Comparison of enzyme-immunoassay of oestrone sulphate in milk with rectal palpation, ultrasonography and farmers' observation for pregnancy diagnosis in seasonal dairy herds in New Zealand

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Abstract

A total of 2139 cows in six commercial, spring-calving New Zealand dairy herds were examined for pregnancy by enzyme-immunoassay of oestrone sulphate in milk, rectal palpation and real-time ultrasonography at 137 to 180 days after the start of mating. The gold standard was based on calving records, observed events such as abortion, or examination of the reproductive tract after slaughter. Sensitivity was 81.8%, 100.0% and 99.9%, and specificity was 81.0%, 91.4% and 90.9% for oestrone sulphate, rectal palpation and ultrasonography, respectively. Oestrone sulphate sensitivity increased in a linear fashion with advancing stage of gestation and reached 96.8% for cows at least 120 days pregnant. Sensitivity and specificity of oestrone sulphate were significantly lower than those of the other two methods were significant (p=0.0001).

In seven additional herds with a total of 967 animals, a pregnancy diagnosis was obtained by oestrone sulphate and farmers' observation. Sensitivity and specificity for these two methods were significantly different at 85.4% vs. 98.6% (p=0.0001), and 80.4% vs. 66.7% (p<0.002), respectively. The sensitivity of oestrone sulphate increased and the specificity of farmers' observation decreased with advancing stage of pregnancy.

Using a partial farm budget, the cost of pregnancy diagnosis by oestrone sulphate was established as NZ$ 6.54 per cow compared to NZ$ 4.34 for rectal palpation and NZ$ 4.60 for ultrasonography. Compared to farmers' observation, oestrone sulphate was more expensive at NZ$ 6.63 vs. NZ$ 6.53 per cow.

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INTRODUCTION TO PRESENTED RESEARCH

Oestrone sulphate and its possible role in pregnancy diagnosis in cattle were initially investigated in the 1970s (Robertson and King, 1979; Heap and Hamon, 1979). Oestrone sulphate is produced by the foetal-maternal unit. It is present in milk and plasma of non-pregnant animals, as well, but its concentration increases as pregnancy advances. Several studies report that after day 100 to 120 of gestation a high proportion of pregnant cows will have oestrone sulphate levels above the highest concentration found in non-pregnant animals. The potential value of oestrone sulphate lies, therefore, in late-stage pregnancy.

With the management of dairy cows prevailing in most New Zealand herds, pregnancy diagnosis is usually carried out four to seven months after the start of the breeding season. The entire herd is commonly presented rather than groups of individual cows at specific times after insemination. Generally, no hormonal treatment is implemented for cows found non-pregnant. Culling and winter feed budgeting are the main management decision based on the results of the pregnancy diagnosis.

A late-stage pregnancy diagnostic method such as oestrone sulphate might be a suitable screening test for seasonal dairy herds in New Zealand. With 21-day submission rates of 75.9% to 86.0% and overall pregnancy rates of 51.5% to 75.0% (Hayes, 1996; Hayes, 1997; Macmillan, 1998) in New Zealand dairy herds, a large proportion of cows can be expected to be at least 100 days pregnant in late lactation. Most herds are enrolled in a periodic herd improvement test scheme, where samples are analysed for milk quality and composition. Milk samples obtained during the autumn herd test could be used for an oestrone sulphate assay. Applied in this way, pregnancy diagnosis by oestrone sulphate would offer a non-invasive method without involving extra labour or time for the herd owner or stress to the cows.

Recent advances in assay techniques have resulted in the replacement of the high-cost, labour intensive radio-immunoassay for oestrone sulphate with the low-cost and faster enzyme-immunoassay. The latter is suitable for the routine processing of large numbers of samples by utilizing micro-titre plate wells and a colour change as endpoint.

Reported sensitivity and specificity of pregnancy diagnosis by oestrone sulphate in cattle ranges from 83.0% to 100.0%, and 80.7% to 100.0%, respectively, with an overall accuracy of around 95% (Hamon et al., 1981; Power et al., 1985; Henderson et al., 1993; Kourletaki-Belibasaki et al., 1995). For oestrone sulphate to have a place in pregnancy diagnosis it will have to show sensitivity and specificity close to other commonly used methods and be cost competitive. In New Zealand, the main methods of pregnancy diagnosis are rectal palpation, real-time ultrasonography and farmers' observations. Progesterone assessment in early gestation is not widely utilised. The main reason for this is that the key purpose of pregnancy diagnosis is identification of non-pregnant cows for culling, and not hormone intervention and re-breeding.

One of the main requirements for the comparison of different diagnostic methods is a perfect control method. In the case of pregnancy diagnosis in cattle, a truly precise gold standard is currently not available. Return-to-oestrus relies on the observer's ability and calving records on the close supervision of animals between the time of pregnancy diagnosis and parturition to detect foetal loss. Hormonal assays can show cow-to-cow and day-to-day variations (oestrone sulphate: Heap et al., 1983; Henderson et al., 1995; progesterone: Booth, 1979), may be influenced by uterine or ovarian abnormalities (progesterone), and concentrations may remain elevated after parturition or foetal loss has occurred (bovine pregnancy-specific protein B: Ruder and Sasser, 1986; Szenci et al., 1996). Clinical examination methods, such as rectal palpation or ultrasonography, rely on the examiner's ability with the possibility of an erroneous diagnosis. Foetal loss, especially when the foetus is absorbed, adds to the problem.

In the absence of a perfect control, investigations into the accuracy of a particular method can only be aimed at relative comparisons rather than establishing absolute values of accuracy. This is achieved by comparing the method under investigation to other relevant methods in the same population. Performing pregnancy diagnosis by all the different methods within a short time period ensures that foetal loss will affect all the methods to the same degree. Investigations must also be carried out in a true sample of the population and under relevant management situations.

Veterinary procedures in farm animals have to be viewed in an economic context. Optimum outcome or high accuracy alone do not guarantee that a particular intervention is the most cost-effective. An economic analysis is necessary to allow a rational comparison. Each method of pregnancy diagnosis involves certain direct costs (for example, cost of test payable to third party, need for equipment, extra labour or time). But the biggest cost connected with pregnancy diagnosis, regardless of method used, is the cost of any misdiagnosis. Management decisions are invariably based on the results of pregnancy diagnosis (for example, culling vs. retaining in the herd), and losses arise from a wrong decision.

Aims and objectives

The aims and objectives of the presented research were:

(i) To establish sensitivity, specificity and positive and negative predictive values of pregnancy diagnosis by an enzyme-immunoassay of oestrone sulphate in milk in seasonal dairy herds in New Zealand.

(ii) To establish sensitivity, specificity and positive and negative predictive values of pregnancy diagnosis by rectal palpation, real-time ultrasonography and farmers' observations.

(iii) To compare all diagnostic methods in an economic model.

The literature relevant to pregnancy diagnosis in cattle is reviewed in Chapter I. Oestrone sulphate in milk for pregnancy diagnosis was compared to rectal palpation and real-time ultrasonography in six commercial, spring-calving dairy herds and this study is described in Chapter II. The second study, comparing pregnancy diagnosis by oestrone sulphate in milk and farmers' observation in seven seasonal dairy herds is presented in Chapter III. In Chapter IV, an economic evaluation of the four methods of pregnancy diagnosis was performed, using the results of Chapters II and III.

CHAPTER I

LITERATURE REVIEW OF PREGNANCY DIAGNOSIS IN CATTLE

History of pregnancy diagnosis

Egyptian papyri from as long ago as 2200-2100 BC contain notes about the effect of urine on wheat and its use for pregnancy diagnosis. Urine from pregnant females suppresses germination and shoot growth of the wheat. Veena and Narendranath (1993) confirmed the validity of this ancient test for cattle. The Asian author Arthaveda in 1200 BC describes reproductive problems such as sterility, abortions and twinning (quoted by Leclainche, 1955) and treatment of sterility is mentioned in Indian texts (quoted by Smithcors, 1957). Despite these references to sterility and obstetrics in cattle in ancient texts, little can be found about pregnancy diagnosis in domesticated animals. Egyptian drawings from about 1500 BC show assisted calvings. Rectal examination for digestive disorders is not only mentioned in a Kahun papyrus from about 1900 BC (Dunlop and Williams, 1996), but also advised by the Roman Vegetius in the 4^{th} century (quoted by Smithcors, 1957), suggesting that the art of palpation had been discovered. The visual examination of urine for diagnostic purposes became popular in the Middle Ages and a text from the 15^{th} century describes the characteristics of urine of pregnant cows (Keil, 1988).

Fleming (1896) reviews a whole range of techniques including oestrus observation, changes in the mammary gland, abdominal distension, rectal and vaginal palpation, ballottement and auscultation of the foetal heartbeat. Rectal palpation for pregnancy diagnosis started to be used in the early 1800's and has been a routine procedure during the 20^{th} century (Momont, 1990). Detailed descriptions of the changes in the pregnant uterus can be found in texts from the 1940's (Hammond, 1948; Benesch and Wright, 1962). In 1927, Ascheim and Zondek described the discovery of the rise in oestrogenic hormones during pregnancy and postulated their use for pregnancy diagnosis, but immunoassays for detecting substances in the peripheral circulation were not developed until the 1960's, about 70 years after the discovery of antibodies in 1890 (van der Lende et al., 1992).

Role of pregnancy diagnosis in dairy cattle herds

Pregnancy diagnosis is an integral part of herd health management programs (Galton et al., 1977; Blood et al., 1978; Heider et al., 1980; Boneschanscher et al., 1982; Oltenacu et al., 1990; Williamson, 1994). Knowing the pregnancy status of animals in a herd allows the herd manager to make decisions about renewed breeding, culling, feeding, and calving. Timely monitoring of herd fertility requires knowing the pregnancy status of each animal. Without this information, the performance of the herd or an individual animal cannot be assessed until calving has taken place, causing a considerable delay. The herd owner's 'peace of mind' is important, as well. This factor may explain why pregnancy diagnosis is often requested at a time during the reproductive cycle when options for interfering are limited or no longer available.

Usage of pregnancy diagnosis services in dairy herds

A survey of 1692 herds in England and Wales carried out in 1979 showed a high number of herds not using veterinary pregnancy diagnosis (42%). About the same number of herds used pregnancy diagnosis in less than half their animals (48%), with only just over 14% of herds utilising pregnancy examination in most of their animals. Most of the pregnancy diagnoses were carried out at 3 to 4 months of gestation (Newton et al., 1982). Esslemont (1995) found not much change to the situation when he surveyed about 18 000 herds in the UK ten years later. Only 15.1% of herds had routine, scheduled veterinary visits, which included pregnancy diagnosis. Several of the remaining herds used pregnancy diagnosis, but not on a routine basis.

A survey of 218 farms in California showed veterinary pregnancy diagnosis being used by 66% of herds. The average gestation length at the time of diagnosis was 76 days, with a quarter of farms examining cows at more than 90 days of gestation (Cowen et al., 1989). Of 213 Canadian farms, 73 were on a herd health scheme that included reproductive examinations of cows frequently or always, while 19% of the 140 herds not using a herd health scheme stated that reproductive examinations were never carried out during veterinary visits (Giger et al., 1994).

Among 72 herds serviced by the Farm Service Clinic, Massey University, New Zealand, in 1997, 41 herds (56.9%) had the whole herd checked for pregnancy (28 herds by a veterinarian and 13 herds by an ultrasound operator), with 19 herds (26.4%) requesting pregnancy diagnosis in cull or suspect animals only (15 herds by veterinarian and 4 herds by ultrasound). Pregnancy diagnosis was carried out between 120 and 190 days after the start of mating. Only a fifth of the herds (15) requested pregnancy diagnosis for primigravid heifers. An abattoir survey in 1973 in Australia found two-thirds of cull cows pregnant, most of them in the second or third trimester. Of the 99 herds of origin, only 6.4% had used pregnancy diagnosis prior to slaughter. The number of cows found pregnant was much higher for herds not using pregnancy diagnosis (Ladds et al., 1975).

Uptake of routine veterinary diagnostic procedures, such as pregnancy diagnosis, is influenced by herd and farm size, calving pattern, and level of education and progressive attitude of the herd manager (Wassell and Esslemont, 1992a; Giger et al., 1994). Economic and convenience aspects may also influence the decision about the usage of pregnancy diagnosis services. The provision of routine visits by veterinary practices is another influencing factor. Most (81.5%) veterinary practices in the UK have been offering routine visits to their clients. However, less than five clients each have been using this service for the majority of the practices (Wassell and Esslemont, 1992b). Giger et al. (1994) found differences in the breadth of herd health services provided to clients between practices.

Decisions based on the result of pregnancy diagnosis

Culling is a major decision based on the results of pregnancy diagnosis. Obtaining information from the farm of origin of slaughtered cows in the UK, Singleton (1996) found that infertility was the main reason for culling in 28.2% of culled animals, making it the highest-ranking single reason overall. Esslemont and Kossaibati (1997), following 50 herds over three seasons in the UK, found that poor fertility was given as the reason for culling in 36.5% of culled animals (9% of total herd). In the Netherlands, poor health and fertility accounted for over half of animals culled in a study by Hogeveen et al. (1992). With an annual culling rate of 12.2% in 247 seasonal Irish herds, infertility was the third most common reason for culling, accounting for 13.2 % of culled animals (Cunningham and Shannon, 1976).

The situation in New Zealand herds is similar. Hayes (1997) reported annual culling because of unsatisfactory reproduction of 5.6% of all cows (with a total culling rate of 15%) in 48 seasonal dairy herds with computerised herd records. Poor reproduction was the highest-ranking reason, accounting for 37.6% of all cull cows. Reports from the 60's and 70's state reproductive disease as the second most common reason for culling, after low production (Bradford, 1968; New Zealand Dairy Board, 1976; Livestock Improvement Corporation, 1985).

Planned calving is enabled by knowing the pregnancy status of animals in a herd. Labour, buildings and feed can be fully utilised. Vaccination programs aimed at protecting the newborn calf rely on knowledge of expected calving dates. The pregnancy status of breeding females can be important for sale and insurance purposes.

Seasonal herds

The breeding period in seasonal New Zealand herds is generally limited to one block of several weeks per year (averages of 75 and 102 days reported by Macmillan and Moller, 1977; Hayes, 1997). Cows that have not conceived during this period are usually not offered further breeding. Pregnancy diagnosis in these herds is often carried out towards the end of the current lactation, forming the basis for culling decisions. In pastoral systems, removal of non-pregnant animals before the start of the limited feed supply period in winter is an important management procedure.

Recently, pregnancy diagnosis six weeks after the end of the artificial insemination period has been advocated in New Zealand to identify non-pregnant, anoestrus cows (Fielden et al., 1980). Hormonal induction of oestrus and subsequent breeding before the end of the mating period can be achieved with this regime. In addition, cows likely to produce calves late and of lower genetic value can be identified.

A high positive predictive value (i.e. no false positive diagnoses) is important for methods of pregnancy diagnosis in seasonal herds. Re-testing of animals with doubtful or unconfirmed diagnoses is an option, as the diagnosis is carried out in a wider time frame.

Of benefit in seasonal herds are methods that can confirm the date of conception to identify bull matings or pregnancies to unrecorded or previous services. Induction of calving is a common management procedure in seasonal herds (Macmillan et al., 1990) and accurate knowledge of the stage of gestation is essential for an induction program. Dry, pregnant cows are often grazed off the farm containing the lactating herd to conserve feed on the pastures available for animals in early lactation. Groups of cows are returned to the main farm when they approach calving. Knowing the expected calving date facilitates this practice, as well.

Non-seasonal herds

Hormonal interference in non-pregnant animals, leading to presentation of these animals for renewed breeding, is an important decision based on the results of pregnancy diagnosis in non-seasonal herds. Warnick et al. (1995) stated "… an important aspect of dairy reproductive management is to decrease the number of cows with prolonged calving intervals by early pregnancy diagnosis and subsequent re-breeding of open cows" (p. 811). While there is no limited breeding period in these herds, the target is to achieve a 365-day calving interval.

Pregnancy diagnosis is ideally carried out less than 42 days after the last service. A cow that has not conceived to the last service can be presented for further breeding at the second cycle in this way, keeping the calving-to-conception interval to a minimum. Pregnancy diagnosis at less than 21 days after insemination would be ideal, if a reliable and convenient test was available. Methods of pregnancy diagnosis used in non-seasonal herds require high specificity and negative predictive value, to maximally identify non-pregnant animals and avoid accidental abortion of pregnant animals when hormone treatment is used. Sexing of foetuses is used in some herds to change the type of bull or semen once the required numbers of high genetic heifer calves have been conceived.

Preferential feeding according to the animal's pregnancy status is not common in non-seasonal herds. There is generally no restriction to the available feed with utilisation of conserved and concentrated feeds.

Current Methods of Pregnancy Diagnosis

Rectal Palpation

Rectal palpation for pregnancy diagnosis has been a routine procedure for more than 100 years (Momont, 1990). The procedure involves introduction of the examiner's hand into the rectum of the restrained cow, identification of the reproductive tract and palpation for signs of pregnancy. Fleming (1896) only mentions palpation of foetal parts, but veterinary textbooks of the 20th century contain more detailed descriptions of manually detectable changes in the pregnant uterus (Hammond, 1948; Benesch and Wright, 1962). These changes include thinning of the uterine wall, fluid fluctuation within the uterus, the chorio-allantoic membrane (membrane slip) and the amniotic vesicle (Hancock, 1962; White et al., 1989a). With advancing pregnancy, placentomes and the foetus can be detected. Size and the appearance of various structures can be used to establish the age of the conceptus.

Palpable changes are present from about day 30 of pregnancy (Zemjanis, 1970). Implantation and organogenesis take place between 30 and 45 days of gestation (Silvia, 1994), giving rise to concerns about risks of rectal palpation during early pregnancy. Ball and Carroll (1963) found that rupture of the amniotic vesicle requires little pressure at 30 to 45 days of gestation.

Several studies were undertaken to assess the degree of foetal loss after rectal palpation in early gestation with varied conclusions. Foetal loss was not affected by membrane slip at 30 to 45 days (Alexander et al., 1995) or palpation at 41 to 45 days of gestation (Drost et al., 1982). Thompson et al. (1994) found that the effect of rectal palpation on the rate of calving was extremely small compared to other factors such as herd, season, and use of hormones. Paisley et al. (1978) concluded that foetal loss could not be ruled out, but the incidence would not be high. Ball (1978) reported a foetal loss rate of 0.7% after rectal palpation, with an overall rate of 10.6%.

Palpating the uterus for fluctuation has been suggested as the safest method for early pregnancy diagnosis by rectal examination, with palpation of the amniotic vesicle and feeling for slip of the chorio-allantoic membrane carrying a higher risk of foetal loss (Abbitt et al., 1978; Beghelli et al., 1986). Based on calving intervals and calving rates, several authors claim a significant difference in foetal loss between cows palpated in early gestation versus control animals not palpated (Franco et al., 1987), early versus late palpation (Abbitt et al., 1978; White et al., 1989b; Warnick et al., 1995), and suggest that the optimal time for rectal palpation is between 51 and 56 days (Vaillancourt et al., 1979; White et al., 1989a; Mohammed et al., 1991; Labèrnia et al., 1996). There was no difference in pregnancy loss between four veterinarians and no association between early or late palpation and pregnancy loss in a study by Lemire et al. (1993). In contrast to this, Abbitt et al. (1978) found a difference in apparent foetal loss between examiners, but no influence of palpating cows once or twice.

Rectal palpation has been implicated in the aetiology of atresia ani or coli (Bellows et al., 1975; Schlegel et al., 1986), although an epidemiological study by von Benda et al. (1978) suggests that, if rectal palpation is causative at all, it would only be so in isolated cases. Transfer of diseases is a concern, as well, especially bovine viral diarrhoea (Lang-Ree et al., 1994; Green, 1994) and enzootic bovine leukosis (Radostits et al., 1994).

Reported sensitivity, specificity, and positive and negative predictive values for pregnancy diagnosis by rectal palpation are summarised in Table 1.1, page 11.

Table 1.1 Reported sensitivity, specificity, positive (PPV) and negative (NPV) predictive values for rectal palpation

Method	No. & Type of Animals	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %	DIC	Control	Reference
	Holstein Heifers	90.7					30 to 45	bPSPB	Alexander et al. 1995
		95.8					40 to 45		
	299 Holstein-Friesian					99	>34	RtO & Calving	Badtram et al. 1991
	266	90.0	52.0	(doubtful 7.8%)			50 to 60	Calving	Ducker et al. 1985
	85			100			42 to 46	Progesterone & repeat RP	Franco et al. 1987
	483	99.7	89.9	(without doubtful diagnosis)			29 to 84	Calving	Hancock 1962
		99.2	87.8	(with doubtful diagnosis)					
	1851 Beef	99.0	69.6	94.9	92.8		70 to 145	Calving	Meacham et al. 1976
	Dairy	98.9	58.6	92.5	90.8		30 to >120	Calving	Reimers et al. 1985
	4558 Dairy					92.5			Reimers et al. 1990
	245 Heifers	89.4	94.4	91.8	92.2		65 to 70	Calving	Roche et al. 1978
	95 Beef	98.0	100	100	97.8		>41	Slaughter	Tierney 1983
	2368 Dairy	99.1	77.2	95.1	95.2		30 to >72	RtO	Warnick et al. 1995
		99.2	79.9				>36	& repeat RP	
		99.3	81.9				> 43	& Calving	
	1624	99.6	49.2	83.3	97.8		30 to 68	Calving	White et al. 1989b
		99.4	67.1	84.6	97.8		>41		
	814 Hereford X	98.5	100	100	82.8	98.7	60 to 137	Slaughter	Yaro et al. 1989
indirect				91-96.2			35 to 42	repeat RP	Abbitt et al. 1978
indirect	565			97.2			33 to 45	repeat RP	Beghelli et al. 1986
indirect				94.0			60 to 90	Calving	Heap et al. 1976
indirect	7477 Cows			94.4			30 to >70	Calving	Vaillancourt et al. 1979

indirect = Study investigated other reproductive problem DIC = Days in calf bPSPB = Bovine pregnancy specific protein B RP = Rectal palpation
RtO = Return to oestrus

Ultrasonography

The reflection of ultrasound waves from an object of interest can be demonstrated by sound or vision. The first method forms the basis of Doppler ultrasonography, used to identify the foetal heartbeat in pregnancy scanning of ewes. Visualisation of the ultrasound reflection can be either by showing the amplitude of the waves (A-Mode) or by transposing the strength of reflection into a picture with grey-scales (B-Mode or real-time). Lindahl (1966) was probably the first to describe the use of ultrasound for pregnancy diagnosis in animals and he found the amplitude-depth presentation (A-Mode) more useful than real-time.

