommendation of  $\geq 28$  days after AI.

By contrast, conducting the milk PAG test during the temporal nadir in milk PAGs from 46-67 days after AI resulted in a lesser overall accuracy of the test outcomes, dropping to 75-80% accuracy, and the possibility of aborting a few pregnancies if prostaglandin F<sub>2</sub> $\alpha$  is administered based on 'not pregnant outcomes' during this period.

Finally, because of the occurrence of pregnancy loss, they recommended that all pregnant cows should be submitted for a pregnancy recheck at 74 days after AI or later, when relative PAG profiles in milk of pregnant cows have rebounded from their nadir.

The study supported the use of milk PAG testing 32-39 days after TAI when PAG levels in pregnant cows are at an early peak and pregnancy outcomes for pregnant cows approach 100% accuracy.

PAG levels detected by these ELISA tests in Paul Fricke's study had a half-life in maternal circulation resulting in a 7-14-day delay in identification of cows undergoing pregnancy loss based on plasma or milk PAG levels compared to trans-rectal ultrasonography. PAG levels remain high for up to 70 days post-calving, precluding its use for pregnancy diagnosis at this stage.

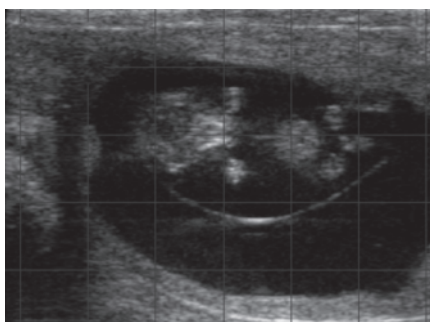
## Comparison of methods

It is difficult to compare the direct cost of the two methods because the cost of an ultrasound diagnosis is extremely variable but in some circumstances they have a very similar direct cost.

The milk test costs in the region of £3.00-£3.50; the ultrasound examination will cost in some cases a similar amount when small numbers of cows are tested. However, the cost of the ultrasound examination becomes very favourable when larger groups are examined. The ultrasound cost per pregnancy diagnosis is determined by the efficiency of the handling facility.

Combining an experienced veterinarian and efficient handling facilities enables 45-64 cows per hour to be examined. This can give per-cow cost ranges from £2.44-£1.70, making ultrasound diagnosis very cost effective.

The main advantage of the hormonal test is its ease of sample collection. The samples



**Fig. 4. The foetus in the image is 49 days old. Accurate gestational stage can be achieved by ultrasound.**

*Images are taken by Easi-Scan, BCF Technology Ltd.*

are taken during milking, eliminating the need for separation and handling of the cow. The only extra time required is to prepare and post the sample. The sampling can be done at the same time as milk recording samples but this restricts the days they are collected, reducing the advantage of the earlier diagnosis.

In addition, restricting the taking of the samples to milk sample day will preclude some of the cows as they may fall outside the early accurate testing window.

In the case of ultrasound diagnosis the result is instant and therefore the positives can be recorded as such, or if negative the cow can be treated immediately so that she can be served as soon as possible. If a routine herd visit is being conducted weekly the negatives are diagnosed quickly and

**Table 1. Comparison of ultrasound and milk test.**

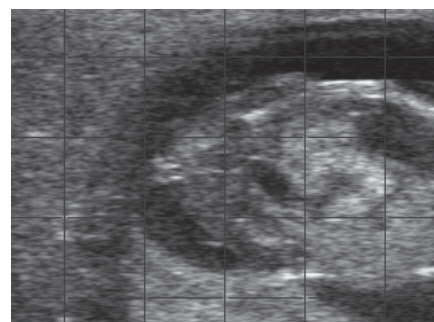
	Ultra-sound	Milk test
Pregnancy determined from (days)	28	28 <sup>1</sup>
Accurate dating of pregnancy	yes	no
Accuracy 32-39 days (%)	98-100	98 <sup>2</sup>
Accuracy 46-67 days (%)	100	78-98 <sup>3</sup>
Accuracy over 74 days (%)	100	98
Viability	yes	no
Diagnosing twins	yes	no
False positive	no	yes <sup>4</sup>
False negatives	no	yes
Determine foetal sex	yes	no
Immediate results	yes	no

<sup>1</sup> Can only be performed from 73 days post calving.

<sup>2</sup> 98% accuracy was after inconclusive results were discarded and not included in the figures.

<sup>3</sup> PAG levels reduce to a temporary nadir during this period.

<sup>4</sup> If a cow conceives but loses the calf the milk test will give a positive reading for a period of time after foetal death.



**Fig. 5. There is a female foetus in this image. Foetal gender determination can be performed when the examination is over 56 days.**

treated on average 2-5 days sooner, this being the time it takes between taking the milk sample and obtaining the result.

Other indirect advantages of the ultrasound diagnosis include the detection of twins, foetal viability, foetal gender determination, reproductive tract health and very accurate detection of the stage of the pregnancy.

In order to get the best out of the ultrasound diagnosis it is imperative that an experienced veterinarian with in-depth knowledge performs the diagnosis. Once the negative pregnancy diagnosis has been determined the cow can be treated on an individual basis with hormonal regimes or advice strategies which can quicken the individual cow's rebreeding and conception times compared to adoption of a blanket re-treatment regime. Treatment of the negatives can take many forms, as determined by the structures seen on the ovary.

Milk testing has a role in small herds where the frequency of the routine visit and the number of cows to be examined are small, therefore the cost of ultrasound diagnosis becomes higher. It is also of benefit where ultrasound may not be an option or countries where the ultrasound machine is expensive and experienced operators are not available.

Both methods can be used where the herd is pregnancy tested at a later stage, over 74 days, and for the routine retesting of all cows just before drying off to confirm they are still in calf. Ultrasound diagnosis has a slight advantage for this diagnosis due to the possibility of the 'false positive' scenario of the PAG levels persisting after foetal loss. Ultrasound can also re-confirm the stage of pregnancy where the cow could have been rebred at a later date than recorded.

Both methods will continue to develop offering more choice and continued benefits for the dairy farmer. As the trend for larger herds continues the cost benefits of both methods will develop as their delivery methods evolve. ■

*References are available from the author on request*