The use of ultrasonography in pregnancy diagnosis of dairy cattle

by Jon Mouncey, Westpoint Veterinary Group, Dawes Farm, Bognor Road, Warnham, West Sussex RH12 3SH, UK.

Reproductive efficiency of dairy cows can greatly influence the profitability of dairy farms. Prolonged intervals from calving to pregnancy lead to economic losses due to delays in beginning or resuming milk production, increased maintenance costs, a decreased rate of replacement and increased depreciation costs.

These costs have been estimated to be between approximately $\pounds 2$ and $\pounds 5$ per day extension over a 365 day calving interval. The variance in the reported costs are dependent on factors such as milk yield per cow, description of lactation curve, milk price, feed costs, calf value and replacement costs. The actual sum of money per day extended calving to pregnancy interval is often disputed amongst dairy farmers, however most agree that there is a significant cost.

Critical control points

Two critical control points for improving reproductive efficiency on dairy farms are:

 Optimising calving to first service.
Early diagnosis of non-pregnancy and timely re-insemination thereafter.

Pregnancy diagnosis is one of the most common and key procedures performed by veterinary surgeons during routine fertility management on dairy farms.

The purpose of examining cows and heifers for pregnancy is to detect those cows that are not pregnant. Detection of the non-pregnant dairy cow or heifer post insemination provides an opportunity to identify individuals that are not pregnant and either decrease the interval between inseminations and therefore reduce the aforementioned costs of extension of calving to pregnancy interval; or alternatively a timely management decision can be made to cull that individual from the herd when economically advanta-



Holsteins in oestrus.

geous. An early accurate diagnosis of non-pregnancy post insemination followed by as short a period of time possible to re-insemination is likely to be an economically profitable strategy for a dairy farm. Fertilisation rates following a correctly timed insemination are approximately 90%. After entering

the uterine lumen on approximately day five post insemination the bovine embryo signals its presence around eight days later, 13 days post insemination.

The bovine embryo secretes interferon tau from trophoblast cells, preventing regression of the corpus luteum by acting on the mechanism which releases prostaglandin F2 alpha and return to oestrus does not occur. In the absence of successful fertilisation or a viable embryo, normal luteolysis of the corpus luteum will occur and the cow or heifer will return to oestrus 18-24 days after insemination.

Early embryonic mortality before 21 days post-insemination occurs in approximately 22% of embryos. Embryonic mortality between 21 and 42 days post insemination and foetal loss thereafter occur at approximately 6% and 5% respectively. Therefore early pregnancy diagnosis prior to 21 days post insemination may be unreliable.

Criteria for the ideal pregnancy diagnosis have previously been described and are as follows. The test should be as early as possible, identify both pregnant and nonpregnant animals correctly, be inexpensive, be simple to conduct under field conditions, determine pregnancy rapidly at the time the test is performed and involve as little additional handling as normally required of the dairy cow or heifer to administer the test.

Economic benefits

The economic benefits of pregnancy diagnosis depend on the factors outlined above, such as the time after insemination when the diagnosis is performed, but also its effects on embryonic loss, the efficiency of oestrus detection, factors that may be affecting oestrus expression on the individual dairy unit and the management or treatment decision made upon finding a non-pregnant individual.

Failure to return to oestrus 18-24 days post insemination may indicate the establishment of pregnancy. This method of oestrus detection is considered to be the most simple, early and inexpensive method of pregnancy diagnosis by many farmers. This assumption does not consider early embryo mortality and is dependent on the efficiency and accuracy of oestrus detection on the dairy unit.

However as labour units per cow are declining and oestrus behaviour has become erratic, short and often even non-existent in Holstein dairy herds, visual observation of oestrus has become particularly difficult. This could lead to the false assumption that some cows – which have either not been detected in oestrus or have not expressed oestrus – might be pregnant.

If oestrus is not detected in these barren cows, presumed pregnant, until the point they are expected to re-calve, the individual is likely to be culled, thus increasing the number of cows culled for failure to conceive.