Real-time ultrasonography for pregnancy diagnosis in animals became popular in the late 1970's and specialised transducers were developed (Peter et al., 1992). Today, a linear transducer is commonly used for pregnancy diagnosis in cattle, with a 3.5 or 5.0 MHz head. This transducer is introduced into the cow's rectum and swept over the structures in, or just cranial to, the pelvic cavity, either with the help of the operator's hand or a moderately rigid support of transducer head and cord.

The structures suggestive of pregnancy have been described by Curran et al. (1986) and Boyd et al. (1988), with the heart beat of the embryo, detectable from 22 days of gestation onwards, being the most definite sign. Accuracy increases as pregnancy progresses, with suggestions that pregnancy diagnosis should be carried out in heifers from 22 or 28 days of gestation onwards (Hughes and Davies 1989; Kastelic et al., 1989) and in cows from 30 to 35 days of gestation onwards (Tainturier et al., 1983; Bonato et al., 1990; Rajamahendran et al., 1994). Hanzen and Laurent (1991) and Badtram et al. (1991) found no difference in accuracy between animals of different ages. The position of the uterus influences accuracy, however, with more false negative diagnoses in animals where the reproductive tract lies cranial to the pelvic inlet (Szenci et al., 1995).

The stage of gestation can be determined by the size of various organs and body parts of the conceptus (Kähn, 1989), with crown-rump-length being the most accurate determinator (White et al., 1985; Hughes and Davies, 1989; Curran et al., 1986). Hughes and Davies (1989) found it possible to correctly detect twin pregnancies at 42 to 49 days of gestation. Müller and Wittkowski (1986) describe sexing of the foetus between 73 and 120 days of gestation with an accuracy of 94%. Pathological conditions identifiable with ultrasound have been described by Kähn and Leidl (1989).

There was no evidence of foetal loss directly attributable to ultrasound examination during early gestation in studies by Hanzen and Laurent (1991), Ball and Logue (1994), and Baxter and Ward (1997). With implantation not being completed until 45 days, scanning cows for pregnancy prior to this carries the risk of animals loosing the conceptus naturally after the pregnancy diagnosis is completed. Re-examination of animals found pregnant at the initial diagnosis is advisable.

Reported sensitivity, specificity, and positive and negative predictive values for pregnancy diagnosis by real-time ultrasonography are summarised in Table 1.2, page 14.

Pregnancy diagnosis using the Doppler method was found useful by Zheng and Wu (1990) with 100% coincidence rate between the Doppler diagnosis and rectal palpation after 51 days of gestation. Several other authors, however, found neither Doppler method nor A-Mode satisfactory, mainly due to poor specificity and length of gestation at which diagnosis was possible (Archibong and Diehl, 1982; Tierney, 1983; Ducker et al., 1985; Cameron and Malmo, 1993; McCaughey and Gilmore, 1990).

Oestrone Sulphate

Oestrone sulphate is produced by the foetal-maternal unit and is the main oestrogen synthesised in placentomes by sulpho-conjugation (Mattiolo et al., 1984). There is also some synthesis by the non-caruncular endometrium and the allanto-chorion (Janszen et al., 1995). Interest in oestrone sulphate started in the 1970's, with its occurrence described in allantoic fluid (Robertson et al., 1978; Robertson and King, 1979) and in the plasma of pregnant pigs and cows (Heap and Hamon, 1979).

Table 1.2 Reported sensitivity, specificity, positive (PPV) and negative (NPV) predictive values for real-time ultrasonography

Method	No. & Type of Animals	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %	DIC	Control	Reference
7.5 MHz	111 (H/ F)	38.1	97.7	95.4	54.6		26-27	repeat US	Szenci et al. 1996
heart beat		89.0	100	100	87.7		33-34	& RP	
		98.1	100	100	97.7		44-45		
		100	100	100	100		53-58		
allantoic fluid		78.1	93.1	93.4	78.1		26-27		
+/- heartbeat		96.3	97.7	98.1	95.6		33-34		
		100	100			100	>39		
5 MHz	471			86.7	75.3		21-45		Lamprecht et al. 1995
	142					97	27-30	RP at 60 days	Bonato et al. 1990
	543			100			>35	RP at 60-90	Chaffaux et al. 1988
	1483				100		>45	days	(similar in 1986)
3.5 MHz	179 (Beef x)	98.8	75	99.4	60	98.4	92-202	Calving	White et al. 1985
5 MHz	85	97.7	87.8	89.6	97.2		26-33	RP at 50-90 days or RtO or Calving	Pieterse et al. 1990
5 MHz	100	82.9	66.7	88.7	55.2		24-26	repeat US	Szenci et al. 1995
		97.4	91.7	97.4	91.7		27-29	& RP at 56-70	
		97.4	95.8	98.7	92		31-33	days	
7.5 MHz	39	80	100	100	57.1		27-32	repeat US & RP	Szenci et al. 1995
		96.6	100	100	90		34-38		
5 MHz	1766 (H/F & BB)	97	74	91	90	91	∅ 40-43	RP30-60 days	Hanzen and Laurent 1991
	57	95	54	88	78	86	<30	later	
	722	95	67	89	84	88	30-39		
	620	98	77	92	94	93	40-49		
	312	98	77	93	95	94	50-59		
	55	97	95	97	95	96	60-70		
3 MHz	320 (Dairy & Beef)	96.9	87.3	94	89.7		∅ 41	RP	Hanzen and Delsaux 1987
	33	92.3	87.5 ?	70.5	75		< 30		
	130	97.8	78.7	92.8	92.8		30-39		
	93	98.6	89.4	97.3	94.4		40-49		
	38	93.9	100	100	71.4		50-59		
	26	93.7	100	100	90.9		> 60		
	299 (H/F)	68.8	71.7	71.1		70.2	23-31	RP at >35 days	Badtram et al. 1991
	22	100	90.1	92.5	100		27-29	RP 3 mths later	Beghelli et al. 1986
5 MHz	85	97.7	87.8	89.6	97.3		26-33	RP	Willemse and Taverne 1989

DIC = Days in Calf   H/F = Holstein/Friesian   BB = Belgian Blue   US = Ultrasonography   RP = Rectal Palpation   RtO = Return to oestrus   ∅ = Average

Oestrone sulphate can be analysed from plasma, serum, faeces and milk of pregnant females of various species, including cattle, buffalo, small ruminants, pigs and horses (Sist et al., 1987a; Fletcher and Worsfold, 1988; Hung and Prakash, 1990; Prakash and Madan, 1993; Szenci et al., 1993; Eissa et al., 1995). When used for pregnancy diagnosis, similar levels of accuracy have been found for plasma (Tainturier et al., 1987), serum (Fletcher and Worsfold, 1988), and faeces (Han Sun Choi, 1987; Sist et al., 1987b). In cows, concentrations in plasma are correlated to those in milk.

It is a hydrophilic substance and over 90% are confined to the whey portion of milk (Heap and Hamon, 1979). Initially, milk analysis involved radio-immunoassays of oestrone after hydrolysis and extraction. Coulson et al. (1981) and Holdsworth and Chaplin (1982) found levels determined by radio-immunoassay of defatted milk similar to those involving hydrolysis and extraction. Henderson et al. (1992) used whole milk successfully.

Enzyme-immunoassays were developed, enabling the processing of large sample numbers in shorter time and with less laboratory equipment. There is a close relation between radio- and enzyme-immunoassays for oestrone sulphate concentrations (Tainturier et al., 1986). The enzyme-immunoassay uses a colour change as endpoint and is based on oestrone sulphate in milk competing with an added oestrone sulphate enzyme conjugate for antibody binding sites. Milk with a high oestrone sulphate concentration will prevent the added enzyme conjugate from binding and the colour change will be less intense.

There is some cross-reaction with oestrone glucuronide and oestrone, but the concentrations of these two hormones is much lower than oestrone sulphate in the maternal circulation during gestation and all three are positively correlated. There is little cross-reaction with progesterone (Tsang et al., 1975; Power et al., 1985).

Non-pregnant cows have circulating oestrone sulphate as well, but concentrations increase as pregnancy progresses (Heap et al., 1983; Tainturier et al., 1986; Henderson, et al., 1994b; Henderson et al., 1995). Henderson et al. (1994a) found oestrone sulphate concentrations in milk measured by enzyme-immunoassay of 0 to 1.3 nmol/l in non-pregnant cows, and of 1.1 nmol/l at 70 to 99 days of gestation and 3.2nmol/l at 140 to 160 days of gestation in pregnant cows. The peak in median oestrone sulphate in milk occurs at 161 to 180 days of gestation at a level of 1ng/ml (Heap and Hamon 1979; Henderson et al., 1993). The highest milk concentration found in non-pregnant cows was 622 pg/ml, and the lowest concentration in pregnant cows at 121 days of gestation was 127 pg/ml in a study by Power et al. (1985).

There is considerable variation in oestrone sulphate levels between and within cows, with transient periods of low concentration possible in pregnant animals, as well as cows presenting with contrasting diagnoses when sampled repeatedly (Heap et al., 1983; Henderson et al., 1993; Henderson et al., 1995). Power et al. (1985) noticed a variation between and within microtitre plates. For pregnancy diagnosis using enzyme-immunoassay of milk, cut-off points between pregnant and non-pregnant cows of 120 pg/ml (Henderson et al., 1993) and 200 pg/ml (Power et al., 1985) have been suggested, with cows ideally more than 100 to 120 days in calf.

Oestrone sulphate concentrations fall rapidly after calving (Tsang et al., 1975; Foote, 1978; Abdo et al., 1991) and can be used to indicate the viability of the foetus (Heap and Hamon, 1979; McCaughey et al., 1982). In cows with a possible *Leptospira hardjo* challenge, oestrone sulphate concentrations decreased after fifteen weeks (Hewitt et al., 1990). However, oestrone sulphate remained high for a few days after induced abortions (Mohamed et al., 1987).

Oestrone sulphate concentration is not influenced by the sex of the foetus (Robertson and King, 1979; Hamon et al., 1981; Bloomfield et al., 1982), birthweight of the calf, or weightloss of the cow during gestation (Bloomfield et al., 1982). Since oestrone sulphate is of placental origin, twin calves may be expected to induce higher oestrone sulphate concentrations due to more placental tissue. Worsfold et al. (1989) and Echternkamp (1992) did find higher oestrone sulphate levels in cows carrying twins, but the increase was not significant and levels could not be used to predict twin pregnancies.

Milk yield or milk composition does not influence oestrone sulphate (Hamon et al., 1981; Bloomfield et al., 1982) and this is reflected in studies by Heap and Hamon (1979) and Henderson et al. (1994b) with only a minor influence of breed or stocking rate. Abdo et al. (1991), however, found a significant difference in oestrone sulphate concentrations between the Swedish Jersey and either the Swedish Red and White or the Swedish Lowland breeds. Dairy cattle management systems prevalent in New Zealand do not influence oestrone sulphate concentrations (Henderson et al., 1994b).

Oestrone sulphate concentrations in milk are slightly increased by freezing (Heap and Hamon 1979) and the sensitivity of the enzyme-immunoassay is increased by incubation of the milk sample at 4°C overnight (Power et al., 1985). Mastitis or oestrus may lead to a wrong pregnancy diagnosis using oestrone sulphate (McCaughey et al., 1982).

Reported sensitivity, specificity, and positive and negative predictive values for pregnancy diagnosis by oestrone sulphate are summarised in Table 1.3, page 18.

Oestrus Observation

Determination of an animal's pregnancy status by observation of its sexual behaviour is probably the oldest method used for pregnancy diagnosis. Return to oestrus after breeding is used as a sign that the cow has not conceived. In seasonal herds, a high milk yield or good body condition compared to the cow's herd mates is also sometimes used as an indication of non-pregnancy. Owner-run herds with no extra farm labour, which generally have herds smaller than the national average, commonly use oestrus observation as their main method for pregnancy diagnosis. Part of the herd, that is classed as 'suspect' by observation or is destined for slaughter, may be presented to the veterinarian for pregnancy diagnosis.

Behaviour during oestrus has been described (Williamson et al., 1972b; Reimers et al., 1985), with a cow standing while being mounted as the most accurate sign. Mounting by the cow herself, blood on the vulva and absent milk letdown are less accurate signs.

Table 1.3 Reported sensitivity, specificity, positive (PPV) and negative (NPV) predictive values for oestrone sulphate

Method	No. & Type of Animals		Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %	DIC	Control	Reference
RIA (whey)	18		83	(none non-	100	3 doubtful		>100	RtO, calving	Hamon et al. 1981
	23		56.5	pregnant)	100	8 doubtful		96-100	RtO, calving	
RIA (milk - defatted)	261		98.4	94.7	97.9	95.9		>126	repeat RP	McCaughey et al. 1982
EIA (milk)	297	150 pg cut-off	99.6	80.7	95.6	97.9	95.3	>120	repeat RP,	Power et al. 1985
		200 pg	99.2	86.0	96.7	96.1	96.0		calving,	
		250 pg	96.7	89.5	97.5	86.4	94.6		slaughter	
RIA (milk -	567		54.2	100	100	29.8		<120	RP,	Henderson et al. 1992
whole)			95.9	100	100	72.5		>120	calving	
EIA	501		62.4	100	100	48.2		<120	not	Henderson et al. 1993
			98.0	100	100	85.4		>120	stated	
EIA	422		85.4	93.8	98.3	60.8		22-178	RP >35 d	Henderson et al. 1995
(Confirm)	Jersey & Friesian		43.1	93.8	86.2	64.7		22-119	& calving	
			97.1	93.8	98.1	91.0		120-178		
? (milk-	53		100	100				110-130	Calving	Kourletaki-
whole)					67.0			100-109	& RtO	Belibasaki et al. 1995
					30.0			90-99		

DIC = Days in calf RIA = Radio-immunoassay EIA = Enzyme – immunoassay RtO = Return to oestrus RP = Rectal palpation

The time frame during which oestrus behaviour is expressed ranges from 2.6 to 26 hours, with an average length of 9.5 to 14 hours (O'Farrell, 1984; Nebel et al., 1992; Stevenson et al., 1996; Walker et al., 1996). During this time, an average of 10 to 14 mounts take place, with only some lasting longer than two seconds (Nebel et al., 1992; Walker et al., 1996). Early morning is the best time to observe cows for oestrus, with one-third to one-half of cows starting oestrus behaviour from midnight onwards (Foote, 1975; Nebel et al., 1992; Stevenson et al., 1996).

The average ability of herdsmen to detect oestrus ranges from 51% to 77% (Williamson et al., 1972a; Esslemont, 1974b; Lehrer et al., 1992; Nebel et al., 1995). Some herdsmen only achieve a 20% detection rate (King et al., 1976; Gaines et al., 1993). These studies used either progesterone profiles or continuous observation to establish true oestrus activity. The 24-day submission rate reflects oestrus detection ability (O'Farrell, 1984; Hayes, 1997), and Esslemont (1992) reports an average 24-day submission rate of 51.9% (range 44.9% to 57.5%) in 91 observed herds.

Pregnant cows may express oestrus behaviour and this can lead to a wrong diagnosis of non-pregnant. Moller et al. (1986) reported pregnancy in 5.6% of 2274 cows that were identified as return to oestrus by the herdsman. Williamson et al. (1972b) observed 8 out of 109 (7.3%) heats in pregnant cows. Using progesterone concentrations on the day of presentation as comparison, between 4.8% and 7.8% of cows identified as expressing oestrus were not in oestrus, with a range of incorrect oestrus detection of 0% to 60% (Reimers et al., 1985; Rajamahendran et al., 1993; Kourletaki Belibasaki et al., 1995).

Between 8.5% and 11.8% of cows presented for breeding were found to have inactive ovaries (Moller et al., 1986; Markusfeld, 1987). Anoestrus after an unsuccessful insemination occurred in 2.1% of 2274 cows in a study by Moller et al. (1986), and Macmillan (1996) reports an incidence of up to 20% of anoestrus in cows inseminated following progesterone treatment. These animals would not be identified as non-pregnant by oestrus observation.

Oestrus detection efficiency can be increased by using combinations of observation and various aids, such as tail paint, rump mounted dye detector

(Kamar®), pedometer, electrical impedance of vaginal mucus, and heifers treated with testosterone (Kilgour et al., 1977; Macmillan and Curnow, 1977; Stevenson and Britt, 1977; Lehrer et al., 1992). Williamson (1980), for example, reports an increase in oestrus detection from 62.1% for observation alone to 96.4% for a combination of observation and tail paint.

A sexually active group needs to be present for cows to show strong oestrus behaviour and Helmer and Britt (1985) suggest that at least 44 cycling cows are needed for two cows to be in oestrus at any time. If the sexually active group consists of more than five to six animals, however, activity may be reduced or the group becomes unstable and splits up (Kilgour et al., 1977; Esslemont et al., 1985). Bulls also inhibit the sexually active group, and oestrus behaviour is suppressed during the feeding of supplements or during yarding of cows for milking (Kilgour et al., 1977). Foote (1975) found that bulls running continuously with cows detected on average 80% of cows in oestrus, with the detection rate decreasing when the bull to cow ratio increased. Mialon et al. (1994) found detection of oestrus using a vasectomised bull for pregnancy diagnosis up to 90 days of gestation as efficient as bovine pregnancy specific protein or progesterone assays.

Most studies on the efficiency of oestrus detection have established the oestrus detection rate for inseminations. Roche et al. (1978), however, looked at oestrus observation for pregnancy diagnosis in 245 heifers and, using the calving records as comparison, found a sensitivity of 94.1%, specificity of 54%, positive predictive value of 65.9%, negative predictive value of 90.7%.

Progesterone

The progesterone concentration in cow's milk is related to the activity of the corpus luteum. If conception does not take place after insemination, progesterone levels will be low 18 to 24 days later, when the subsequent oestrus will normally occur. A high progesterone concentration at this time indicates the presence of a corpus luteum, which is likely to be related to pregnancy (Laing and Heap, 1971). Robertson and Sanda (1971) used plasma progesterone levels for pregnancy diagnosis in several species.

Progesterone tests using milk have been available for pregnancy diagnosis in Germany and the UK since 1975 (Foote, 1978; Booth, 1979). Especially larger herds took up the testing service offered by the Milk Marketing Board in the UK, with 5.6% of all herds using it in 1977 (Booth, 1979). Cow-side tests have become available, and some of these have been reviewed by Nebel (1988) and Smale (1991). Future developments may include immunosensors in the milking unit (van der Lende et al., 1992).

Milk is tested on one or more days in the 18 to 24 day interval after insemination, though two samples (e.g. on Day 21 and 24) did not significantly increase overall accuracy in studies by Heap et al. (1976) and Pennington et al. (1985). Since progesterone secretion is not related to the conceptus, it is only an indirect sign of pregnancy. Most non-pregnant animals are identified correctly, and these can then be targeted for oestrus observation and repeat breeding. False positive diagnoses occur in cows with an abnormally long inter-oestrus interval or a persistent corpus luteum.

In addition, the interpretation of progesterone levels is based on the assumption that the cow was in oestrus when insemination took place. An animal inseminated in dioestrus would be in dioestrus again with an active corpus luteum, and therefore high progesterone levels, at the time of testing. Measuring progesterone concentrations on the day of insemination and 18 to 24 days later increases sensitivity by confirming oestrus at the time of breeding (Günzler et al., 1975). With pregnancy diagnosis taking place prior to implantation, apparently false positive diagnoses will occur due to embryonic mortality. Karagiannidis (1990) found this to be the main cause for a difference in diagnoses between days 21 to 24 and days 60 to 90, accounting for 63.6% of the disagreements. Insemination during the luteal phase, a persistent corpus luteum with or without uterine infection, and irregular oestrus cycles accounted for 20%, 12.7%, and 3.7%, respectively.

Breed has no effect on the accuracy of milk progesterone for pregnancy diagnosis (Pennington et al., 1976), though there is variation between herds (Booth, 1979). Milkfat also influences progesterone concentrations (Günzler et al., 1975).

Reported sensitivity, specificity, and positive and negative predictive values for pregnancy diagnosis using progesterone are summarised in Table 1.4, page 23. Sasser and Ruder (1987) presented a review of studies using progesterone for pregnancy diagnosis.

Other methods

Bovine pregnancy specific protein B (bPSPB)

Laster (1977) isolated a protein from the endometrium and uterine flushings of cows 15 days after insemination, which could not be detected in other body tissues. Further work showed this pregnancy specific protein to be secreted by binucleate cells of the trophoblastic ectoderm or placenta (Reimers et al., 1985; O'Connor, 1994; Xie et al., 1991). A radio-immunoassay was developed in the 1980's (Sasser et al., 1986). Reported sensitivity and specificity range from 60.2% and 91.3%, respectively, at 24 days after insemination, to 99.0% to 100.0% and 90.2% to 94.7%, respectively, between 25 to 35 days after insemination (Maurer et al., 1985; Humblot et al., 1988a). Positive and negative predictive values of 74% to 90.0% and 97.8% to 100.0% in cows 28 days after insemination have been reported (Humblot et al., 1988b; Mialon et al., 1994).

Bovine PSPB has a long half-life of approximately 7 days and is found in the post-partum period for up to 9 weeks (Ruder and Sasser, 1986; Sasser et al., 1991). High levels were also found in cows with endometritis or degenerating foetal tissues (Maurer et al., 1985). It is, therefore, less suitable for the detection of early embryonic death, and false positive results may occur in cows tested less than 80 days after their last calving (Ruder and Sasser, 1986; Sasser et al., 1991; Zoli et al., 1992; Szenci et al., 1996). Age or parity of the cow does not influence bPSPB levels, but breed may have an effect (Maurer et al., 1985). Cows carrying twins showed significantly higher levels than those with a single conceptus (Vasques et al., 1993). Radio-immunoassays generally are costly and fairly labour-intensive, making them less suitable for large scale sample processing.

Table 1.4 Reported sensitivity, specificity, positive (PPV) and negative (NPV) predictive values for progesterone

Method	No. & Type of Animals	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %	DIC	Control	Reference
RIA	213	100	54.5	79.5	100		20	RP at 62-76 DIC	Günzler et al. 1975
RIA	150-170 dairy cows			78-86	88-100		21, 28 & 42		
				80	100		24	RP at 60-90 DIC	Heap et al. 1976
RIA (plasma)	245	95.6	61.5	73.6	93.1	5 d-f	21	calving & RtO	Roche et al. 1978
?	2000 dairy cows			84.5	97		18-24	RP & RtO	Booth et al. 1979
RIA	82			95.2	94.7		38 & 42	calving	Laing et al. 1979
RIA	3014			76.9	93.8		23	RP & RtO	Gowan et al. 1982
RIA	11 herds	(allowed diagnosis 'doubtful')		77.4	97.2	85.8	21	RP or RtO	Pennington et al. 1985
				83.5	95.3	83.4	24		
				84.2	91.7	83.7	21 & 24		
RIA	1974	(allowed diagnosis 'doubtful')		88.6	94		21-24	calving	Reimers et al. 1985
RIA	2975	97.9	43.5	64.9	96	7.6% d-f	21-23	RP at 40-60 DIC	Beghelli et al. 1986
	247	98.3	34.1	62.7	95.4	7.3% d-f	0 & 21-23		
ELISA				67.3	93.9	75.8	21	RP	Nebel et al. 1987
RIA	10 herds			75	94.5	80.5	21	or RtO	
RIA	175 heifers & cows		56.5	67.2	98		24	RP & RtO	Humblot et al. 1988a
RIA	512			81.3	97.1		21-24	RP at 60-90 DIC	Karagiannidis 1990
EIA (cowside)	119	93.1	39.3	59.3	85.7		0 & 21	RP at 50-90 DIC,	Pieterse et al. 1990
RIA	119	86.2	47.5	64.9	96		0 & 21	RtO or calving	
?	4558			88.6	93.9		21	?	Reimers et al. 1990
EIA	50			65.5-68	90-91	76.5-78.6	21	RP at 60 DIC	Dionysius 1991
EIA	Cows			76.5	90.6		18-24		
				91.6	100		25-90		
	Buffalo			71.4	81.8		18-24	RP at 45-50 DIC	Gupta et al. 1991
RIA	472			72	94		21	RP	Rajamahendran et al. 1993
EIA	274	100	72.5	83.1	100	6.6% d-f	21	RtO & calving	Kourletaki-Belibasaki et al. 1995

DIC = Days in calf RIA = Radio-immunoassay EIA = Enzyme-immunoassay RP = Rectal palpation RtO = Return to oestrus d-f = doubtful diagnosis

Bovine pregnancy-associated glycoprotein (bPAG)

Another protein found in the peripheral circulation of pregnanct cows is bPAG or bPAG1 and a radio-immunoassay is used for its detection. Reported sensitivity ranges from 81.8% at 26 to 27 days after insemination to 100% at over 33 days after insemination. Specificity of 54.5% in early pregnancy to 93% for cows at least five weeks pregnant has been reported. Positive and negative predictive values ranged from 69.2% to 97% and 70.5% to 100% in these studies (Zoli et al., 1992; Skinner et al., 1996; Szenci et al., 1996). bPAG does persist for some time in the post-partum period, as well, and false positive diagnoses may occur in cows sampled before 100 days after calving. Ectors et al. (1996) suggests that bPAG may be useful to monitor placental abnormalities, embryonic mortality and abortion.

Early pregnancy factor (EPF)

The paternal part of the developing embryo would be expected to stimulate an immune response in the pregnant female. For implantation to take place, immunosuppression is necessary and EPF is thought to achieve this. EPF inhibits the rosette formation between T lymphocytes and red blood cells and hence the rosette inhibition titre is used to detect it (Morton, 1976 quoted by Sasser and Ruder, 1987). Two fractions have been identified: EPF-A produced by the oviduct during oestrus and pregnancy, and EPF-B produced by the ovary during pregnancy (Morton et al., 1980). EPF-B (or ovum factor or zygotin) requires a signal from the fertilised ovum (Koch et al., 1983). It has been found to be present throughout pregnancy in the sow, but was only found between 72 hours and 16 weeks after insemination in the ewe (Morton, 1979 quoted by Shaw and Morton, 1980). Nancarrow et al. (1981) has described its use in cows and reviews are given by Shaw and Morton (1980) and Koch and Ellendorff (1985).

The rosette inhibition titre is very time consuming. Threlfall and Bilderbeck (1999), using an early conception factor (ECF) dipstick test on serum samples 6 to 15 days after insemination, achieved sensitivity, specificity, and positive and negative predictive values of 91.0%, 46.4%, 59.2% and 85.8%, respectively. Using milk, higher values were achieved. Much lower values are reported by Adams and Jardon (1999), using a similar test at 3 to 7 days after breeding.

Re-testing of cows diagnosed pregnant with a very early test like this is advisable, as pregnancies will be lost due to early embryonic death.

Miscellaneous methods

In the presence of a corpus luteum, a non-luteolytic dose of prostaglandin $F_{2\alpha}$, causes luteal oxytocin release leading to milk ejection after a quarter has been drained of milk. This method achieved a higher positive predictive value than progesterone assay at 18 to 22 days after insemination (72.3% vs. 68.6%). The negative predictive value was slightly lower (93.5% vs. 100%; Labussiere et al., 1992).

Nuclear magnetic resonance spectra of the cervical mucus showed a 98% and 94% effectiveness in pregnancy diagnosis at 5 to 50 days after breeding (Merilan, 1982). Electronic probe measurements of the cervical-vaginal mucus did not proof useful to detect oestrus and was found to be labour-intensive (Cavestany and Foote, 1985). Electrical conductance of ovaries and uterine horns varied depending on months of gestation (Petrov, 1994).

Externally conducted electrocardiograms will detect on average 4 out of 5 pregnancies, with low detection rates in the first two trimesters. This method is more suitable for assessing foetal health than diagnosing pregnancy (Bosc and Chupin, 1975; Mansfeld and Grunert, 1989).

Measuring serum alkaline phosphatase activity for pregnancy diagnosis in early gestation cows has also been reported (Wu XianShu et al., 1993).

Conclusion

Pregnancy diagnosis should form an integral part of herd reproductive management. The available and commonly used methods of pregnancy diagnosis, like rectal palpation, real-time ultrasonography, and progesterone and oestrone sulphate assays, each have advantages and disadvantages in terms of stage of gestation when applicable, reliability and accuracy, cow welfare, equipment or training needed, costs, and information detail provided, and the literature review has shown this. There is a constant quest for refining existing methods and developing alternatives. Major advances have been made with the development of immunoassays, especially enzyme-based assays that allow processing of large number of samples. The inherent difficulty with establishing accuracy of pregnancy diagnosis lies in the absence of a reliable gold standard or control, and the reviewed studies demonstrate this by employing various, and often concurrent, techniques. Foetal loss, which results in an apparent number of misdiagnoses, adds to the problem.

Acceptance of pregnancy diagnosis by the herd owner or manager as the important management tool it is depends on the accuracy, convenience and cost-effectiveness of the procedure. To establish the true value of a method, it has to be tested in the particular management situation in which it would be applied. The seasonal, grass-based production system prevalent in New Zealand dairy herds is unique in many aspects and places its own requirements on pregnancy diagnosis. No study was found that compared oestrone sulphate with rectal palpation, ultrasonography, and farmers' observation in New Zealand dairy herds. Extrapolating data from other studies would not do justice to the methods under investigation. The literature review has stimulated a desire to contribute to the knowledge about methods of pregnancy diagnosis as applicable under New Zealand management systems.

CHAPTER 2

COMPARISON OF MILK OESTRONE SULPHATE WITH RECTAL PALPATION AND REAL-TIME TRANSRECTAL ULTRASONOGRAPHY FOR PREGNANCY DIAGNOSIS

Introduction

Pregnancy diagnosis is considered an essential component of reproductive management (Williamson, 1994). Rectal palpation is one of the main methods used in cattle and real-time ultrasonography has been used extensively since the late 1970's. Both methods are invasive and require skilled examiners, with some countries restricting the procedure to qualified veterinarians. Alternative methods of pregnancy diagnosis utilising hormone concentrations in milk would avoid potential cow discomfort and the need for skilled people (at point of sampling). Oestrone sulphate in milk, with a reported high positive predictive value (Hamon et al., 1981; McCaughey et al., 1982; Power et al., 1985; Henderson et al., 1992; Henderson et al., 1993; Henderson et al., 1995), is potentially suited to the seasonal dairy system in New Zealand, where pregnancy diagnosis is commonly carried out several weeks after the end of the breeding period and the main decision based on the results of pregnancy diagnosis is culling. Most farms are enrolled in periodical herd milk testing (for example, 87.2% of herds in the 1996-97 season, with 89.6% of cows tested at least once; Livestock Improvement Corporation, 1997a).

For oestrone sulphate to have a place in pregnancy diagnosis, it must have accuracy similar to currently used methods. Materials, methods and presentation of results of studies looking at sensitivity and specificity for rectal palpation, ultrasonography and oestrone sulphate vary widely, making a comparison based on reported data impossible. The most profound difference lies in the method of control used, varying from calving records (Meacham et al., 1976; Vaillancourt et al., 1979; White et al., 1985; White et al., 1989b) to repeat examinations by the same (Abbitt et al., 1978; Beghelli et al., 1986; Szenci et al., 1996) or a different method (McCaughey et al., 1982; Chauffaux et al., 1988; Bonato et al., 1990; Hanzen and Laurent, 1991; Alexander et al., 1995), to combinations of control methods including 'return to oestrus' (Power et al., 1985; Pieterse et al., 1990; Badtram et al., 1991; Henderson et al., 1995; Kourletaki-Belibasaki et al., 1995; Warnick et al., 1995). No study was found that compares all three methods of pregnancy diagnosis.

The current study was aimed at assessing the accuracy of oestrone sulphate in milk for pregnancy diagnosis in dairy cattle, while comparing it to rectal palpation and ultrasonography. The study was not aimed at developing or refining an oestrone sulphate test, but to evaluate a currently marketed testing kit. Equally, the study did not intend to establish definitive values for sensitivity and specificity, but to compare the three methods relative to each other in the same cow population.

Materials and Methods

Pregnancy status of cows in six commercial, spring-calving dairy herds in the Manawatu region of New Zealand was established using rectal palpation, ultrasonography and oestrone sulphate concentrations in milk. Herds were chosen from those clients of the Massey University Farm Service Clinic who annually presented their entire herd for pregnancy diagnosis and who had an accurate recording system in place. Pregnancy diagnosis was carried out between 137 and 180 days after the start of mating. Results for the three methods were obtained within eight days of each other in herds 1, 3, 4 and 5. In herds 2 and 6, milk collection took place 35 and 34, and 19 and 42 days before rectal palpation and ultrasonography, respectively. Table 2.1, page 29, shows the dates of examination in relation to the start of artificial insemination, start of natural mating and end of mating for the herds involved. The ear tag number was used to identify cows at all examinations and for follow up.

Rectal palpation

Cows in the six herds were palpated by one of two veterinarians, with the palpator's identity recorded for each cow. Cows were allocated haphazardly to either of the two palpators in that, after entering the examination area, whichever cow stood in front of the palpator was examined by that person. A diagnosis of pregnant, non-pregnant or recheck was recorded by an assistant.

Table 2.1 Dates of breeding management and pregnancy diagnosis and days between events

Dates of Events	Herd Number 1	2	3	4	5	6
Start of artificial insemination (AI)	20-Oct-96	20-Oct-96	25-Oct-96	21-Oct-96	29-Oct-96	10-Oct-96
Start of natural mating (NM)	05-Dec-96 [#]	no NM	13-Dec-96	01-Dec-96	07-Dec-96	09-Dec-96
End of mating	01-Feb-97	01-Jan-97	10-Jan-97	07-Jan-97	20-Jan-97	06-Jan-97
Rectal palpation	09-Apr-97	10-Apr-97	14-Mar-97	11-Apr-97	23-Apr-97	08-Apr-97
Ultrasonography	16-Apr-97	17-Apr-97	21-Mar-97	03-Apr-97	18-Apr-97	24-Mar-97
Milk collection	09-Apr-97	06-Mar-97	18-Mar-97	03-Apr-97	21-Apr-97	05-Mar-97
Days Between Events						
Start of AI to rectal palpation	171	172	140	172	176	180
Start of AI to ultrasonography	178	179	147	164	171	165
Start of AI to milk collection	171	137	144	164	174	146
End of mating to rectal palpation	67	99	63	94	93	92
End of mating to ultrasonography	74	106	70	86	88	77
End of mating to milk collection	67	64	67	86	91	58

[#] Two cows served by natural mating before this date; 38 cows served with AI after this date

Real-time ultrasonography examination

An experienced local commercial ultrasonography operator examined cows in the six herds by transrectal real-time ultrasonography using an Aloka SSD500 with a 3.5 MHz linear transducer. In most cows only the probe was introduced rectally, supported by an extender[1]. A diagnosis of pregnant, non-pregnant or recheck was recorded by the operator's assistant.

Oestrone sulphate determination

Milk collection

For analysis of oestrone sulphate, milk collected during a routine herd improvement test was used in herds 3 and 5. A composite milk sample of approximately 250 ml was taken from each cow at two consecutive milkings with in-line collectors by technicians of the Livestock Improvement Corporation (LIC). The samples were transferred into 150 ml plastic pots containing 200 μl of 10% Bronopol[2]. Samples were stored at ambient temperature during collection and transport to the local LIC office. Further storage and transport to the National Milk Analysis Centre, Hillcrest, was at 4° to 7° Celsius.

In herds 1, 2, 4, and 6, milk was collected manually at one milking. Before cup attachment a composite milk sample of 10 to 30 ml was taken of each cow into 30 ml plastic pots containing 100 μl of 10% Bronopol. All four quarters were used for the sample in most cows. Mastitis, blind teats, dry quarters or nervous animals were reasons for not sampling a particular quarter. The cow's eartag number was written on the lid of the sample container. Adhesive labels showing herd and cow identification were applied to the samples after milking had finished. Storage and transport of samples were the same as above.

Milk analysis

Oestrone sulphate concentrations of whole milk samples were established by enzyme-immunoassay at the National Milk Analysis Centre, Hillcrest, using the

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[1] An extender consists of a semi-rigid tube to support the probe head and adjacent 40 cm of the lead

[2] Bronopol Boots. The Boots Company, Nottingham, United Kingdom

Confirm™ pregnancy test[3]. Prior to the test all reagents, samples and plates were warmed to room temperature. 100 µl of milk was mixed with 300 µl of diluted horseradish peroxidaseconjugate, and 200 µl of this mix transferred into the EIA plate. After incubation of the plates at 2° to 8° Celsius for two hours, plates were emptied and washed four times with 250 µl wash solution. After adding 100 µl of substrate solution, plates were incubated at room temperature for 30 minutes, before 100 µl of a stop solution was applied. The optical density (OD) was read at 490nm in an EIA plate reader. OD results of the samples were compared with the cow cut-off standard for each well. Samples with OD readings below the standard were declared 'confirmed pregnant', samples with OD readings above the standard were declared 'not confirmed pregnant'. The cut-off standard was set at 10 pg oestrone sulphate in the well, equivalent to 200 pg oestrone sulphate per millilitre of milk.

Gold Standard

Cows remaining in the herd

For cows remaining in the herd until the end of gestation, the herds' calving records were used for confirmation of pregnancy status in the first instance. Further information on the actual event was sought from the owner for cows with no entry in the herd records or cows with discrepancies between the recorded calving outcome and one of the test results.

Owners were asked to record abortions where observed, but no special inspection of cows or their environment took place. Owners were also asked to record premature calvings and cases where induction of calving was utilised.

Cull cows

The author and/or one of two technicians were present at the abattoir when cows were slaughtered. Ear tag number and relating slaughter line number were recorded after jugular sticking. Once the viscera had been removed by the slaughtermen, the reproductive tract was palpated. The foetal curved crown-rump length (CRL)[4] was

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[3] ICP (Immuno-Chemical Products Ltd), P.O. Box 1607, Auckland, New Zealand

[4] Following the body outline from forehead to tailbase (Lyne, 1960)

measured through the uterine wall in pregnant cows. Reproductive tracts appearing non-pregnant on palpation were individually bagged and labelled, and later incised to establish a definite pregnancy status. The pregnancy status of tracts from *condemned* carcasses was established by palpation only.

Cows sold

No gold standard result could be obtained from these cows.

Calculation of conception dates

a) Cows with full term pregnancies

Calculated conception dates were defined as calving date minus 282 days[5] for cows that remained in the herd and had a calf at full term. These calculated conception dates were compared to recorded service dates. Where the discrepancy between recorded service date and calculated conception date was twelve days or less, the recorded service date was taken as actual conception date. Where the discrepancy was 13 days or more and the calculated conception date fell into the natural mating period for that farm, this calculated date was used as actual conception date. Where the discrepancy was 13 to 21 days and the calculated conception date fell into the artificial insemination period for that farm, the recorded service date was used as actual conception date. For cows with completely disagreeing recorded service and calculated conception dates, the actual conception date was omitted.

b) Cows with induced parturitions

Initially, the conception date was calculated as induced calving date minus 282 days. The last recorded service was used as actual conception date for cows where this was later than the calculated conception date. Where the last recorded service date fell before the calculated conception date, multiples of 21 days were added to the last service date, until the new service date was later than the calculated conception date. Where the difference between calculated conception date and last

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[5] Based on average gestation length in New Zealand dairy cattle established by Ward and Castle (1947) and Macmillan and Curnow (1976). The Livestock Improvement Corporation uses 282 days gestation length to calculate Planned Start of Calving.

recorded service date was seven days or less, the calculated conception date was used as actual conception date.

c) Cows with abortions

The last recorded service date was used as the actual conception date for cows with a recorded abortion.

d) Cows with premature calvings

The last recorded service date was used as the actual conception date for cows with a premature calving.

e) Cows pregnant at slaughter

The curved CRL (see page 31) of the foetus was measured through the uterine wall. Using the formula of Richardson et.al. (1990), a conception date was calculated from the CRL and compared to recorded service dates. The closest recorded service date was used as actual conception date where the discrepancy between calculated conception date and service date was twelve days or less. Where the discrepancy was 13 days or more, the calculated conception date was used as actual conception date if it fell into the period of natural mating for that farm. The conception date was omitted where the calculated conception date fell into the artificial insemination period and no service date had been recorded within twelve days or less. For two cows, no CRL was recorded and the actual conception date was omitted.

Calculation of fertility parameters

21-day submission rate

All cows that had calved at least 42 days before the planned start of mating for each herd were included in the 21-day submission rate. The rate was calculated as the number of cows served on or before the target date (planned start of mating plus 21 days) divided by the total number of cows eligible for service during those 21 days, times 100 (MAFF, 1984; Williamson, 1998).

Pregnancy rate

The gold standard results were used for final pregnancy status. Calculations were based on all cows with a service date, including those without a result for the gold standard and cull cows. For pregnancy rate calculation, it was assumed that cows conceived to their last service.

Pregnancy rate to first service

This is defined as the number of first services which result in a pregnancy and was calculated as number of cows pregnant to their first service divided by the total number of cows receiving a first service, multiplied by 100 (MAFF, 1984; Williamson, 1998).

Overall pregnancy rate

This is defined as number of cows pregnant expressed as percentage of total number of services and was calculated as number of cows pregnant, divided by the total number of services multiplied by 100 (MAFF, 1984).

Services per pregnancy

This was calculated as the total number of services given, divided by the total number of pregnant cows (MAFF, 1984). Services to cull cows were included.

Percent non-pregnant of cows served

This was calculated as number of non-pregnant cows (based on gold standard result) at the end of the breeding period expressed as a percentage of cows in the herd at the start of the breeding period (Williamson, 1998). Only cows with a gold standard result were included.

Comparison and statistics

Results were grouped into correct diagnosis pregnant (a), incorrect diagnosis pregnant (b), correct diagnosis non-pregnant (c), and incorrect diagnosis non-pregnant (d). Sensitivity was calculated as $[a \div (a+d)] \times 100$, specificity as $[c \div (c+b)] \times 100$, positive predictive value as $[a \div (a+b)] \times 100$, and negative predictive value as $[c \div (c+d)] \times 100$.

Sensitivity and specificity for the three methods were compared statistically by fitting a binomial generalised linear model using the repeated measures facility in Proc Genmod of the SAS system, with cows grouped into ten-day periods for days in calf or days since last service. The same model was used to compare the probability of a correct diagnosis for each cow depending on either herd or diagnosis method, and to compare the two veterinarians. Successive models were fitted and changes in deviance assessed to establish whether the Herd-Test interaction was significant. A chi-square test (Microsoft Excel) was used to compare oestrone sulphate sensitivity and specificity of the two herds where milk collection had taken place much earlier than rectal palpation and ultrasonography to those where all three methods were carried out within eight days of each other. A chi-square test was also used to test for differences in oestrone sulphate results between manual and in-line milk collection.

Oestrone sulphate as screening test

Data was analysed under the theoretical scenario of using oestrone sulphate as a screening test. Cows diagnosed as non-pregnant by oestrone sulphate were assumed to have undergone re-examination by either rectal palpation or ultrasonography. That is, cows declared pregnant by oestrone sulphate were regarded as such. For cows declared non-pregnant by oestrone sulphate, the diagnosis of either rectal palpation or ultrasonography was used (in two scenarios). Sensitivity, specificity, and positive and negative predictive values were calculated as described above.

Results

Numbers examined

Table 2.2, page 36, shows the total number of cows in each herd, the number of cows for which pregnancy diagnosis results were available and the number of cows with a result for all three diagnostic methods and the gold standard. There were 2402 cows present in the six herds at the start of the study. About 10% of cows were lost from the study because of incomplete results for diagnostic methods and / or gold standard. About one-third (76/263) of losses arose from a combination of no follow-up of culled cows (31 cases), sale of cows (39 cases; 33 from herd 5) and deaths (six cases; pregnancy status was established during a post-mortem examination in two other deaths).

Table 2.2 Number of cows with results for each herd

Herd	Total number of cows in herd	Cows with result for all three methods		Cows with full set of results*		Total number of cows culled from herd		Cows with full set of results of culled cows		Total number of cows retained in herd		Cows with full set of results of retained cows	
	No.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	759	730	96.2	718	94.6	51	6.7	37	72.5	708	93.3	681	96.2
2	302	257	85.1	257	85.1	63	20.9	46	73.0	239	79.1	211	88.3
3	442	415	93.9	410	92.8	85	19.7	76	87.4	357	80.8	334	93.6
4	249	221	88.8	206	82.7	51	20.5	30	58.8	198	79.5	176	88.9
5	285	266	93.3	233	81.8	43	15.1	10	23.3	242	84.9	223	92.1
6	365	320	87.7	315	86.3	67	18.4	54	80.6	298	81.6	261	87.6
Total	**2402**	**2209**	**92.0**	**2139**	**89.1**	**360**	**15.0**	**253**	**70.3**	**2042**	**85.0**	**1886**	**92.4**

* Full set of results = Result for all three diagnostic methods and gold standard

Just under 90% of cows with full results were pregnant. For the majority of pregnant cows the gold standard was derived from calving records (96.1%). For three-quarters of non-pregnant cows the gold standard was derived from examination of the reproductive tract after slaughter. Just over one tenth of cows were culled from the herds and 70% of these were non-pregnant. Most cows remaining in the herds were pregnant (97.1%). Table 2.3, page 38, shows the number of cows pregnant or non-pregnant and the source of the gold standard result for each herd.

Sensitivity and specificity

Overall results

Overall sensitivity, specificity, and positive and negative predictive values for all cows in the six herds are shown in Table 2.4, page 39.