Culling costs

The cost of culling a dairy cow is estimated to be £750 and total costs due to fertility culls are likely to be much greater than 13% on farms employing poor oestrus detection and depending on return to cyclicity as a means of pregnancy diagnosis.

Approximately 6-10% of cows exhibit behavioural oestrus during a normal pregnancy. Intrauterine insemination of pregnant cows can result in termination of pregnancy.

In these instances if the cow returns to oestrus after iatrogenic attrition of pregnancy and at best is re-inseminated and becomes pregnant within 21 days the incurred cost is estimated to be £94.50, assuming £4.50 per days extension in calving to pregnancy interval.

Equally these 6-10% of cows exhibiting behavioural oestrus during a normal pregnancy may be subsequently culled as barren with greater associated costs plus the value of a pregnancy.

In summary, efficient and accurate oestrus detection in cows and heifers profoundly influences the reproductive performance and profitability of dairy herds as one missed *Continued on page 19* Continued from page 17 oestrus event without intervention may cost approximately \pounds 189, assuming \pounds 4.50 per days extension in calving to pregnancy interval; however non-return to oestrus, should be considered an unreliable indicator of pregnancy and pregnancy loss.

Laboratory testing

Various laboratory tests have been developed for early pregnancy diagnosis in dairy cattle. These tests have the advantage of being minimally invasive as the hormones or proteins they rely on can be detected in milk, however they do have some disadvantages.

Oestrone sulphate for example, can be detected in plasma and milk in pregnant cows by 105 days, and concentrations are significantly lower in the non-pregnant animal.

However it is not cost effective to wait 105 days post-insemination to determine the pregnancy status of a dairy cow and other methods can diagnose pregnancy accurately much sooner, for example rectal palpation at day 30 post insemination, offering a potential saving of 70-75 days over oestrone sulphate.

Early pregnancy factor (EPF) can be detected in milk of pregnant cattle three days post insemination, although after eight days more accurate results can be obtained.

This test would enable fertilisation failure post insemination to be identified within eight days and allow prostaglandin F2a to be administered resulting in a rapid return to re-insemination, however a dipstick cow side test has to date been too unreliable.

Bovine pregnancy specific glycoprotein B (bPSPB) is produced by the binucleate cells of the trophoblastic ectoderm thus indicating the presence of a viable embryo.

bPSPB also has the added advantage of being detectable in milk at 24 days post-insemination, however its

Portable ultrasound in use.





Fig. 1. Left, a male foetus and, right, a cystic structure on an ovary. The images were taken with Easi-Scan, BCF.

half life is many months and as such can be detected after embryonic and foetal death and after normal parturition resulting in a false positive pregnancy diagnosis.

Milk progesterone concentrations are high during dioestrus and pregnancy and low around the time of oestrus. The optimum time for sampling has been shown to be 24 days after service. Milk progesterone sampling has the advantage of being relatively cheap, minimally invasive, and can be performed as a cow side test. The accuracy of milk progesterone testing for confirmation of pregnancy at day 24 post insemination is reported to be 85% and 100% for confirmation of non-pregnancy.

However as a single test 24 days post insemination there are a number of reasons for false positive pregnancy diagnosis. These include incorrect timing of insemination, persistent corpus luteum associated with endometritis, cystic ovarian disease, short return to oestrus interval and late embryonic death.

The greatest benefit to be gained in a single test 24 days post insemination is a low progesterone result as a confident diagnosis of non-pregnancy can be made. A low progesterone value indicates only that the animal is either 2.0-2.5 day's pre- or post-oestrus or indeed in oestrus at time of sampling.

Milk progesterone sampling has been deemed labour intensive by some and so less attractive for the practical farmer. In-line milking parlour biosensors are therefore currently being developed for application in automatic milking systems.

Transrectal palpation

Pregnancy diagnosis by transrectal palpation of the uterus was reported to be first recorded in the 19th Century and is now considered the most widely used method of pregnancy diagnosis in dairy cattle.