No pregnant cow was misdiagnosed as non-pregnant by rectal palpation. Two cows were incorrectly diagnosed as non-pregnant by ultrasonography and 346 by oestrone sulphate (see Table 2.5, page 39). Rectal palpation was marginally more sensitive than ultrasonography (p=0.06), and both methods showed higher sensitivity than oestrone sulphate (p=0.0001).

All three methods diagnosed non-pregnant cows as pregnant, but specificity of both rectal palpation and ultrasonography was significantly higher than that of oestrone sulphate (p=0.0001), with no significant difference between rectal palpation and ultrasonography (p=0.38) (see Table 2.4, page 39). Ten cows were incorrectly diagnosed as pregnant by all three methods and a further ten cows by two of the three methods (see Table 2.5, page 39).

The positive predictive value of oestrone sulphate was close to that of rectal palpation and ultrasonography, but the negative predictive value of oestrone sulphate was very low at 35.1%.

Table 2.3 Numbers of pregnant or non-pregnant cows with full set of results* and derivation of gold standard

Herd	Total	Cows pregnant on gold standard		Pregnant cows with gold standard derived from calving records		Cows non-pregnant on gold standard		Non-pregnant cows with gold standard derived from abattoir	
	No.	No.	%	No.	%	No.	%	No.	%
1	718	642	89.4	639	99.5	76	10.6	34	44.7
2	257	216	84.0	211	97.7	41	16.0	41	100.0
3	410	356	86.8	331	93.0	54	13.2	49	90.7
4	206	201	97.6	175	87.1	5	2.4	4	80.0
5	233	222	95.3	216	97.3	11	4.7	4	36.4
6	315	270	85.7	261	96.7	45	14.3	45	100.0
Total	**2139**	**1907**	**89.2**	**1833**	**96.1**	**232**	**10.8**	**177**	**76.3**

* Full set of results = Result for all three diagnostic methods and gold standard

Table 2.4 Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c), incorrect non-pregnant (d), sensitivity, specificity, and positive (PPV) and negative (NPV) predictive value for the three methods

Method	a	b	c	d	Sensitivity	Specificity	PPV	NPV
Oestrone sulphate	1560	44	188	347	81.8	81.0	97.3	35.1
Rectal palpation	1907	20	212	0	100.0	91.4	99.0	100.0
Ultrasonography	1905	21	211	2	99.9	90.9	98.9	99.1

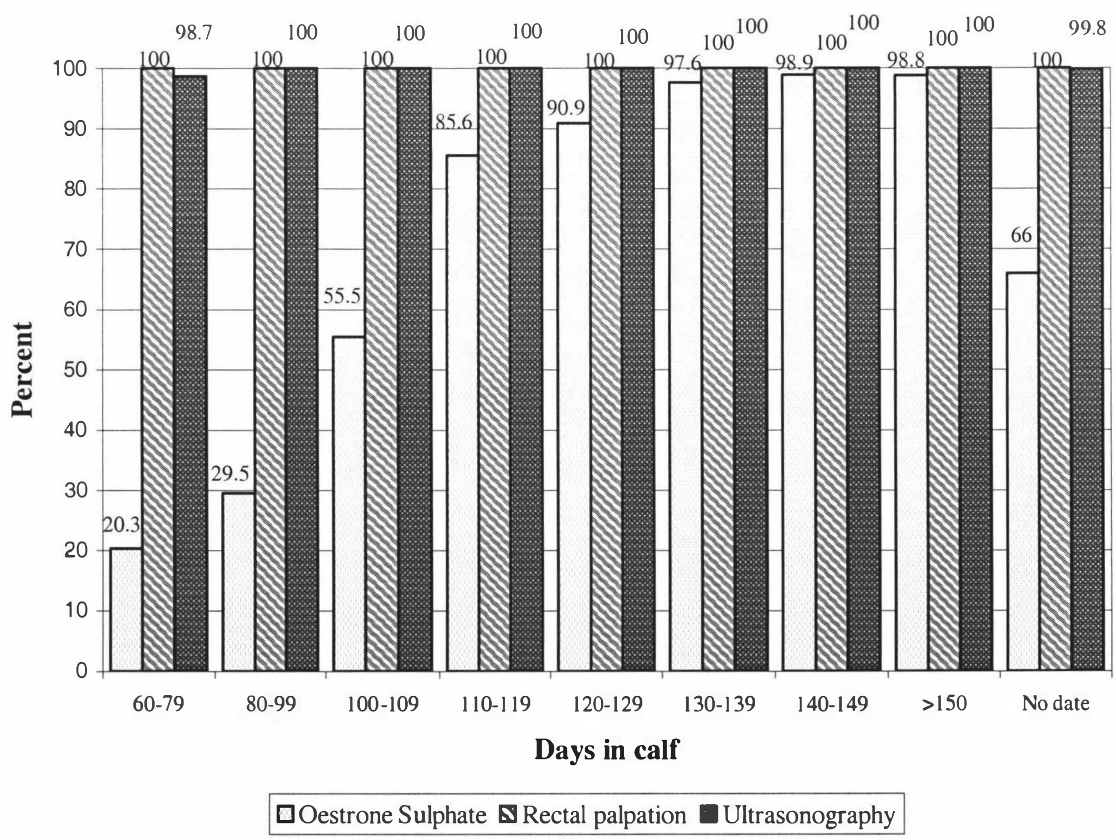
Table 2.5 Comparison of diagnosis results for pregnant (P) and non-pregnant (MT) cows

	Number of cows	Oestrone sulphate	Rectal palpation	Ultrasonography
Pregnant cows	1559	P	P	P
	1	P	P	MT
	0	P	MT	P
	0	P	MT	MT
	0	MT	MT	MT
	0	MT	MT	P
	1	MT	P	MT
	346	MT	P	P
Non-pregnant cows	177	MT	MT	MT
	4	MT	MT	P
	3	MT	P	MT
	4	MT	P	P
	10	P	P	P
	3	P	P	MT
	3	P	MT	P
	28	P	MT	MT

Results by days in calf or since last service.

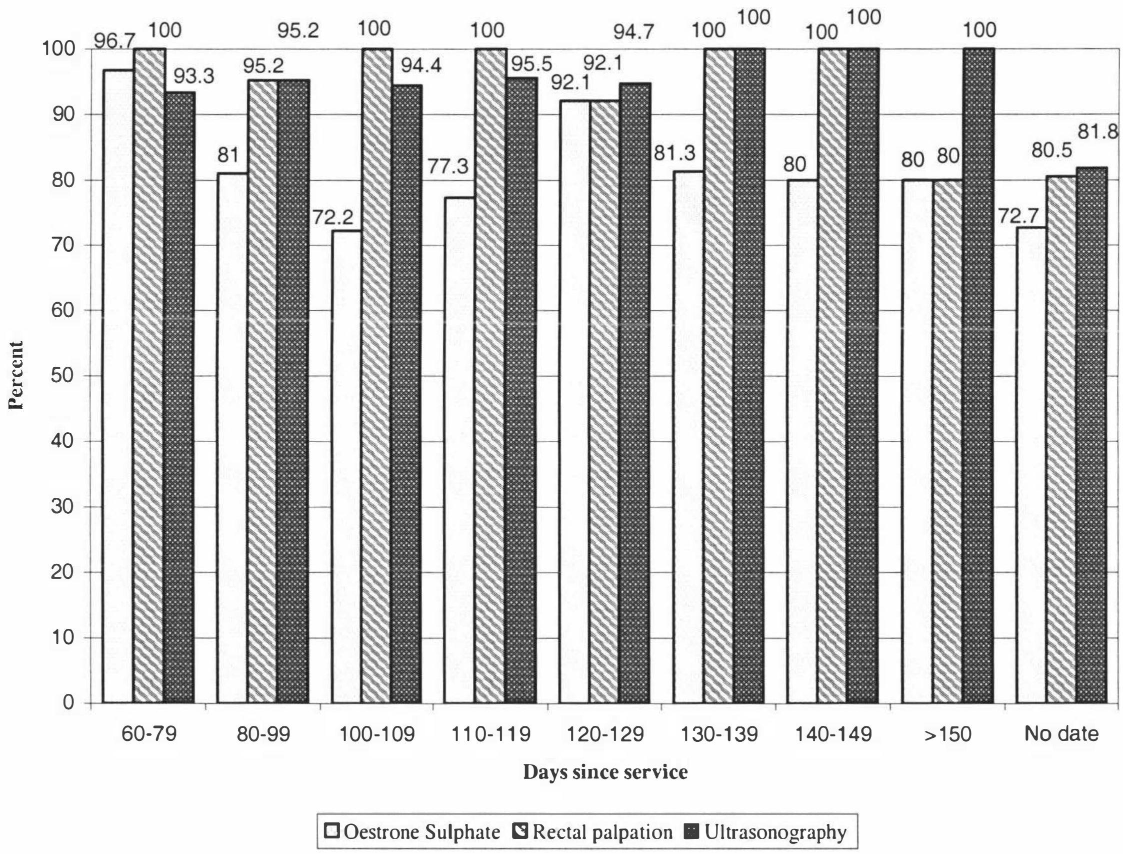
Sensitivity, specificity, and positive and negative predictive values were calculated with cows grouped according to their stage of gestation at the time of milk collection and for cows at least 120 days pregnant or 120 days since their last service (Tables 2.6, page 43, 2.7, page 44, and 2.8, page 44). Oestrone sulphate sensitivity increased in a linear fashion with advancing stage of gestation, reaching a plateau for cows 140 or more days in calf, with no significant increase in sensitivity beyond this stage (Figure 2.1, below). For cows at least 120 days pregnant, oestrone sulphate sensitivity was improved by 15% (96.8% vs. 81.8%), but was still inferior to rectal palpation or ultrasonography (p=0.0001). Days in calf had no influence on the sensitivity of either rectal palpation or ultrasonography and there was no significant difference between these two methods.

Figure 2.1 Sensitivity of the three methods according to days in calf at time of milk collection



Specificity of oestrone sulphate varied across days since last service, but no particular pattern was obvious (Figure 2.2, below). Specificity for rectal palpation and ultrasonography was fairly constant. Specificity for all three methods was improved for cows tested at least 120 days since their last service, but days since last service did not affect the probability of a correct diagnosis for non-pregnant cows.

Figure 2.2 Specificity of the three methods according to days since last service at time of milk collection



The positive predictive value of all three methods was improved for this subset of cows. The negative predictive value for ultrasonography was reduced by 0.5% (Figures 2.3 and 2.4, page 42).

Figure 2.3 Positive predictive value of the three methods according to days in calf at time of milk collection

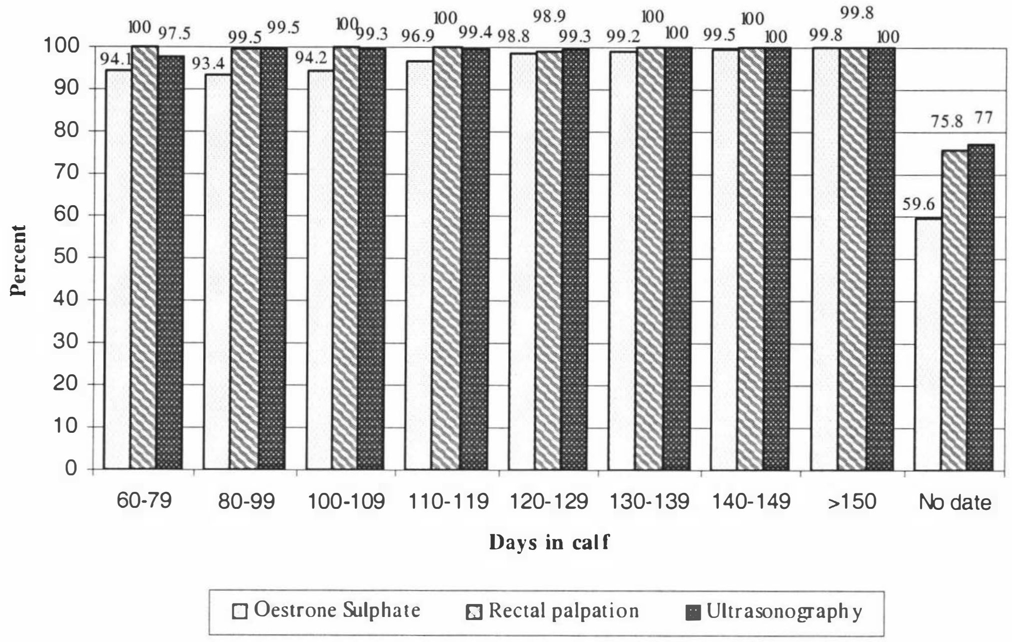


Figure 2.4 Negative predictive value of the three methods according to days since last service at time of milk collection

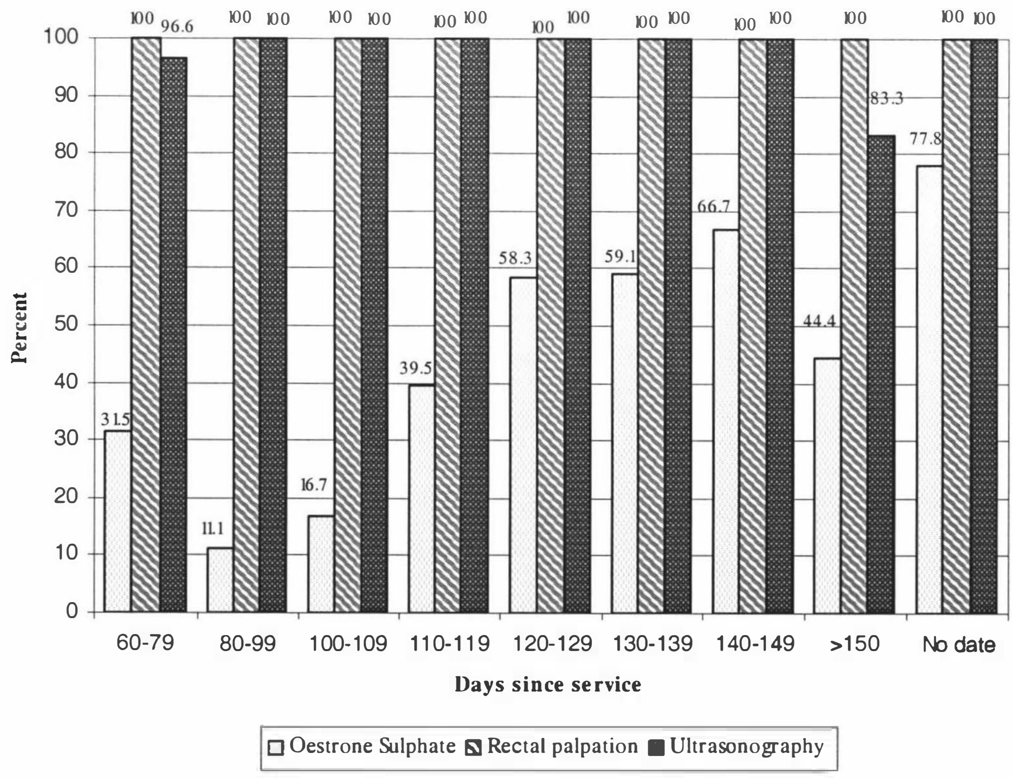


Table 2.6 Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c) and incorrect non-pregnant (d) for the three methods according to days in calf or since last service at time of milk collection

Days in calf / since last service	Method	a	b	c	d
60-79	109 cows: 79 pregnant, 30 non-pregnant				
	Oestrone sulphate	16	1	29	63
	Rectal palpation	79	0	30	0
	Ultrasonography	78	2	28	1
80-99	214 cows: 193 pregnant, 21 non pregnant				
	Oestrone sulphate	57	4	17	136
	Rectal palpation	193	1	20	0
	Ultrasonography	193	1	20	0
100-109	164 cows: 146 pregnant, 18 non-pregnant				
	Oestrone sulphate	81	5	13	65
	Rectal palpation	146	0	18	0
	Ultrasonography	146	1	17	0
110-119	202 cows: 180 pregnant, 22 non-pregnant				
	Oestrone sulphate	154	5	17	26
	Rectal palpation	180	0	22	0
	Ultrasonography	180	1	21	0
120-129	312 cows: 274 pregnant, 38 non-pregnant				
	Oestrone sulphate	249	3	35	25
	Rectal palpation	274	3	35	0
	Ultrasonography	274	2	36	0
130-139	388 cows: 372 pregnant, 16 non-pregnant				
	Oestrone sulphate	363	3	13	9
	Rectal palpation	372	0	16	0
	Ultrasonography	372	0	16	0
140-149	193 cows: 188 pregnant, 5 non-pregnant				
	Oestrone sulphate	186	1	4	2
	Rectal palpation	188	0	5	0
	Ultrasonography	188	0	5	0
≥ 150	433 cows: 428 pregnant, 5 non-pregnant				
	Oestrone sulphate	423	1	4	5
	Rectal palpation	428	1	4	0
	Ultrasonography	427	0	5	1
No date	124 cows: 47 pregnant, 77 non-pregnant				
	Oestrone sulphate	31	21	56	16
	Rectal palpation	47	15	62	0
	Ultrasonography	47	14	63	0

Table 2.7 Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c), incorrect non-pregnant (d), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for cows at least 120 days in calf or since their last service

Method	a	b	c	d	Sensitivity	Specificity	PPV	NPV
Oestrone sulphate	1221	8	56	41	96.8	87.5	99.3	57.7
Rectal palpation	1262	4	60	0	100.0	93.8	99.7	100.0
Ultrasonography	1261	2	56	1	99.9	96.9	99.8	98.4

Table 2.8 Comparison of diagnosis results for pregnant cows at least 120 days in calf and non-pregnant cows at least 120 days since their last service at the time of milk collection (P = pregnant, MT = non-pregnant)

	Number of cows	Oestrone sulphate	Rectal palpation	Ultrasono-graphy
Pregnant cows	1220	P	P	P
	1	P	P	MT
	0	P	MT	P
	0	P	MT	MT
	0	MT	MT	MT
	0	MT	MT	P
	0	MT	P	MT
	41	MT	P	P
Non-pregnant cows	53	MT	MT	MT
	1	MT	MT	P
	1	MT	P	MT
	1	MT	P	P
	0	P	P	P
	2	P	P	MT
	0	P	MT	P
	6	P	MT	MT

Results by herds

Sensitivity, specificity, and positive and negative predictive value for each herd are shown in Table 2.9, page 46. Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c) and incorrect non-pregnant (d) diagnoses for each herd are shown in Table 2.10, page 47. There were significant differences in the likelihood of a correct diagnosis of pregnant between herds, even after differences in days in calf had been taken into account ($p<0.001$). Pregnant cows in herd 2 were least likely to be diagnosed correctly. Probability for a correct diagnosis increased in the order of herd 4 and 6 (no difference), 1, 3, and 5 ($p<0.001$). The herd-test interaction was significant ($P<0.003$), but the effect was small in comparison with the major effects like difference between oestrone sulphate and the other two methods, the effect of days in calf and the overall difference between herds. Similar results were obtained when including only cows that were at least 120 days in calf in the analysis.

Initial analysis showed a significant difference in specificity between herds ($p<0.001$), with the probability of a correct (i.e. non-pregnant) diagnosis being lowest in herd 5, slightly better in herds 3 and 4, and best in herds 1,2 and 6. Further analysis, to establish whether this was due to a herd influence (i.e. all three methods affected) or a herd-test interaction, showed that the effectiveness of oestrone sulphate, relative to the other two methods, varied significantly between herds ($p<0.01$). Oestrone sulphate was significantly less accurate (i.e. more likely to give an incorrect diagnosis of pregnant) in herds 3 and 5 ($p<0.01$), and marginally less accurate in herd 1 ($p=0.057$). All three methods were less likely to give a correct (i.e. non-pregnant) diagnosis in herd 4 ($p<0.01$), but there was no difference in overall effectiveness between rectal palpation and ultrasonography ($p=0.67$).

Table 2.9 Sensitivity, specificity, and positive and negative predictive values for oestrone sulphate (OS), rectal palpation (RP), and ultrasonography (US) for each herd.

Herd	Sensitivity			Specificity			Positive predictive value			Negative predictive value		
	OS	RP	US	OS	RP	US	OS	RP	US	OS	RP	US
1	85.8	100.0	100.0	84.2	92.1	92.1	97.9	99.1	99.1	41.3	100.0	100.0
2	60.2	100.0	100.0	95.1	95.1	95.1	98.5	99.1	99.1	31.2	100.0	100.0
3	84.8	100.0	99.7	63.0	87.0	88.9	93.8	98.1	98.3	38.6	100.0	98.0
4	88.1	100.0	99.5	80.0	80.0	60.0	99.4	99.5	99.0	14.3	100.0	75.0
5	98.6	100.0	100.0	27.3	72.7	81.8	96.5	98.7	99.1	50.0	100.0	100.0
6	67.0	100.0	100.0	97.8	97.8	93.3	99.5	99.6	98.9	33.1	100.0	100.0

Herd 2 and 6: Milk collection approximately one month prior to rectal palpation and ultrasonography
Herd 3 and 5: Milk collection during routine herd test
Herd 1, 2, 4 and 6: Manual milk collection

Table 2.10 Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c) and incorrect non-pregnant (d) for each method and herd

Herd	Method	a	b	c	d
1	718 cows: 642 pregnant, 76 non-pregnant				
	Oestrone sulphate	551	12 [a]	64	91
	Rectal palpation	642	6 [a]	70	0
	Ultrasonography	642	6 [a]	70	0
2	257 cows: 216 pregnant, 41 non-pregnant				
	Oestrone sulphate	130	2	39	86
	Rectal palpation	216	2	39	0
	Ultrasonography	216	2	39	0
3	410 cows: 356 pregnant, 54 non-pregnant				
	Oestrone sulphate	302	20 [b]	34	54
	Rectal palpation	356	7 [b]	47	0
	Ultrasonography	355	6 [b]	48	1
4	206 cows: 201 pregnant, 5 non-pregnant				
	Oestrone sulphate	177	1 [c]	4	24
	Rectal palpation	201	1 [c]	4	0
	Ultrasonography	200	2 [c]	3	1
5	233 cows: 222 pregnant, 11 non-pregnant				
	Oestrone sulphate	219	8 [d]	3	3
	Rectal palpation	222	3 [d]	8	0
	Ultrasonography	222	2 [d]	9	0
6	315 cows: 270 pregnant, 45 non-pregnant				
	Oestrone sulphate	181	1	44	89
	Rectal palpation	270	1	44	0
	Ultrasonography	270	3	42	0

[a] Five cows diagnosed incorrect pregnant by all three methods
[b] Three cows diagnosed incorrect pregnant by all three methods
[c] One cow diagnosed incorrect pregnant by all three methods
[d] One cow diagnosed incorrect pregnant by all three methods

Early vs. late milk collection

Milk was collected about one month before rectal palpation and ultrasonography in herds 2 and 6 and this is reflected in the low oestrone sulphate sensitivity for all cows in these two herds. For cows at least 120 days in calf, oestrone sulphate sensitivity for herds 2 and 6 was statistically not different to herds 1, 3, 4 and 5 ($p=0.11$). Specificity for herds 2 and 6 was statistically not different from the other four herds, regardless whether all cows ($p=0.17$) or cows at least 120 days since their last service ($p=0.42$) were considered.

Manual vs. herd test milk collection

Oestrone sulphate sensitivity was lower in herds 1, 2, 4 and 6 where milk was collected manually compared to herds 3 and 5 where collection took place at a routine herd test ($p<0.001$ for all cows and $p=0.34$ for cows at least 120 days in calf). Specificity for manual vs. routine herd test collection showed a reversed picture with herds 3 and 5 being inferior to the other four herds ($p<0.001$).

Comparison of breeds

The breed was known for a total of 1943 cows. Just over three-quarters were pure Friesians (1473, 75.8%). Fifty:fifty Friesian:Other crosses accounted for 11.3% (220) and crosses with more than 50% Friesian blood for 11.4% (221). Pure or crossbred Jerseys accounted for 1.2% (24). Herd 1 had five Ayrshire or Ayrshire crosses (0.3%), but these were not included in the analysis. With sensitivity of rectal palpation and ultrasonography being 100% or close to it, no difference between breeds would be expected. Oestrone sulphate sensitivity was not different between breeds ($p>0.25$) and there was no significant difference when comparing Friesian and Friesian crosses with Jerseys ($p>0.26$). Comparison of breeds for cows at least 120 days in calf was not possible due to the small number of Jerseys. Similarly, specificity could not be compared, as there were only two non-pregnant Jerseys.

Comparison of veterinarians

A sensitivity of 100% for rectal palpation excludes a difference between the two veterinarians who carried out the diagnosis. Specificity did vary between the two veterinarians, with an overall specificity of 94.4% for veterinarian A and 89.2% for veterinarian B, but the difference was not significant ($p>0.12$).

Table 2.11, page 49, shows the specificity achieved by each veterinarian in each herd. Modelling the probability of an incorrect diagnosis showed significant differences between herds ($p<0.01$), veterinarians ($p<0.01$) and a marginal herd-veterinarian interaction ($p=0.063$). The herd-veterinarian interaction appeared strongest in those herds where veterinarian A achieved 100% specificity. In these three herds, the specificity achieved by veterinarian B differed significantly from veterinarian A ($p<0.001$). In the three herds where veterinarian A did not achieve 100% specificity, there was no significant difference between veterinarians ($p>0.2$).

Of the six cows incorrectly diagnosed by veterinarian A, three had been incorrectly diagnosed as pregnant by all three methods, and the other three by two of the three methods. Of the 14 cows incorrectly diagnosed as pregnant by veterinarian B, seven were incorrectly diagnosed by all three methods and a further four by two of the three methods.

Table 2.11 Specificity achieved by the two veterinarians in each herd

			Without likely abortions		No. of non-pregnant cows examined	
Herd	**Vet A**	**Vet B**	**Vet A**	**Vet B**	**Vet A**	**Vet B**
1	94.1	90.2	97.1	100.0	34	41
2	100.0	87.5	100.0	87.5	25	16
3	93.5	76.2	96.8	85.7	31	21
4	100.0	75.0	100.0	100.0	1	4
5	60.0	83.3	80.0	83.3	5	6
6	100.0	97.1	100.0	97.1	10	35

Oestrone sulphate as screening test

A sensitivity of 100% was achieved for all herds when assuming that rectal palpation had been used for re-examination. With ultrasonography for re-examination, a sensitivity of 100% was achieved in all bar one herd. Specificity ranged from 27.3% to 95.6% and did not differ between scenario one (rectal palpation for re-examination) or scenario two (ultrasonography for re-examination). Specificity was lower than using oestrone sulphate as the only test in all herds. Table 2.12, page 50, shows sensitivity, specificity, and positive and negative predictive values for the six herds.

Table 2.12 Values for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c), and incorrect non-pregnant (d) using oestrone sulphate as screening test and re-examination of cows diagnosed non-pregnant with either rectal palpation or ultrasonography

Herd	Method	Sensitivity	Specificity	PPV	NPV	a	b	c	d	No. of incorrect pregnant diagnoses by OS (b)	Cows requiring re-examination No.	%
1	OS & RP	100.0	82.9	98.0	100.0	642	13	63	0	12	155	21.6
	OS & US	100.0	82.9	98.0	100.0	642	13	63	0			
2	OS & RP	100.0	92.7	98.6	100.0	216	3	38	0	2	125	48.6
	OS & US	100.0	90.2	98.2	100.0	216	4	37	0			
3	OS & RP	100.0	55.6	93.7	100.0	356	24	30	0	20	88	24.7
	OS & US	99.7	59.3	94.2	97.0	355	22	32	1			
4	OS & RP	100.0	80.0	99.5	100.0	201	1	4	0	1	28	13.6
	OS & US	100.0	60.0	99.0	100.0	201	2	3	0			
5	OS & RP	100.0	27.3	96.5	100.0	222	8	3	0	8	6	2.6
	OS & US	100.0	27.3	96.5	100.0	222	8	3	0			
6	OS & RP	100.0	95.6	99.3	100.0	270	2	43	0	1	133	42.2
	OS & US	100.0	93.3	98.9	100.0	270	3	42	0			

OS = Oestrone sulphate RP = Rectal palpation US = Ultrasonography

Fertility parameters

Table 2.13, page 52, shows the fertility parameters for the six herds. Twenty-one-day submission rates were between 62.3% and 88.4% (77.5% for all cows). The first service pregnancy rate ranged from 43.4% to 57.1% (mean 51.8%) and overall pregnancy rate was 46.7% to 60.5% (mean 55.2%). Between 1.65 and 2.14 services were needed per pregnancy (mean 1.83). The lowest percent 'non-pregnant of cows served' was 6.0, the highest 20.3 (mean 12.4).

Abnormal gestation lengths

Abortion was noticed between diagnosis by rectal palpation and ultrasound in one cow each from herds 2 and 6. Milk collection had taken place more than one month earlier in these two herds and was regarded as unaffected by the abortion. A further 25 cows were recorded with abortions between completion of all diagnostic tests and calving. Rectal palpation and ultrasound had correctly diagnosed all 25 cows as pregnant. Oestrone sulphate had correctly diagnosed 20 cows as pregnant. Seven premature calvings were recorded by herd owners (see Appendix I).

In herd 1, three cows were suspected as having aborted because they started to produce milk. Two foetuses were found in the winter paddock. For the analysis, the gold standard for these three cows was entered as non-pregnant. Both rectal palpation and ultrasound had diagnosed all three cows as pregnant, whereas oestrone sulphate had declared two as pregnant.

Induction of calving was carried out in 108, 10, 12, 25, and 56 cows in herds 1, 2, 3, 5, and 6, respectively, from end of July onwards. Herd 4 did not induce calving (see Appendix II).

Table 2.13 Fertility parameters for the six trial herds

Herd	Total number of cows in herd	Number of cows with service recorded	21-day submission rate (%)	First service pregnancy rate (%)	Overall pregnancy rate (%)	Services per pregnancy	Percent non-pregnant of cows served
1	759	720	62.3	47.5	54.2	1.85	11.9
2	302	279	88.4	55.2	56.8	1.76	20.3
3	442	409	79.6	52.3	57.3	1.75	14.9
4	249	238	77.8	57.1	60.5	1.65	6.6
5	285	260	81.5	55.0	55.7	1.8	6.0
6	365	348	86.9	43.4	46.7	2.14	14.8

Estimation of stage of gestation using Crown-Rump-Length (CRL)

Based on gestational age derived from recorded service dates and slaughter date, the formula by Richardson et al. (1990) confirmed the calculated gestational age (+/- 13 days) for 60% of cows compared to 46% using the formula by Arthur et al. (1982). The formula by Richardson et al. estimated gestational age best up to a CRL of 55 cm, corresponding to 193 days of gestation. Their formula overestimated gestational age by an average of 27.5 days for CRLs of 64 to 78 cm. The formula by Thomsen (1975) was better for these late stage pregnancies, but less accurate for the first two trimesters. Arthur's formula was best up to 52 cm CRL or 182 days of gestation. For more advanced pregnancies, deducting 13% of the CRL gave more accurate estimates for Arthur's formula.

Discussion

Oestrone sulphate

Enzyme-immunoassay of milk oestrone sulphate for pregnancy diagnosis was inferior to rectal palpation and ultrasound in this study. Specificity of oestrone sulphate was about 10% lower than the other two methods, and did not reach the 100% reported in previous studies (Heap and Hamon, 1979; Henderson et al., 1992; Henderson et al., 1993; Henderson et al., 1994a; Henderson et al., 1994b; Kourletaki-Belibasaki et al., 1995). Counting all incorrect diagnoses of pregnant, 19.0% (44/232) of non-pregnant cows had a false positive result with oestrone sulphate. For cows where only oestrone sulphate gave an incorrect pregnant diagnosis, 12.1% (28/232) had a false positive result. This compares to 6% reported by Henderson et al. (1995) using the same test kit. In practical terms, the lower than expected specificity and positive predictive value would lead to non-pregnant cows being carried over the winter and fed unnecessarily.

In herds 1, 3 and 5, only oestrone sulphate showed a poorer specificity compared to the other herds. Five of the ten cows diagnosed pregnant by all three methods, which subsequently failed to calve, were from herd 1. However, since the other two methods would be equally affected by these likely unobserved abortions, it does not serve as a satisfactory reason for the moderate specificity of oestrone sulphate in this herd. Herd 5 sold most of its non-pregnant cows. These animals were lost to follow-up, resulting in a small number of known non-pregnant cows, with a major impact of one false pregnant diagnosis. No apparent reason can be found for the low specificity in herd 3.

Milk was collected during herd improvement tests in herd 3 and 5, but how this should affect specificity is not obvious. The number of herds is too small to establish whether the apparent difference between manual vs. in-line collection is a true effect. If it were, using milk samples collected for herd improvement testing for oestrone sulphate analysis may not be suitable. This would seriously impair the practicality of pregnancy diagnosis based on milk. Although oestrone sulphate is a hydrophilic substance with over 90% confined to the whey portion of whole milk (Heap and Hamon, 1979), it has been shown that whole milk can be used reliably for assessment (Holdsworth and Chaplin, 1982; Henderson et al., 1992} and that milk composition has no influence (Hamon et al., 1981; Bloomfield et al., 1982). If manual milk sampling resulted in a reduction of milkfat great enough to increase the relative oestrone sulphate concentration, thereby influencing specificity, an effect on sensitivity would be expected, as well.

Only after 140 days of gestation did oestrone sulphate reach a sensitivity comparable to that of rectal palpation and ultrasonography. Sensitivity for all cows, irrespective of stage of gestation, was lower (81.8% vs. 85%) than reported by Henderson et al. (1995) using the same test kit, despite cows in that study being sampled from an earlier stage of gestation (22 days onwards). For cows at least 120 days pregnant, the sensitivity of 96.8% was similar to other studies (Heap and Hamon, 1979; Power et al., 1985; Henderson et al., 1992; Henderson, 1994a; Henderson et al., 1995), although higher sensitivities of 98% to 100% were achieved in studies by Tainturier et al. (1986) and Henderson et al. (1993 and 1994b). Cut-off points between 120 and 200 pg/ml were used in these studies, while the current study used a cut-off point of 200 pg/ml.

Sensitivity would have been improved by lowering the cut-off point. However, this would have increased the number of false positive diagnoses, thereby decreasing specificity even further. Power et al. (1985) showed a decreased specificity when the cut-off was lowered from 250 to 150 pg/ml, and suggested using two cut-off points: 150 pg/ml for a non-pregnant diagnosis (98% negative predictive value) and >250 pg/ml for a pregnant diagnosis (98% positive predictive value), with re-examination of cows with values between the two.

Low sensitivity of oestrone sulphate may in part be due to the within-cow variance of concentration with period of high levels interrupted by transient periods of low concentration (Bloomfield et al., 1982; Heap et al., 1983; Power et al., 1985; Tainturier et al., 1986; Henderson et al., 1993; Henderson et al., 1995). The transport and cooling of milk samples in this study probably increased sensitivity (Heap and Hamon, 1979; Power et al., 1985). Power et al. (1985) noticed between plate and low within plate variance, a finding supported by Henderson et al. (1995) using the same test kit as in this study. Mastitis and oestrus can lead to an incorrect diagnosis of pregnancy (McCaughey et al., 1982). Milk was not taken from quarters with clinical mastitis at manual milk collection in this study. No record was taken of cows in oestrus on the day of sampling.

No differences in oestrone sulphate accuracy were found between Friesians, Friesian crosses or Jerseys and Jersey crosses. This is in agreement with studies comparing Jersey with Friesian cows (Heap and Hamon, 1979) and Karan Swiss cows with Murrah buffalo (Hung and Prakash, 1990). In contrast, Abdo et al. (1991) found significantly lower oestrone sulphate levels between 101 and 200 days of gestation in Swedish Jerseys compared to Swedish Red and White and Swedish Lowland cattle. Prakash and Madan (1993) reported differences between Karan Swiss, Karan Friesian, Sahiwal and Murrah buffalo.

Oestrone sulphate as screening test

If oestrone sulphate were to be used as a screening test, with only cows not confirmed pregnant examined by a different method, a specificity or positive predictive value of 100% would be essential. These criteria were not met by the test used in this study. The incorrect pregnant results by oestrone sulphate were compounded by any false positive diagnosis by either rectal palpation or ultrasonography in cows re-examined by these methods, resulting in a lower specificity in five of the six herds compared to using oestrone sulphate as the only test. Sensitivity and negative predictive value were increased markedly by the combined screening and re-examination. This was the result of the high sensitivity of the two methods used for re-examination.

Rectal palpation and ultrasonography have advantages over oestrone sulphate in addition to the higher sensitivity and specificity shown in this study. Both methods are independent of lactation status. The examiner can establish the gestational age or confirm the service date, sex the foetus (Müller and Wittkowski, 1986; Müller et al., 1986), confirm normality of the pregnancy, establish ovarian and uterine status and pathology in non-pregnant animals (Paisley et al., 1978; Yaro et al., 1989; Kähn and Leidl, 1989), and detect twin pregnancies (Hughes et al., 1989). Oestrone sulphate concentration is increased in cows with twin pregnancies, but the increase is not large enough to be used diagnostically (Worsfold et al., 1989; Echternkamp, 1992). It has been suggested that repeated sampling to establish an oestrone sulphate profile can detect abnormal pregnancies (McCaughey et al., 1982; Heap et al., 1983; Mohamed et al., 1987; Hewitt et al., 1990). Regional averages of days-in-milk were 218 to 228 for the 1996-97 season (national average 223 days; (Livestock Improvement Corporation, 1997a). In years with adverse weather conditions and poor grass availability days-in-milk may be reduced as, for example, in the 1994-95 season when some regions only achieved an average of 175 days in milk (Livestock Improvement Corporation, 1995). Repeat sampling to establish an oestrone sulphate profile can, therefore, be difficult in New Zealand dairy herds.

Rectal palpation

Considering its wide use as a method of pregnancy diagnosis, reliable information on the accuracy of rectal palpation cannot be obtained easily (Arthur et al., 1983). Several authors assume 100% accuracy (Abbitt et al., 1978; Paisley et al., 1978; Vaillancourt et al., 1979; Gowan et al., 1982). It also is commonly used as control or standard for studies examining the accuracy of other methods, e.g.

progesterone and ultrasonography (Günzler et al., 1975; Heap et al., 1976; Heap and Hamon, 1979; Pennington et al., 1985; Beghelli et al., 1986; Hanzen and Delsaux, 1987; Chaffaux et al., 1988; Humblot et al., 1988a; Willemse and Taverne, 1989; Bonato et al., 1990; Pieterse et al., 1990; Gupta, 1991; Badtram et al., 1991; Hanzen and Laurent, 1991; Rajamahendran et al., 1993), or establishing the rate of foetal loss (Weigelt et al., 1988; Labèrnia et al., 1996).

The sensitivity of 100% for rectal palpation achieved in this study supports its role as one of the main methods of pregnancy diagnosis and has not been reached in any other study as far as the author is aware. Specificity of 100% was not reached. However, the achievement of maximum sensitivity is probably more important than maximum specificity, as the misdiagnosis of a pregnant cow with subsequent culling would incur a greater loss than the feeding of a non-pregnant cow during the dry period. In dairy herds in the Northern Hemisphere, where hormonal interference is common for cows diagnosed non-pregnant, high sensitivity is again more important than high specificity. The overall specificity of 91.4% compares favourably to other studies that report specificity below 70% (Meacham et al., 1976; Ducker et al., 1985; Reimers et al., 1985; White et al., 1989b). Two studies achieved 81.9% and 89.9%, respectively (Warnick et al., 1995; Hancock, 1962). Specificity of 100% is reported by Tierney (1983) and Yaro et al. (1989), both in beef animals. The assumption that the ten cows incorrectly diagnosed as pregnant by all three methods aborted, would increase overall specificity to 95.7%.

The positive predictive value of 99% is also higher than that reported in above studies, with the exception of the two studies by Tierney (1983) and Yaro et al. (1989). The negative predictive value of 100% exceeds the 82.9% to 97.8% reported in other studies (Meacham et al., 1976; Roche et al., 1978; Tierney, 1983; Reimers et al., 1985; White et al., 1989b; Yaro et al., 1989; Warnick et al., 1995).

The examiner's ability does influence the accuracy of rectal palpation. Veterinarian B achieved a lower specificity than veterinarian A in some herds, although the difference was not significant across the six herds. Specificity was worst for both veterinarians in the two herds that had a small number of non-pregnant animals, where one wrong diagnosis had a comparatively large impact. Specificity was higher for cows at least 120 day since their last service, which may reflect increased confidence on the examiner's part to declare a cow non-pregnant when a considerable time has elapsed since the last service. However, rectal palpation took place between 92 and 99 days after the end of mating in those herds where specificity was below 100%. One would expect an experienced examiner to be able to distinguish between a cow potentially three months or more pregnant and a non-pregnant cow, even if the latter had an enlarged uterus due to multiparity. Abbitt et al. (1978) found a difference between clinicians in apparent foetal loss, which would have included inaccurate diagnoses.

Ultrasonography

This study confirms the value of transrectal, real-time ultrasonography for pregnancy diagnosis in cattle. The sensitivity of 99.9% is very high and the declaration of two pregnant cows as non-pregnant may have been a recording error. White et al. (1985) reported a sensitivity of 98.8%, and 100% was achieved by Beghelli et al. (1986) and Szenci et al. (1996). Other studies examining cows at more than 60 days of gestation achieved a sensitivity of 93.7% to 97% (Hanzen and Delsaux, 1987; Hanzen and Laurent, 1991).

The specificity of 90.9% is higher than the 75% reported by White et al. (1985), who examined cows between 92 and 202 days of gestation. Re-calculation without the ten cows that were misdiagnosed by all three methods results in a specificity of 95.3%, which is comparable to that achieved by Hanzen and Laurent (1991). Specificity of 100% is reported in few studies (Hanzen and Delsaux, 1987; Szenci et al., 1995; Szenci et al., 1996). Specificity was poorest in herds 4 and 5, which had very few non-pregnant animals, resulting in a large impact of one wrong diagnosis.

The positive predictive value of 98.9% is higher than the 86.7% to 97% reported by Pieterse et al. (1990), Hanzen and Laurent (1991) and Lamprecht et al. (1995), although higher (99.4% to 100%) positive predictive values have been achieved (White et al., 1985; Hanzen and Delsaux, 1987; Szenci et al., 1996).

A negative predictive value as high as 99.1% was not achieved in above studies, with the exception of the studies by Beghelli et al. (1986), Chaffaux et al. (1988), and Szenci et al. (1996).

There was no difference between ultrasound operators in a study by Badtram et al. (1991), but the sensitivity of one operator declined over time. The ultrasound examiner did not show any decline in accuracy in the current study. On the contrary, the higher sensitivities were achieved in the second, fourth, fifth and sixth herd examined and specificity was highest in the fifth herd.

Factors affecting accuracy of all three methods

Probability of a correct diagnosis

Specificity was poor for all three methods in herd 4. One batch of cull cows from this herd had been missed at the abattoir, resulting in only five known non-pregnant cows. The one or two wrong diagnoses made by the three methods had a major impact as a result of this.

Sensitivity varied between herds for all three methods, suggesting that it may be more difficult to reach a correct diagnosis in some cows or herds, regardless of method used. The worst herd, herd 2, had a high 'non-pregnant of cows served' rate, but it is not clear how this may affect probability of reaching a correct diagnosis. There is no obvious factor in handling facilities, cow temperament or management, to explain the observed differences. The probability of a correct pregnant diagnosis was highest in herds 1, 3 and 5. This is almost a reverse picture of the specificity achieved in these herds and may offer an explanation. Overestimating the number of pregnant cows will result in high sensitivity, but low specificity. But again, there is no obvious reason why this pattern should emerge in these herds. No suggestion has been made in previous studies that a different level of production or metabolic rate could influence oestrone sulphate concentrations and that, therefore, different cut-off points may have to be applied to different herds. Henderson et al. (1994b) found that neither New Zealand management systems nor stocking rate had a significant influence on oestrone sulphate concentrations.

Identification of cows

Throughput of animals during rectal palpation, ultrasonography and milk collection was as close to normal working conditions as possible. Wrong identification and / or recording of cows may have occurred. However, the study did not set out to establish absolute values, but to test the three diagnostic methods under field conditions. Mistakes in recording or identification would probably occur when applying any of the methods as a routine diagnostic procedure.

Foetal loss

Abortion is likely to have occurred in the ten cows that were diagnosed pregnant by all three methods but failed to calve. The ten cows diagnosed pregnant by two of the methods, which subsequently failed to calve, possibly suffered foetal loss. Adding these 20 cows to the 27 observed abortions would give a foetal loss rate of 2.5% (47/1907).

Unobserved foetal loss does occur in the New Zealand dairy herds. The likelihood of finding an aborted foetus is lower in grazed than in housed animals. In New Zealand, pregnant dry cows are commonly grazed away from the main milking farm with stock often checked not more than once a day. Hayes (1997) found that only 11 of 157 herds recorded abortions. In these herds, 0.8% of unrecorded cows were believed to have aborted because they started to lactate and the incidence of premature calving was 1.2%. In a study by Forar et al. (1996), only 20% of foetal losses were observed with the remainder being detected on repeat pregnancy diagnosis.