This method of diagnosis, although invasive, is relatively cheap and simple to perform once appropriate training has been undertaken. Various structures of the bovine reproductive tract can be palpated per rectum to assist in pregnancy diagnosis including corpus lutea, amniotic vesicle, chorioallantois, placentomes and the foetus.

These various structures can be palpated at different times from insemination to pregnancy diagnosis. Accuracy of pregnancy by transrectal palpation from day 35 of gestation has been estimated to be 95%, however this will vary between operators. Manual rupture of the amniotic vesicle can cause termination of pregnancy in cattle and there remains controversy over the risk of iatrogenic pregnancy loss by examining cows early in gestation by transrectal palpation.

The viability of the embryo cannot be assessed by transrectal palpation alone. The risk of pregnancy loss can be high at 35-42 days of gestation when cows are diagnosed pregnant by transrectal palpation and transrectal ultrasonography.

If most cows within a dairy herd are submitted for pregnancy diagnosis at this time it may not be possible to distinguish between spontaneous losses which might have occurred 'normally' and those losses which are iatrogenic. There may also be an instance when an embryo or foetus may not be viable or is dead and is palpated and diagnosed pregnant, with subsequent pregnancy loss thereafter. Although invasive, transrectal palpation of the uterus for pregnancy diagnosis remains relatively accurate, cheap and simple to perform, with manual skills rather than equipment required.

It does, however, provide a solid grounding in the skills of uterine and ovarian palpation necessary for transrectal ultrasound for pregnancy diagnosis and ultrasonographic examination of ovarian structures.

It is therefore likely to continue to be a valued and cost efficient means of pregnancy diagnosis where acquisition of ultrasound technology has been deemed cost prohibitive or is unavailable. The use of real-time Bmode transrectal ultrasonography is now widely used within the UK for pregnancy diagnosis and in dairy cattle. There are several advantages of transrectal ultrasonography over transrectal palpation alone including the ability to detect pregnancy and non-pregnancy earlier, with greater accuracy and with less iatrogenic pregnancy loss.

Furthermore, foetal number, gender and viability can be assessed and evaluation can be made in the case of a non-pregnant diagnosis to aid reproductive management decisions.

Although still invasive and rate of pregnancy loss is significant in studies using ultrasound to assess pregnancy loss, the technique has not been identified as a direct cause of pregnancy loss and is deemed less invasive than transrectal palpation by some authors, however there is the risk of iatrogenic trauma to the rectum by the transducer.

The cost of equipment required for transrectal ultrasonography in recent years has reduced considerably and where a source of electricity and suitable viewing conditions were once required, ultrasonographic equipment has now become more portable, battery operated and is now commonplace in most large animal veterinary surgeon's vehicles within the UK.

Almost all UK veterinary students are now taught how to perform transrectal ultrasonography for pregnancy diagnosis as it has become so widespread and expected by dairy clients, however in the author's opinion uterine and ovarian palpation skills remain a cornerstone from which transrectal ultrasonography skills grow.

Regular routine pregnancy diagnosis may also allow early identification of potential costly reproductive issues. For example if there is a sudden drop of pregnancy rate from one month's visit to the next which might highlight issues, for example, with insemination technique.

Conclusion

In the author's opinion, the most cost effective means of diagnosis of non-pregnancy is milk progesterone testing at days 19 and 24 post insemination. However, for positive pregnancy diagnosis transrectal ultrasonography is cost effective from day 30 post insemination.

A re-check diagnosis at day 55-60 post insemination is to be recommended, given the risk of embryonic death after initial pregnancy diagnosis, with the added advantage that foetal sexing (Fig. 1) can be performed at this stage. Frustratingly, these results are similar to that found by Oletnacu et al. (1990), 20 years previously, with the exception that pregnancy diagnosis could be performed five days earlier using transrectal ultrasonography.

References are available from the author on request.