A rate of 2.5% is similar to or lower than foetal loss rates as reported in other studies (Hancock, 1962; Kummerfeld et al., 1978; Vaillancourt et al., 1979; Reimers et al., 1985; Screenan and Diskin, 1986; Weigelt et al., 1988; Thurmond et al., 1990; Lemire et al., 1993; Mee et al., 1994; Mohammed et al., 1991; Warnick et al., 1995; Bouchard et al., 1998). It should be noted that when examining foetal loss, the rates derived depend on the methods used and the gestation period during which observations took place, as demonstrated by Beghelli et al. (1986). Estimation of foetal loss using progesterone assay, for example, does not distinguish between true loss and, for example, cows that were inseminated during the luteal phase or had cystic ovarian disease. Rates derived from using rectal palpation and ultrasonography may incorporate misdiagnoses by the examiner and loss caused by these invasive methods.

Most authors agree that the highest rate of foetal loss occurs in the first trimester of gestation (Hancock, 1962; Ball, 1978; Reimers et al., 1985; Moller et al., 1986; Screenan and Diskin, 1986; Allen, 1997). Labèrnia et al. (1996) concluded that each extra day between breeding and pregnancy diagnosis reduced pregnancy loss by a ratio of 0.97. Cows in this study were a minimum of 58 days in calf at the time of the first pregnancy diagnosis.

Moller et al. (1986) reported a lower foetal loss rate of 1.4% from day 58 of gestation onwards in New Zealand dairy cows. However, that study relied on observation of return to oestrus. Bulman and Lamming (1979) reported that 14 of 802 (1.75%) cows lost their calf between 120 and 260 days of gestation, and Beal et al. (1992) derived a loss rate of 1.5% between 45 and 65 days of gestation.

Fertility and management parameters of study herds

Because the accuracy of the oestrone sulphate test changes with stage of gestation, it was important that fertility in the study herds was typical for the New Zealand seasonal calving herd. The study herds had fertility parameters comparable to other New Zealand seasonal calving herds.

21-day submission rate

The target for 21-day submission rate is 90% in the Dairyman program (Massey University), and rates of 75.9% to 86% are commonly achieved (Hayes, 1996; Hayes, 1997). The mean 21-day submission rate for the study herds was 79.6% with two of the six herds falling below this level. The poorest rate, of 62.3%, was in the largest herd of the study (759 cows).

First service and overall pregnancy rate

The Dairyman (Massey University) target is 60% for both first service and overall pregnancy rate. The study herds had rates below this target, but were comparable to other New Zealand herds. Previous studies have shown first service pregnancy rates of 48.5% to 71.0% (Hayes, 1996; Hayes 1997; Macmillan 1998) in New Zealand seasonal herds. Macmillan et al. (1984) found that the pregnancy rate to first service decreases with increasing herd size and this is reflected in the study herds. All services pregnancy rates of 51.5% to 75.0% reported in previous studies (Hayes, 1996; Hayes, 1997) are in agreement with the rates in the herds of this study with the exception of herd 6 which showed a low overall pregnancy rate of 46.7%.

Services per pregnancy

All but one study herd were above the Dairyman (Massey University) target of 1.7 services per pregnancy, but all herds fell within the range of 1.2 to 2.4 services per pregnancy reported in previous New Zealand studies (Fielden et al., 1980; Hayes, 1996; Macmillan, 1998).

Breeding period

The total breeding period ranged from 73 to 104 days in the study herds, with artificial insemination being used for the first 41 to 60 days of this period. Herd 2 used AI for its entire breeding period of 73 days. These periods are similar to findings by Macmillan and Moller (1977) and Hayes (1996) of total breeding periods between 72 to 108 days. AI was used for periods of 36 to 43 days in their studies.

Induction rate

Induction rates varied widely in the study herds (3.4% to 20.7%). The target rate is less than 10% (Williamson, 1998), and average induction rates of 5.9%, 7.8% and 11.3% have been reported with a maximum of 26.1% (Macmillan et al., 1990; Hayes, 1996). Herd 1 had just expanded to almost double its previous herd size and herd 6 had a poor overall pregnancy rate, which may explain the high induction rates in these two herds.

Cull rates

The cull rates of four of the study herds were below the target cull rate of 20% commonly stated by farmers and reported rates of 17.6% to 19.0% (Bradford, 1968; New Zealand Dairy Board, 1976; Macmillan and Moller, 1977). The cull rates of herds 2 and 4 were just above these averages. The lowest cull rate was in herd 1, which had just expanded.

Percent non-pregnant of cows served

The mean rate of non-pregnant cows of all cows served in this study was higher than mean rates of 6.5% to 10.7% reported by others (Morris et al., 1994; Hayes, 1997; Crabb, 1998; Macmillan, 1998). A partial reason for this higher rate is the inclusion of only cows with a gold standard result in this study. This reduced the total number of eligible cows in the herd, with non-pregnant cows forming a higher proportion. Non-pregnant rates observed in the study herds were similar to those reported by Morris et al. (1994) and Hayes (1997).

Estimating gestational age using Crown-Rump-Length (CRL)

The formula by Richardson et al. (1990) gave the best overall estimate of gestational age. Their formula was derived from straight CRL measurements using a rigid frame, while the curved CRL was measured through the uterine wall in this study. In a study by Lyne (1960), the difference between curved and straight CRL was on average 23.8%. Application of a similar correction factor to the curved CRL taken in the current study resulted in underestimation of gestational age for most foetuses.

Conclusion

The results of this study indicate that enzyme-immunoassay of oestrone sulphate, based on a single milk sample taken in March to April, is inferior to rectal palpation or ultrasonography for pregnancy diagnosis in spring-calving New Zealand dairy cows. The specificity achieved for oestrone sulphate was much lower than expected considering the results of other studies. A high specificity would be essential if oestrone sulphate were to be used as a screening test, with cows diagnosed non-pregnant re-examined by either rectal palpation or ultrasonography. The sensitivity of oestrone sulphate did not reach levels comparable to the other two methods until cows were at 140 days or more of gestation. Other studies achieved this level of sensitivity about twenty days earlier. The lower accuracy of oestrone sulphate is unlikely to be offset by the non-invasive, convenient nature of this method. In addition, oestrone sulphate is not capable of providing additional information that was regarded as beneficial by most participating farmers, such as stage of pregnancy, and normal or pathological conditions of the reproductive tract or pregnancy. The independence on stage of lactation of rectal palpation and ultrasonography will also be of benefit in short milking seasons.

Highly accurate results were achieved for both rectal palpation and real-time transrectal ultrasonography. Findings compare favourably with other studies and support the value of these two methods for pregnancy diagnosis.

CHAPTER III

COMPARISON OF MILK OESTRONE SULPHATE AND FARMERS' OBSERVATIONS FOR PREGNANCY DIAGNOSIS

Introduction

Non-return to oestrus after breeding is probably the oldest method of pregnancy diagnosis and is still used in the analysis of the reproductive performance of dairy herds. Studies about the use of pregnancy diagnosis report between 19% and 42% of herds as having no veterinary pregnancy diagnosis carried out at all, with the majority of the remaining herds presenting only some of their cows for pregnancy diagnosis (Newton et al., 1982; Cowen et al., 1989; Giger et al., 1994). While investigating cows found pregnant at slaughter, Ladds et al. (1975) found that only 6.4% of the herds of origin had used veterinary pregnancy diagnosis. Of the herds serviced by the Farm Service Clinic of Massey University, where this study was carried out, 16.7% did not request pregnancy diagnosis at all and 26.4% presented only part of their herd for examination. These findings suggest that a considerable proportion of herd owners rely on observation of returns to oestrus to determine a cow's pregnancy status.

Oestrus detection rates can vary from 20% to 77%, resulting in a high number of non-pregnant cows being assumed to be pregnant (Williamson et al., 1972a; Esslemont, 1974b; King et al., 1976; Lehrer et al., 1992; Gaines et al., 1993; Nebel et al., 1995). Oestrus observation only incurs the cost of the observer's time, without any costs payable to a third party. For pregnancy diagnosis using milk oestrone sulphate to be attractive for these herds, it would have to show sensitivity, specificity and predictive values considerably higher than those achieved by farmers' observation in order to justify its cost.

Materials and Methods

Pregnancy status of cows in seven commercial, spring calving dairy herds in the Manawatu was established using farmers' observation and oestrone sulphate concentrations in milk. Herds were chosen from those clients of the Massey University Farm Service Clinic who had presented none or only a number of cows for pregnancy diagnosis over the previous years, and who had an effective recording system in place. Pregnancy diagnosis by oestrone sulphate was carried out between 132 and 191 days after the start of mating. Farmers gave a final list of their opinion on the pregnancy status of their cows between 114 and 206 days after the start of mating. Milk collection took place in early March in herds 9 and 12, and in early to mid-April in the other five herds. Table 3.1, page 67, shows the dates of examination in relation to the start of artificial insemination, start of natural mating and end of mating for the herds involved. The ear tag number was used for identifying milk samples, following up cows and recording farmers' observations.

Farmers' observation

The person responsible for each of the seven herds provided a list with the pregnancy status of each cow in their herd, based on that person's observations such as return to oestrus, body condition and milk yield. An opinion of pregnant, non-pregnant or suspect was recorded. Cows were denoted 'suspect' where normally a second opinion from a veterinary surgeon or ultrasound operator would have been sought. Farmers were asked to provide the list before the results of the oestrone sulphate test were known.

Oestrone sulphate determination

Milk collection

For analysis of oestrone sulphate, milk collected during a routine herd improvement test was used in herds 10, 11 and 13. A composite milk sample of approximately 250 ml was taken from each cow at two consecutive milkings with in-line collectors by technicians of the Livestock Improvement Corporation (LIC).

Table 3.1 Dates of breeding management and pregnancy diagnosis and days between events

	Herd Number						
Dates of Events	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Start of Artificial Insemination (AI)	18-Oct-96	29-Oct-96	25-Oct-96	11-Oct-96	15-Oct-96	10-Oct-96	17-Oct-96
Start of Natural Mating	02-Dec-96	01-Dec-96	06-Dec-96	01-Dec-96[a]	28-Nov-96	15-Nov-96[c]	25-Nov-96
End of Mating	01-Jan-97	31-Dec-96	05-Jan-97	23-Jan-97	08-Jan-97[b]	20-Dec-96	24-Dec-96
Farmers' Observation	31-Mar-97	15-Apr-97	15-Apr-97	05-May-97	29-Apr-97	01-Feb-97	26-Feb-97
Milk Collection	02-Apr-97	04-Apr-97	06-Mar-97	20-Apr-97	14-Apr-97	04-Mar-97	08-Apr-97
Days between Events							
Start of AI to farmer's observation	164	168	172	206	196	114	132
Start of AI to milk collection	166	157	132	191	181	145	173
End of mating to farmers' observation	89	105	100	102	111	43	64
End of mating to milk collection	91	94	60	87	96	74	105

[a] 2 cows naturally mated 25th and 28th November

[b] 7 cows mated after that until 27th January

[c] 16 cows served with AI between 13th and 28th November in addition to natural mating

The samples were transferred into 150 ml plastic pots containing 200 µl of 10%Bronopol[6]. Samples were stored at ambient temperature during collection and transport to the local LIC office. Further storage and transport to the National Milk Analysis Centre, Hillcrest, was at 4° to 7° Celsius.

In herds 7, 8, 9 and 12, milk was collected manually at one milking. Before cup attachment, a composite milk sample of 10 to 30 ml was taken of each cow into 30 ml plastic pots containing 100 µl of 10% Bronopol. All four quarters were used for the sample in most cows. Mastitis, blind teats, dry quarters or nervous animals were reasons for not sampling a particular quarter. The cow's eartag number was written on the lid of the sample container. Adhesive labels showing herd and cow identification were applied to the samples after milking had finished. Storage and transport of samples were the same as above.

Milk analysis

Oestrone sulphate concentrations of whole milk samples were established by enzyme-immunoassay at the National Milk Analysis Centre, Hillcrest, using the Confirm™ pregnancy test[7]. Prior to the test all reagents, samples and plates were warmed to room temperature. 100 µl of milk was mixed with 300 µl of diluted horseradish peroxidaseconjugate, and 200 µl of this mix transferred into the EIA plate. After incubation of the plates at 2° to 8° Celsius for two hours, plates were emptied and washed four times with 250 µl wash solution. After adding 100 µl of substrate solution, plates were incubated at room temperature for 30 minutes, before 100 µl of a stop solution was applied.

The optical density (OD) was read at 490 nm in an EIA plate reader. OD results of the samples were compared with the cow cut-off standard for each well. Samples with OD readings below the standard were declared 'confirmed pregnant', samples with OD readings above the standard were declared 'not confirmed pregnant'.

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[6] Bronopol Boots. The Boots Company, Nottingham, United Kingdom

[7] ICP (Immuno-Chemical Products Ltd), P.O. Box 1607, Auckland, New Zealand

The cut-off standard was set at 10 pg oestrone sulphate in the well, equivalent to 200 pg oestrone sulphate per millilitre of milk.

Gold Standard

Cows remaining in the herd

For cows remaining in the herd until the end of gestation, the herds' calving records were used for confirmation of pregnancy status in the first instance. Further information on the actual event was sought from the owner for cows with no entry in the herd records or cows with discrepancies between calving outcome and one of the test results.

Owners were asked to record abortions where observed, but no special inspection of cows or their environment took place. Owners were also asked to record premature calvings and cases where induction of calving was utilised.

Cull cows

The author and/or one of two technicians were present at the abattoir when cows were slaughtered. Ear tag number and relating slaughter line number were recorded after jugular sticking. Once the viscera had been removed by the slaughterman, the reproductive tract was palpated. The foetal curved crown-rump length (CRL)[8] was measured through the uterine wall in pregnant cows. Reproductive tracts appearing non-pregnant on palpation were individually bagged and labelled, and later incised to establish a definite pregnancy status. The pregnancy status of tracts from condemned carcasses was established by palpation only.

Cows sold

No gold standard result could be obtained from these cows.

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[8] Following the body outline from forehead to tailbase (Lyne, 1960)

Calculation of conception dates

a) Cows with full term pregnancies

Calculated conception dates were defined as calving date minus 282 days[9] for cows that remained in the herd and had a calf at full term. These calculated conception dates were compared to recorded service dates. Where the discrepancy between recorded service date and calculated conception date was twelve days or less, the recorded service date was taken as actual conception date.

Where the discrepancy was 13 days or more and the calculated conception date fell into the natural mating period for that farm, this calculated date was used as actual conception date. Where the discrepancy was 13 to 21 days and the calculated conception date fell into the artificial insemination period for that farm, the recorded service date was used as actual conception date. For cows with completely disagreeing recorded service and calculated conception dates, the actual conception date was omitted.

b) Cows with induced parturition

Initially, the conception date was calculated as induced calving date minus 282 days. The last recorded service was used as actual conception date for cows where this was later than the calculated conception date. Where the last recorded service date fell before the calculated conception date, multiples of 21 days were added to the last service date, until the new service date was later than the calculated conception date. Where the difference between calculated conception date and last recorded service date was seven days or less, the calculated conception date was used as actual conception date.

c) Cows with abortions

The last recorded service date was used as the actual conception date for cows with a recorded abortion.

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[9] Based on average gestation length in New Zealand dairy cattle established by Ward and Castle (1947) and Macmillan and Curnow (1976). The Livestock Improvement Corporation uses 282 days gestation length to calculate Planned Start of Calving.

d) Cows with premature calvings

The last recorded service date was used as the actual conception date for cows calving prematurely.

e) Cows pregnant at slaughter

The curved CRL (see page 69) of the foetus was measured through the uterine wall. Using the formula of Richardson et al. (1990), a conception date was calculated from the CRL and compared to recorded service dates. The closest recorded service date was used as actual conception date where the discrepancy between calculated conception date and service date was twelve days or less.

Where the discrepancy was 13 days or more, the calculated conception date was used as actual conception date if it fell into the natural mating period for that farm. The conception date was omitted where the calculated conception date fell into the artificial insemination period and no service date had been recorded within twelve days or less. For five cows, no CRL was recorded and the actual conception date was omitted.

Calculation of fertility parameters

21-day submission rate

All cows that had calved at least 42 days prior to the planned start of mating for each herd were included in the 21-day submission rate. The rate was calculated as the number of cows served on or before the target date (planned start of mating plus 21 days) divided by the total number of cows eligible for service during those 21 days, times 100 (MAFF, 1984; Williamson, 1998).

Pregnancy rate

The gold standard results were used for final pregnancy status. Calculations were based on all cows with a service date, including those without a result for the gold standard and cull cows. For pregnancy rate calculation, it was assumed that cows conceived to their last service.

Pregnancy rate to first service

This is defined as the number of first services which result in a pregnancy and was calculated as number of cows pregnant to their first service divided by the total number of cows receiving a first service, multiplied by 100 (MAFF, 1984; Williamson, 1998).

Overall pregnancy rate

This is defined as number of cows pregnant expressed as percentage of total number of services and was calculated as number of cows pregnant divided by the total number of services, multiplied by 100 (MAFF, 1984).

Services per pregnancy

This was calculated as the total number of services given, divided by the total number of pregnant cows (MAFF, 1984). Services to cull cows were included.

Percent non-pregnant of cows served

This was calculated as number of non-pregnant cows (based on gold standard result) at the end of the breeding period expressed as a percentage of cows in the herd at the start of the breeding period (Williamson, 1998). Only cows with a gold standard result were included.

Comparison and statistics

Results were grouped into correct diagnosis pregnant (a), incorrect diagnosis pregnant (b), correct diagnosis non-pregnant (c), and incorrect diagnosis non-pregnant (d). Sensitivity was calculated as $[a \div (a+d)] \times 100$, specificity as $[c \div (c+b)] \times 100$, positive predictive value as $[a \div (a+b)] \times 100$, and negative predictive value as $[c \div (c+d)] \times 100$.

Sensitivity and specificity for the two methods were compared statistically by fitting a binomial generalised linear model using the repeated measures facility in Proc Genmod of the SAS system, with cows grouped into ten-day periods for days in calf or days since last service. The same model was used to compare the probability of a correct diagnosis for each cow depending on either herd or diagnostic method.

Successive models were fitted and changes in deviance assessed to establish whether the Herd-Test interaction was significant. A chi-square test (Microsoft Excel) was used to compare oestrone sulphate sensitivity and specificity of the two herds where milk collection had taken place in March to those where milk had been collected in April. A chi-square test was also used to test for differences in oestrone sulphate results between manual and in-line milk collection.

Results

Numbers examined

Of the 1049 cows present in the seven herds at the start of the study, just under 8% were lost to the study because of incomplete results. Lack of a diagnosis by farmers' observation caused the loss of only one cow. About one-third (24/82) of losses arose from a combination of no follow-up of culled cows (two cases), sale of cows (18 cases) and deaths (four cases). All non-pregnant cows from herd 7 were lost to follow-up, preventing calculation of specificity and negative predictive value for this herd. About one in four culled animals had incomplete results compared to one in twenty cows retained in the herds. Table 3.2, page 74, shows the total number of cows in each herd, the number of cows with results and the number culled or retained in the herds.

Ninety percent of cows with full results were pregnant and for most of these cows (94.8%) the gold standard was derived from calving records. For about 80% of non-pregnant cows, the gold standard was derived from examination of the reproductive tract after slaughter. Most of the cows remaining in the herds were pregnant (97.6%) and about two-thirds (63.1%) of the culled animals were non-pregnant. Table 3.3, page 75, shows the number of cows pregnant or non-pregnant and the source of the gold standard result for each herd.

Table 3.2 Number of cows with results for each herd

Herd	Total number of cows in herd	Cows with result for both methods		Cows with full set of results*		Total number cows culled from herd		Cows with full set of results of culled cows		Total number cows retained in herd		Cows with full set of results of retained cows	
	No.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
7	108	100	92.6	96	88.9	9	8.3	0	0	99	91.7	96	97.0
8	140	140	100.0	140	100.0	15	10.7	15	100.0	125	89.3	125	100.0
9	116	108	93.1	102	87.9	22	19.0	13	59.1	94	81.0	89	94.7
10	184	173	94.0	167	90.8	40	21.7	34	85.0	144	78.3	133	92.4
11	217	201	92.6	198	91.2	39	18.0	32	82.1	178	82.0	166	93.3
12	82	78	95.1	77	93.9	13	15.9	12	92.3	69	84.1	65	94.2
13	202	192	95.0	187	92.6	28	13.9	16	57.1	174	86.1	171	98.3
Total	**1049**	**992**	**94.6**	**967**	**92.2**	**166**	**15.8**	**122**	**73.5**	**883**	**84.2**	**845**	**95.7**

* Full set of results = Result for both diagnostic methods and gold standard

Table 3.3 Numbers of cows with full set of results* that were pregnant or non-pregnant and derivation of gold standard

Herd	**Total**	**Pregnant on gold standard**		**Pregnant cows with gold standard derived from calving records**		**Non-pregnant on gold standard**		**Non-pregnant cows with gold standard derived from abattoir**	
	No.	No.	%	No.	%	No.	%	No.	%
7	96	96	100.0	96	100.0	0	0	-	-
8	140	125	89.3	124	99.2	15	10.7	14	93.3
9	102	91	89.2	89	97.8	11	10.8	11	100.0
10	167	147	88.0	131	89.1	20	12.0	18	90.0
11	198	174	87.9	157	90.2	24	12.1	15	62.5
12	77	70	90.9	65	92.9	7	9.1	7	100.0
13	187	167	89.3	163	97.6	20	10.7	12	60.0
Total	**967**	**870**	**90.0**	**825**	**94.8**	**97**	**10.0**	77	**79.4**

* Full set of results = Result for both diagnostic methods and gold standard

Sensitivity and specificity

Overall results

Overall sensitivity, specificity, and positive and negative predictive values for all cows in the seven herds are shown in Table 3.4, page 77.

Farmers' observations misdiagnosed twelve pregnant cows as non-pregnant (Table 3.5, page 77), but were still significantly more sensitive than oestrone sulphate, even when cows recorded as 'suspect' were counted as misdiagnosis ($p=0.0001$).

Both methods diagnosed non-pregnant cows as pregnant, but specificity of oestrone sulphate was significantly higher than farmers' observation ($p<0.002$). Five cows were incorrectly diagnosed as pregnant by both methods (see Table 3.5, page 77).

Positive predictive value of oestrone sulphate was marginally better than that of farmers' observation, but the negative predictive value of oestrone sulphate was much lower, even compared to farmers' observation where 'suspect' cows were counted as misdiagnosis.

Table 3.4 Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c), incorrect non-pregnant (d), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for both methods

Method	a	b	c	d	Sensitivity	Specificity	PPV	NPV
Oestrone sulphate	743	19	78	127	85.4	80.4	97.5	38.0
Farmers' observation †	836	26	52	12	98.6	66.7	97.0	81.3
Farmers' observation ‡	836	45	52	34	96.1	53.6	94.9	60.5

Table 3.5 Comparison of diagnosis results for pregnant (P), non-pregnant (MT) and suspect (S) cows

	Number of cows	Oestrone sulphate	Farmers' observation
Pregnant cows	720	P	P
	5	P	MT
	18	P	S [a]
	7	MT	MT
	116	MT	P
	4	MT	S [b]
Non-pregnant cows	45	MT	MT
	21	MT	P
	12	MT	S [c]
	5	P	P
	7	P	MT
	7	P	S [d]

[a] 17 cows from herd 13, one from herd 11
[b] One cow from herd 13, one from herd 8, two from herd 7
[c] Ten cows from herd 13, one each from herd 10 and 11
[d] All 7 cows from herd 13

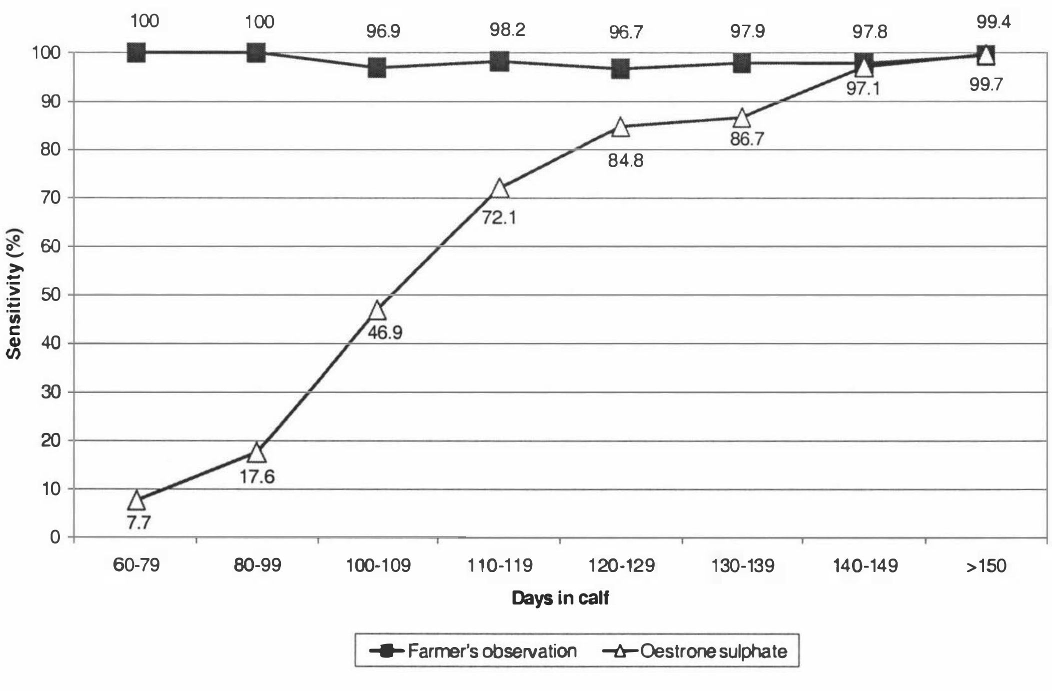
† Excluding cows recorded as 'suspect'
‡ Cows recorded as 'suspect' regarded as wrong diagnosis (i.e. pregnant cows recorded as 'suspect' counted as incorrect non-pregnant diagnosis and non-pregnant cows recorded as 'suspect' counted as incorrect pregnant diagnosis)

Results by days in calf or since last service

Sensitivity, specificity, and positive and negative predictive values were calculated with cows grouped according to their stage of gestation at the time of milk collection, and for cows at least 120 days pregnant or 120 days since their last service (Tables 3.6, page 81, 3.7, page 82, and 3.8, page 82). Cows recorded as 'suspect' by farmers' observation were not included in this analysis.

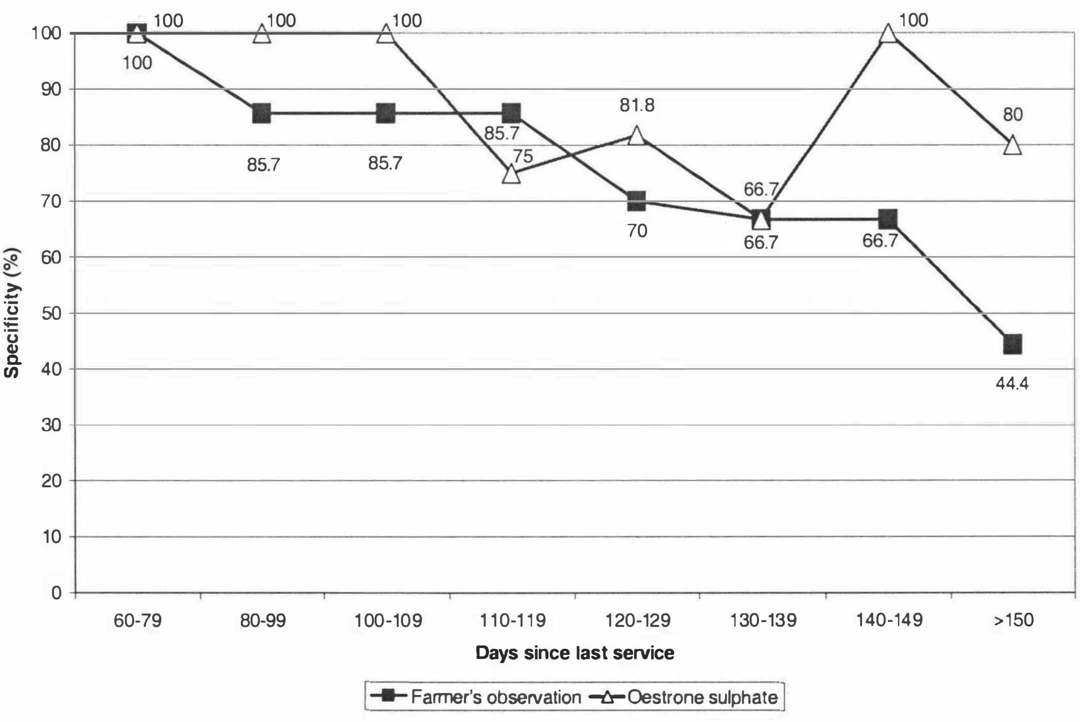
Days in calf significantly affected the sensitivity of oestrone sulphate ($p=0.0001$), but not farmers' observation. The sensitivity of farmers' observation was fairly constant. Oestrone sulphate sensitivity increased in a linear fashion with advancing stage of gestation, reaching a level similar to farmers' observation for cows 140 or more days in calf (Figure 3.1, below). For cows at least 120 days pregnant, the sensitivity of oestrone sulphate was improved by 9.9% over earlier testing (95.3% vs. 85.4%), but was still inferior to farmers' observation. For the group of cows where no conception date could be established, sensitivity of oestrone sulphate and farmers' observation was 73.1% and 100%, respectively.

Figure 3.1 Sensitivity of the two methods according to days in calf at the time of milk collection



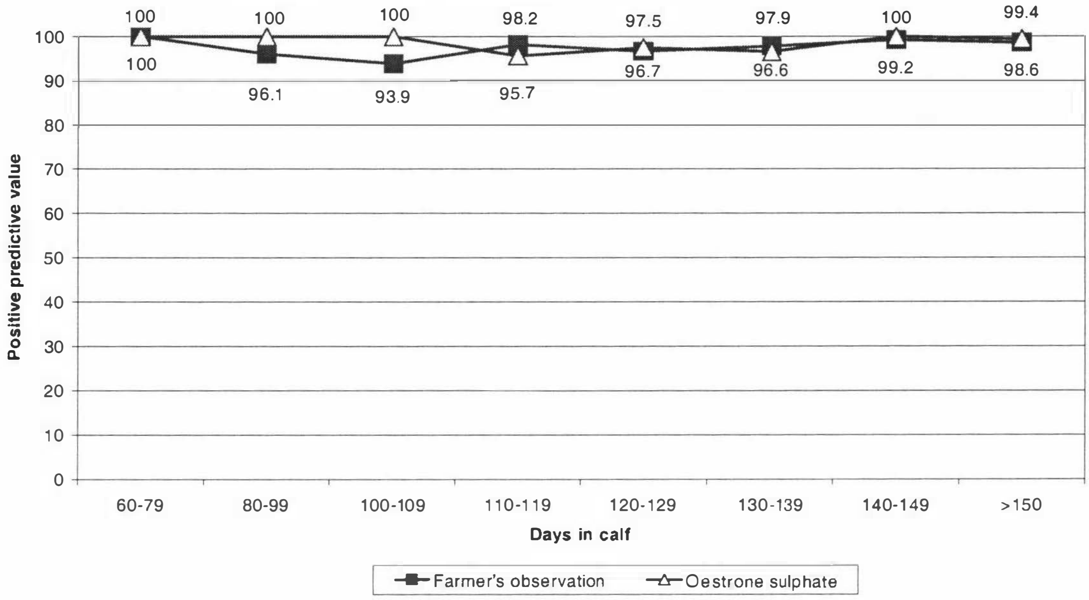
Specificity of oestrone sulphate varied across days since last service and appeared to fall for cows not served for at least 110 days. Beyond 120 days since last service, the specificity of farmers' observation appeared to fall consistently, reaching a low point of 44.4% for cows five months or more after their last service (Figure 3.2, below). Overall, increasing days since last service marginally decreased the probability of a correct non-pregnant diagnosis for both methods (p=0.06). Specificity for cows at least 120 days since their last service was slightly lower for both oestrone sulphate and farmers' observation compared to specificity for all cows. For the group of cows where no conception date could be established, specificity of oestrone sulphate and farmers' observation was 58.3% and 28.6%, respectively.

Figure 3.2 Specificity of the two methods according to days since last service at the time of milk collection



The positive predictive value of oestrone sulphate was better than farmers' observation for cows less than 110 days pregnant. For cows with longer gestations, farmers' observation showed a positive predictive value equal to oestrone sulphate (Figure 3.3, page 80). The positive predictive value of both methods was improved for cows at least 120 days in calf or since their last service.

Figure 3.3 Positive predictive value of the two methods according to days in calf at the time of milk collection



The negative predictive value of oestrone sulphate did not exceed 50% for cows less than 150 days since their last service. Farmers' observation showed a continuous decline the longer cows were from their last service, with a moderate recovery of the negative predictive value for cows at least 150 days since their last service (Figure 3.4, below). Looking at all the cows which had not been served for at least 120 days, the negative predictive value for oestrone sulphate was improved, but farmers' observation showed a marked drop (63.0% vs. 81.3%).

Figure 3.4 Negative predictive value of the two methods according to days since last service at time of milk collection

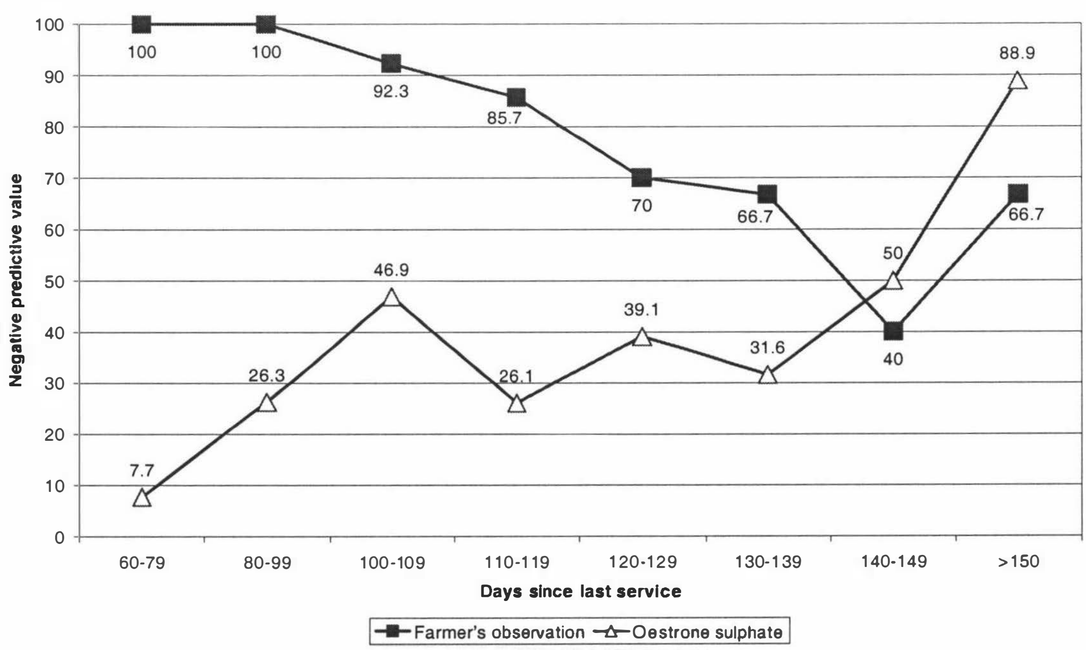


Table 3.6 Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c) and incorrect non-pregnant (d) for both methods according to days in calf or since last service at time of milk collection (excluding cows recorded as 'suspect' by farmers' observation)

Days in calf / since last service	Method	a	b	c	d
60-79	14 cows: 13 pregnant, 1 non-pregnant				
	Oestrone sulphate	1	0	1	12
	Farmers' observation	13	0	1	0
80-99	66 cows: 51 pregnant, 15 non pregnant				
	Oestrone sulphate	9	0	15	42
	Farmers' observation	49	2	12	0
100-109	47 cows: 32 pregnant, 15 non-pregnant				
	Oestrone sulphate	15	0	15	17
	Farmers' observation	31	2	12	1
110-119	69 cows: 61 pregnant, 8 non-pregnant				
	Oestrone sulphate	44	2	6	17
	Farmers' observation	54	1	6	1
120-129	103 cows: 92 pregnant, 11 non-pregnant				
	Oestrone sulphate	78	2	9	14
	Farmers' observation	87	3	7	3
130-139	107cows: 98 pregnant, 9 non-pregnant				
	Oestrone sulphate	85	3	6	13
	Farmers' observation	94	2	4	2
140-149	141cows: 137 pregnant, 4 non-pregnant				
	Oestrone sulphate	133	0	4	4
	Farmers' observation	132	1	2	3
≥ 150	370 cows: 360 pregnant, 10 non-pregnant				
	Oestrone sulphate	359	2	8	1
	Farmers' observation	355	5	4	2
No date	50 cows: 26 pregnant, 24 non-pregnant				
	Oestrone sulphate	19	10	14	7
	Farmers' observation	21	10	4	0

Table 3.7 Comparison of diagnosis results for pregnant cows at least 120 days in calf and non-pregnant cows at least 120 days since their last service at the time of milk collection (P = pregnant, MT = non-pregnant, S = suspect)

	Number of cows	Oestrone sulphate	Farmers' observation
Pregnant cows	642	P	P
	5	P	MT
	8	P	S [a]
	5	MT	MT
	26	MT	P
	1	MT	S [b]
Non-pregnant cows	13	MT	MT
	9	MT	P
	5	MT	S [c]
	2	P	P
	4	P	MT
	1	P	S [d]

[a] All 8 cows from herd 13
[b] From herd 13
[c] Four cows from herd 13, one from herd 11
[d] From herd 13

Table 3.8 Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c), incorrect non-pregnant (d), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for cows at least 120 days in calf or since their last service

Method	a	b	c	d	Sensitivity	Specificity	PPV	NPV
Oestrone sulphate	655	7	27	32	95.3	79.4	98.9	45.8
Farmers' observation†	668	11	17	10	98.5	60.7	98.4	63.0
Farmers' observation‡	668	17	17	19	97.2	50.0	97.5	47.2

† Excluding cows recorded as 'suspect'
‡ Cows recorded as 'suspect' regarded as wrong diagnosis (i.e. pregnant cows recorded as 'suspect' counted as incorrect non-pregnant diagnosis and non-pregnant cows recorded as 'suspect' counted as incorrect pregnant diagnosis)

Results by herd

Sensitivity, specificity, and positive and negative predictive value for each herd are shown in Table 3.9, page 84. Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c) and incorrect non-pregnant (d) for each herd are shown in Table 3.10, page 85.

There were small, but significant differences in the probability of a correct diagnosis for pregnant cows between herds, even when differences in days in calf had been taken into account (p=0.0001). Herds 11 and 13 showed a significantly higher probability than the other five herds (p=0.0001), suggesting that a pregnant diagnosis was more common in these two herds regardless of the true status of the cow. The Herd-Test interaction was significant (p=0.0001), but the effect was much smaller than the major effects of difference between tests, days in calf and overall herd difference. Similar results were obtained when including only cows at least 120 days in calf in the analysis. Oestrone sulphate achieved a higher sensitivity than farmers' observation only in herd 13.

There were significant differences between herds for both methods in the probability of a correct diagnosis for non-pregnant cows (p=0.0001) even after days since last service had been taken into account. Herds 9 and 12 showed the most accurate results, followed by herds 8 and 10, while herds 11 and 13 were the hardest to achieve accurate diagnoses in. This is almost a mirror image of differences in sensitivity. Herd 7 did not have any non-pregnant cows with full results, so specificity could not be established for this herd. The Herd-Test interaction was not significant (p>0.15). However, grouping the herds into their three layers of accuracy (Layer one = herds 9 and 12; layer two = herds 8 and 10; layer three = herds 11 and 13) showed a marginal Layer-Test interaction (p=0.085). In layers one and two, there was no significant difference between the two methods but in layer three, oestrone sulphate was more accurate in diagnosing non-pregnant cows than farmers' observation. This did not change the observation that both methods were less accurate in these two herds compared to the other four herds.

Table 3.9 Sensitivity, specificity, and positive and negative predictive values for oestrone sulphate (OS) and farmers' observation (FO) for each herd.

	Sensitivity			Specificity			Positive predictive value			Negative predictive value		
Herd	**OS**	**FO** [†]	**FO** [‡]	**OS**	**FO** [†]	**FO** [‡]	**OS**	**FO** [†]	**FO** [‡]	**OS**	**FO** [†]	**FO** [‡]
7	90.6	98.9	96.9	-	-	-	100.0	100.0	100.0	-	-	-
8	80.0	99.2	98.4	86.7	93.3	93.3	98.0	99.2	99.2	34.2	93.3	87.5
9	41.7	97.8	97.8	100.0	100.0	100.0	100.0	100.0	100.0	17.2	84.6	84.6
10	93.2	97.3	97.3	90.0	68.4	65.5	98.6	96.0	95.3	64.3	76.5	76.5
11	96.0	98.8	98.3	70.8	21.7	20.8	96.0	90.5	90.0	70.8	71.4	62.5
12	68.6	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	24.1	100.0	100.0
13	99.4	98.7	88.0	60.0	66.7	11.1	95.4	99.3	89.1	92.3	50.0	10.0

Herd 9 and 12: Milk collection approximately one month prior other herds
Herd 10, 11 and 13: Milk collection during routine herd test
Herd 7, 8, 9 and 12: Manual milk collection

[†] Excluding cows recorded as 'suspect'
[‡] Cows recorded as 'suspect' regarded as wrong diagnosis (i.e. pregnant cows recorded as 'suspect' counted as incorrect non-pregnant diagnosis and non-pregnant cows recorded as 'suspect' counted as incorrect pregnant diagnosis)

Table 3.10 Values for correct pregnant (a), incorrect pregnant (b), correct non-pregnant (c) and incorrect non-pregnant (d) diagnoses for each method and herd

Herd	Method	a	b	c	d
7	96 cows: 96 pregnant, 0 non-pregnant				
	Oestrone sulphate	87	0	0	9
	Farmers' observation	93	0	0	1
8	140 cows: 125 pregnant, 15 non pregnant				
	Oestrone sulphate	100	2	13	25
	Farmers' observation	123	1	14	1
9	102 cows: 91 pregnant, 11 non-pregnant				
	Oestrone sulphate	38	0	11	53
	Farmers' observation	89	0	11	2
10	167 cows: 147 pregnant, 20 non-pregnant				
	Oestrone sulphate	137	2	18	10
	Farmers' observation	143	6	13	4
11	198 cows: 174 pregnant, 24 non-pregnant				
	Oestrone sulphate	167	7	17	7
	Farmers' observation	171	18	5	2
12	77 cows: 70 pregnant, 7 non-pregnant				
	Oestrone sulphate	48	0	7	22
	Farmers' observation	70	0	7	0
13	187 cows: 167 pregnant, 20 non-pregnant				
	Oestrone sulphate	166	8	12	1
	Farmers' observation	147	1	2	2

The owner of herd 13 recorded almost one-fifth (18.7%) of his cows as 'suspect', about half of which were pregnant, the other half non-pregnant. Herds 7 and 9 did not use this option at all, and only one or two cows were recorded as 'suspect' in the remaining four herds (see Table 3.5, page 77).

Early vs. late milk collection

Milk was collected about one month earlier in herds 9 and 12 than in the other five herds and this is reflected in the low oestrone sulphate sensitivity for all cows in these two herds. But even for cows at least 120 days in calf, oestrone sulphate sensitivity was significantly poorer for the two herds with early milk collection compared to the other five herds (86.3% vs. 96.5%; $p<0.0001$). Specificity for herds 9 and 12 was significantly better than the other five herds when analysing results from all cows (100% vs. 75.9%; $p=0.02$). The sample size was too small to validly compare specificity for cows more than 120 days since their last service.

Manual vs. herd test milk collection

Milk was collected in-line during a routine herd test in herds 10, 11 and 13. Sensitivity in these three herds was significantly better than in the four herds with manual milk collection (96.3% vs. 71.5%; $p<0.0001$), even when only cows at least 120 days in calf were considered (97.6% vs. 91.7%; $p<0.0004$). The difference remained significant ($p<0.03$) when comparing the three herds with in-line collection with herds 7 and 8 only (herds 9 and 12 showed low sensitivity compared to the other herds – see above). Specificity in herds 10, 11 and 13 was significantly poorer than in the other four herds (73.4% vs. 93.9%; $p<0.02$).

Comparison of breeds

Of the cows with a full set of results, the breed was known for 871. Over half of these were pure Friesians, originating mainly from herds 7, 11 and 13. All Ayrshire cows were in herd 9, representing about one-eighth of cows. Herd 12 had only pure Jersey cows (except one), accounting for 90% of all Jerseys. Table 3.11, page 87, shows the distribution of breeds in the study herds. The sensitivity of the diagnostic methods in pure Friesian cows was significantly higher than that for pure Jerseys, Ayrshires or the various cross-breeds ($p<0.0001$).

Table 3.11 Distribution of breeds amongst the study herds and contribution of each breed to total number of cows

Breed	**Ayrshire**		**Friesian**		**Friesian : Jersey cross**[a]		**Friesian cross**[b]		**Jersey**		**Jersey cross**[c]	
Herd	No.	% §	No.	% §	No.	% §	No.	% §	No.	% §	No.	% §
7	-	-	91	18.3	-	-	5	4.4	-	-	-	-
8	-	-	1	0.2	6	18.2	59	51.8	6	8.5	53	94.6
9	101	100	-	-	-	-	-	-	-	-	-	-
10	-	-	99	20	8	24.2	23	20.2	1	1.4	3	5.4
11	-	-	136	27.4	10	30.3	20	17.5	-	-	-	-
12	-	-	1	0.2	-	-	-	-	64	90.1	-	-
13	-	-	168	33.9	9	27.3	7	6.1	-	-	-	-
Total	**101**	**11.6**¶	**496**	**56.9**¶	**33**	**3.8**¶	**114**	**13.1**¶	**71**	**8.2**¶	**56**	**6.4**¶

[a] Fifty:fifty cross
[b] Over 50% Friesian blood
[c] Over 50% Jersey blood

§ Contribution of cows in herd to that particular breed
¶ Contribution of that particular breed to total number of cows.

Even when only cows at least 120 days in calf were considered, diagnostic methods in pure Friesians showed higher sensitivity than the other pure or cross-breeds ($p<0.008$), with the exception of pure Friesians versus Jersey-cross-Friesians where the difference was only marginal ($p=0.08$). Comparing pure and cross-bred Friesians together against the other pure or cross-bred cows showed a significantly higher sensitivity for diagnostic methods in the former ($p<0.006$), except when compared to Jersey-cross cows at least 120 days in calf ($p=0.23$). There was also no difference in outcomes between Friesian-crosses and Jersey-crosses, regardless of stage of gestation ($p=0.2$). The sensitivity of methods in Ayrshires at all stages of pregnancy was significantly lower than that for all other pure or cross-bred cows ($p<0.0001$). When considering breed outcomes on diagnostic methods on cows beyond 120 days of gestation, however, there was no difference ($p=0.18$ to $p=0.8$). The number of non-pregnant animals was too small to establish differences in specificity between breeds.

Fertility parameters

Table 3.12, page 89, shows the fertility parameters for the six herds. Twenty-one day submission rates were between 68.6% and 96.3% (mean 80.2% for all cows). The first service pregnancy rate ranged from 53.4% to 68.0% (mean 61.7%) and overall pregnancy rates were 57.1% to 69.3% (mean 63.5%). Between 1.44 and 1.75 services were needed per pregnancy (mean 1.58). The lowest percent non-pregnant of cows served was 0, the highest 11.8 (mean 9.1).

Abnormal gestation lengths

A total of five cows were recorded with abortions between completion of the diagnostic tests and calving in herds 8, 10 and 13. Farmers' observation had correctly diagnosed four of these five cows as pregnant, with one declared 'suspect'. Oestrone sulphate had correctly diagnosed four cows as pregnant. The only recorded premature calving took place in herd 7. Induction of calving was carried out in herds 7, 11 and 12 only, with one, four, and seven cows induced, respectively, from mid-August onwards. Oestrone sulphate had declared ten of these twelve cows non-pregnant. Table 3.13, page 90, shows the details of abortions, and premature and induced calvings.

Table 3.12 Fertility parameters for the seven trial herds

Herd	Total number of cows in herd	Number of cows with service recorded	21-day submission rate (%)	First service pregnancy rate (%)	Overall pregnancy rate (%)	Services per pregnancy	Percent non-pregnant of cows served
7	108	100	96.3	68.0	69.3	1.44	0
8	140	140	87.2	64.3	65.8	1.52	10.7
9	116	100	72.8	61.0	63.9	1.56	10.3
10	184	178	74.8	53.9	57.9	1.73	11.8
11	217	208	84.2	53.4	57.1	1.75	11.8
12	82	82	93.2	67.1	63.2	1.58	8.6
13	202	180	68.6	63.9	67.4	1.48	10.5

Table 3.13 Date of abnormal gestation event and diagnosis made by oestrone sulphate (OS) and farmers' observation (FO) for abortions, and premature and induced calvings (P = Pregnant, MT = Non-pregnant, S = Suspect)

Herd	Type of abnormal gestation	Date	Cow ID	Gold standard	OS Diagnosis	FO Diagnosis
7	Premature	06-Jul	11848488	P	P	P
8	Aborted	03-Jul	11924657	P	P	P
	Aborted	10-Jul	8577818	P	P	P
10	Aborted	04-May	7045245	P	MT	P
13	Aborted	15-May	9663114	P	P	S
	Aborted	15-Jun	3758284	P	P	P
7	Induced	03-Oct	9982400	P	MT	P
11	Induced	14-Aug	11989429	P	P	P
	Induced	07-Sep	11989430	P	P	P
	Induced	06-Sep	7068059	P	MT	P
	Induced	07-Set	10048292	P	MT	P
12	Induced	23-Aug	6720259	P	MT	P
	Induced	24-Aug	12011450	P	MT	P
	Induced	26-Aug	10022261	P	MT	P
	Induced	28-Aug	9221872	P	MT	P
	Induced	28-Aug	6720264	P	MT	P
	Induced	29-Aug	10022281	P	MT	P
	Induced	29-Aug	9221883	P	MT	P

Discussion

Oestrone sulphate

Determination of milk oestrone sulphate by enzyme-immunoassay was less sensitive than farmers' observation for pregnancy diagnosis in all but one herd in this study. The overall sensitivity of 85.4% was similar to that reported by Henderson et al. (1995) using the same test kit. Oestrone sulphate concentration increases with advancing pregnancy (Heap et al., 1983; Tainturier et al., 1986; Henderson et al., 1994b; Henderson et al., 1995) and this was reflected in the linear increase of sensitivity for cows in later stages of gestation. It has been suggested that cows should ideally be 100 to 120 days in calf when using oestrone sulphate for pregnancy diagnosis (Power et al., 1985; Henderson et al., 1993).

For cows at least 120 days pregnant, oestrone sulphate sensitivity was improved by almost ten percent, but was still inferior to farmers' observation in this study. It did only reach a level comparable to farmers' observation for cows at least 140 days in calf. The sensitivity of 95.3% achieved for cows 120 or more days pregnant was similar to other studies (Heap and Hamon, 1979; Power et al., 1985; Henderson et al., 1992; Henderson et al., 1994a; Henderson et al., 1995), although higher sensitivities of 98% to 100% have been reported (Tainturier et al., 1986; Henderson et al., 1993; Henderson et al., 1994b). The influence of cut-off points and other factors such as transport, oestrus and mastitis on oestrone sulphate sensitivity have been discussed above in Chapter II, page 55.

Specificity of oestrone sulphate was superior to farmers' observation across all herds, but, when comparing herd by herd, it was only better in three of the six herds in which specificity could be established. Almost one in five (19.6%) non-pregnant cows were incorrectly diagnosed as pregnant with the milk test, against about one in four (26.8%) for farmers' observation. A specificity for oestrone sulphate of 100% has been reported in other studies (Heap and Hamon, 1979; Henderson et al., 1992; Henderson et al., 1993; Henderson et al., 1994a; Henderson et al., 1994b; Kourletaki-Belibasaki et al. 1995). This was achieved in herds 9 and 12, but the farmers' observation accomplished the same specificity in these two herds. A specificity of

100% would be highly desirable to avoid unnecessary feeding of non-pregnant cows over the winter and loss of potential milk yield when cows fail to calve.

In herds 9 and 12, milk was collected about one month earlier than in the other herds. This explains the low sensitivity in these two herds, with more cows in the early stages of pregnancy and, therefore, lower oestrone sulphate concentrations. Even for cows at least 120 days in calf however, the sensitivity was lower than in the other herds. There is no obvious explanation for this, especially since the fertility parameters in these two herds were comparable to the other five herds. None of the non-pregnant cows misdiagnosed as pregnant by both methods originated from herds 9 or 12. It is interesting to note, though, that herd 9 consisted of only Ayrshire cows and herd 12 of only pure-bred Jerseys. The difference between breeds is discussed below. The specificity achieved in these two herds reached the maximum of 100%. The low sensitivity may in part be contributing to this, with an overestimation of non-pregnant cows.

Sensitivity of oestrone sulphate appeared to be higher, and specificity lower in those herds where milk samples were drawn from in-line collectors during a herd improvement test. The most practical approach for using oestrone sulphate for pregnancy diagnosis would be to combine it with the routine herd testing of milk, but these results suggest that this technique may increase the number of false positive diagnoses. However, the apparent difference may be a reflection of the overall herd difference observed (with herds 11 and 13 showing lower specificity for both methods than the other herds) rather than a true difference between collecting methods. It has been shown that milk composition has no influence on oestrone sulphate analysis (Hamon et al., 1981; Bloomfield et al., 1982) and a possible different composition of a milk sample collected manually before cluster attachment to one collected with in-line collectors should not affect the test result.

Marked differences between breeds were observed. It is difficult to establish whether this is truly a breed difference or a reflection of differences between herds. All the Ayrshire cows originated from one herd (herd 9) and most of the pure-bred Jersey cows from another (herd 12) which leads to the confounding of herd and breed effects. Both breeds have a higher butterfat content than Friesians, but as mentioned above, milk composition was not found to have an influence, and Heap and Hamon (1979) found no difference between Friesian and Jersey cows. Abdo et al. (1991), however, did observe a lower oestrone sulphate concentration in the milk of Swedish Jersey cows during the second trimester of pregnancy compared to other Swedish breeds. So it may be possible that the high butterfat content does influence oestrone sulphate as a hydrophilic substance, leading to a reduced sensitivity. With the Jersey and Ayrshire breeds accounting for a fair proportion of New Zealand dairy cattle (16% in the 1996/97 season; Livestock Improvement Corporation, 1997a), this would have implications for using oestrone sulphate for pregnancy diagnosis.

Farmers' observation

Sensitivity of farmers' observation was high and exceeded the 94.1% reported by Roche et al. (1978) . The maximum of 100% was achieved in the smallest herd. As farmers' observation relies on a definite sign of non-pregnancy, like return to oestrus, the stage of gestation should not have any influence on sensitivity and this was indeed observed in this study. An incorrect diagnosis of non-pregnant may arise from pregnant cows expressing oestrus, which occurred in 7.3% and 5.6% of cows in two studies (Williamson, et al., 1972b; Moller et al., 1986). A recording of 'suspect' may have arisen from perceived oestrus behaviour, but only 0.6% to 4.2% of pregnant cows were declared 'suspect' in three of the herds (excluding herd 13). The high sensitivity achieved by farmers' observation suggests that oestrus behaviour was not wrongly identified to any large extent. This is in contrast to studies where between 4.8% and 11.8% of cows identified as expressing oestrus either were in dioestrus or had inactive ovaries (Reimers et al., 1985; Moller et al., 1986; Markusfeld, 1987; Rajamahendran et al., 1993; Kourletaki-Belibasaki et al., 1995). The negative predictive value achieved by farmers' observation in this study was below the 90.7% reported by Roche et al. (1978).

Specificity of farmers' observation was inferior to oestrone sulphate, but overall was better than the 54% achieved in the study by Roche et al. (1978). Counting cows recorded as 'suspect' as misdiagnoses, however, gave a specificity similar to that found by Roche et al. The positive predictive value achieved in this study exceeded that observed by Roche et al. by far (97.0% vs. 65.9%). Low specificity reflects an overestimation of the number of pregnant animals. Since a definite sign such as return to oestrus, is needed to identify non-pregnant animals, this might be expected.

Specificity was considerably different between herds, with the smallest herd achieving 100%, together with the third smallest herd (herd 9). The ability of herdsmen to detect oestrus varies widely (Williamson et al., 1972a; Esslemont, 1974b; King et al., 1976; Lehrer et al., 1992; Gaines et al., 1993; Nebel et al., 1995) and it is interesting to note that specificity was poorest in the three largest herds in this study, which were either managed by two partners, or the owner and a stockman. Less time may be spent observing cows in larger herds and employed labour may be less interested in accurate observations than herd owners. Moller et al. (1986) found that 22.1% of cows returning to oestrus at 21 days after insemination were not detected.

The consistent decline in the specificity with increasing interval from the last service date may be explained by decreasing attention to an animal that has not returned to oestrus after one or two normal inter-oestrus intervals. If inadequate attention is payed to an animal, the likelihood of it being observed in oestrus is small, as shown in a study by McDougall (1994) where 60% of oestrus events were not detected before the planned start of mating. Farmers using oestrus observation for pregnancy diagnosis may not be aware of the incidence of late returns to oestrus, reported to be between 3.8% at 36 to 49 days after last service to 22.7% between 28 and 75 days after breeding, and mainly attributed to embryonic or foetal death (New Zealand Dairy Board, 1976; Wijeratne, 1973; Kummerfeld et al., 1978; Moller et al., 1986). Equally, they may concentrate their observations on the likely time a cow would return, but may not be aware that the inter-oestrus interval is not 17 to 24 days in all cows (Esslemont and Bryant, 1974; Esslemont, 1974b).

The rate of foetal loss is highest in the first trimester of gestation (Hancock, 1962; Ball, 1978; Reimers et al., 1985; Moller et al., 1986; Screenan and Diskin, 1986; Allen, 1997), but between 2% and 10.6% of cows are reported to have suffered foetal loss between 42 and 260 days of gestation (Thurmond et al., 1990; Mee et al., 1994; Bouchard et al., 1998). Screenan and Diskin (1986) observed foetal loss in 5.8% of cows after 42 days, i.e. after the second normal inter-oestrus interval.

Prolonged anoestrus after an unsuccessful insemination was found to occur in 2.1% of cows in a study by Moller et al. (1986), and in up to 20% of cows inseminated after progesterone treatment (Macmillan, 1996). Farmers' observation would not identify these cows as non-pregnant.

The owner of herd 13 recorded a large percentage of cows as 'suspect', not discriminating between truly pregnant or non-pregnant cows. His observations were mainly based on high body condition and milk production of particular cows relative to their herdmates. Whereas this may give a true indication of the pregnancy status in some animals, it is not a reliable sign, as reflected in the relatively poor sensitivity, specificity and negative predictive value achieved in this herd. Using oestrone sulphate for pregnancy diagnosis would appear beneficial in this herd.

Factors affecting accuracy of both methods

Probability of a correct diagnosis

The probability of correctly diagnosing pregnant cows was highest for both methods in herds 11 and 13. Specificity showed the converse, with it being lowest in these two herds. An overestimate of pregnant cows would lead to a reduced specificity, but why both methods showed this herd pattern is not obvious. Herd 13 had a low 21-day submission rate and herd 11 showed the lowest pregnancy rates, but both these factors would be expected to have a negative effect on sensitivity, not specificity. Both herds had a high percentage of cows with full results and had high numbers of cows non-pregnant, i.e. one misdiagnosis was not likely to have a greater impact than in the other herds. Two of the likely abortions occurred in herd 11 and additional unnoticed foetal loss could explain these observed results.

Identification of cows

Cows appeared to be well known by the farmers and wrong identification was unlikely in these relatively small herds relying on oestrus observation for pregnancy diagnosis. Milk samples may have been wrongly identified, but the collection during a herd test was a reflection of the practical conditions under which the test might be applied. Manual milk collection took place in the smaller, owner-operated herds and was completed without any pressure to achieve fast throughput during milking.

Foetal loss

Abortion is likely to have occurred in the five cows that were diagnosed pregnant by both methods but failed to calve. Adding these cows to the six recorded abortions and premature calvings would give a foetal loss rate of 1.3%, slightly below the rate observed in New Zealand dairy cattle by Moller et al. (1986) . Two studies found a foetal loss rate of below two percent (Bulman and Lamming, 1979; Beal et al., 1992), but a rate of 2.5% or above is more commonly reported (Hancock, 1962; Kummerfeld et al., 1978; Vaillancourt et al., 1979; Reimers et al., 1985; Screenan and Diskin, 1986; Weigelt et al., 1988; Thurmond et al., 1990; Lemire et al., 1993; Mee et al., 1994; Mohammed et al., 1991; Warnick et al., 1995; Bouchard et al., 1998).

Fertility and management parameters of study herds

Because oestrone sulphate concentration increases with advancing gestation, it was important that fertility and breeding management in the study herds accurately reflected the situation in the New Zealand seasonal dairy cattle population. The study herds had fertility parameters comparable to other New Zealand seasonal calving herds.

21-day submission rate

Two herds surpassed the 21-day submission rate target of 90% in the Dairyman program (Massey University). Rates in four of the remaining five herds were within or close to the range of 75.9% to 86% found by Hayes (1996 and 1997) . The poorest rate was in the second but largest herd, in which the pregnancy status of a large proportion of cows was recorded as 'suspect'.

First service and overall pregnancy rate

The Dairyman (Massey University) target of 60% for both first service and overall pregnancy rate was exceeded in five herds. The two herds not achieving this target were still within the range of first service pregnancy rates of 48.5% to 71% and overall pregnancy rates of 51.5% to 75% reported in seasonal dairy herds in New Zealand (Hayes, 1996; Hayes 1997; Macmillan, 1998).

Services per pregnancy

Only two herds exceeded the Dairyman (Massey University) target of 1.7 services per pregnancy, but both herds fell within the range of 1.2 to 2.4 services per pregnancy reported in previous New Zealand studies (Fielden et al., 1980; Hayes, 1996; Macmillan, 1998).

Breeding period

The total breeding period ranged from 63 to 104 days in the study herds, with artificial insemination being used for the first 33 to 51 days. These periods are similar to total breeding periods of 72 to 108 days and artificial insemination periods of 36 to 43 days reported by Macmillan and Moller (1977) and Hayes (1996) .

Induction rate

Induction rates varied from 1% to 10% in the three herds that induced parturition. These rates are acceptable, with the target being up to 10% (Williamson, 1998) and reported rates 5.9% to 11.3% (Macmillan et al., 1990; Hayes, 1996).

Cull rate

The cull rate in all but one of the study herds was below the target cull rate of 20% often stated by farmers and reported rates of 17.6% to 19.0% (Bradford, 1968; New Zealand Dairy Board, 1976; Macmillan and Moller, 1977). Herd 10, with a cull rate just above target, had a mastitis problem during the period of the study, which increased the number of cows culled.

Percent non-pregnant of cows served

Four out of six of the study herds had a mean percentage of cows non-pregnant of cows served similar to the mean rates of 6.5% to 10.7% reported in New Zealand dairy herds (Morris et al., 1994; Hayes, 1997; H. Crabb, personal communication, 1998; Macmillan, 1998). Two herds experienced a slightly higher percentage. In herd 7, where the few cows believed to be non-pregnant were lost to follow-up, a percentage of zero resulted from including cows with a gold standard result only. This way of calculating the rate also increased the percentage in the other herds, as the exclusion of cows with incomplete results reduced the total number of cows on which the calculation was based, leading to a greater impact of non-pregnant cows.

Conclusion

The results of this study indicate that pregnancy diagnosis by oestrone sulphate enzyme-immunoassay may be valuable in some herds currently using oestrus observation. Sensitivity of oestrone sulphate was on average lower than that of farmers' observation. And, as in the study described in Chapter II, it did not reach its plateau level until about twenty days later than expected at 140 days of gestation. Specificity of oestrone sulphate, although on average higher than that of farmers' observation, was below the expected level with about one in five non-pregnant cows incorrectly diagnosed as pregnant.

A high accuracy was achieved with farmers' observation in four of the seven herds and oestrone sulphate would have offered no advantages over the currently used method. The three larger herds in the study would have benefited from an alternative method of pregnancy diagnosis. However, it is arguable whether oestrone sulphate should be employed rather than a more accurate method like rectal palpation or ultrasonography. Farmers' preferences and perceptions of advantages or disadvantages of the different methods (for example, the need to yard cows for rectal examination) would influence this decision.

CHAPTER IV

ECONOMIC EVALUATION OF FOUR DIFFERENT METHODS OF PREGNANCY DIAGNOSIS IN SEASONAL NEW ZEALAND DAIRY HERDS

Introduction

Economic analysis of veterinary procedures or intervention has gained increasing attention in farm animal practice over the last twenty years. In the case of diagnostic tests, the most accurate method is not necessarily the most cost-effective. The direct and indirect costs may make a slightly less accurate method more profitable. Pregnancy diagnosis involves the cost of the test procedure itself plus immediate costs to enable the diagnostic test to be carried out (labour, facilities).

But the largest indirect cost is associated with cows diagnosed incorrectly as pregnant or non-pregnant, with subsequent management decisions, in particular culling, based on the diagnosis. Two methods of similar overall accuracy may result in different total costs, if one method has a poorer sensitivity or specificity than the other.

Different economic models have been suggested to cost veterinary procedures. Simple models are sufficient in less complex situations and are useful in a number of practical scenarios where there is a choice of management regimes. More complex models, like the simulation and optimising (or dynamic programming) models, incorporate the biophysical changes inherent to farm animal production, but require very detailed analyses and may not be suitable to make general recommendations (DeLorenzo et al., 1992; Williamson, 1994; Enevoldsen et al., 1995).

The partial farm budget predicts the return of a decision or procedure that may have economic implications (Sprecher et al., 1989). It takes into account the additional income generated from the proposed procedure, the costs of that procedure, the savings made, and the potentially lost income (Williamson, 1994).

Only parameters that are likely to change as a direct result of the decision are included and it is particularly suitable for endemic diseases or for decisions whether to introduce a procedure or not (Erb, 1984).

Another example of a fairly simple model is the decision analysis or decision tree. This method does require knowledge of the risk or probability of a particular outcome and is particularly suitable for sporadic or epidemic diseases. The layout of the decision tree provides a good visual guide to the decision options and their likely economic impact (Erb, 1984; Fetrow et al., 1985; Pitcher and Galligan, 1990; Williamson, 1994). In both the partial farm budget and the decision analysis, initial calculations are usually based on default values. One or more parameters are then changed to find threshold values or compute indifference curves (Fetrow et al., 1985; Pitcher and Galligan, 1990).

Based on the findings of the studies described in Chapters II and III, a partial farm budget was used to financially compare the different methods of pregnancy diagnosis. The impact of changing some parameters was established in a sensitivity analysis. An example of a decision tree for comparing the different diagnostic methods is also provided.

Materials and methods

A partial farm budget was created using a computer spreadsheet (Microsoft Excel). Total cost of pregnancy diagnosis by oestrone sulphate was compared with pregnancy diagnosis by rectal palpation or ultrasonography (Figure 4.1, page 101). In a second model, oestrone sulphate was compared with farmers' observation (Figure 4.2, page 102). Total cost of pregnancy diagnosis per cow was established, both inclusive and exclusive of opportunity cost. The value for one or two parameters was then changed to establish threshold values for the breakeven point when oestrone sulphate would be more cost-effective than the other methods.

Figure 4.1 Spreadsheet used to calculate cost of pregnancy diagnosis by oestrone sulphate*, rectal palpation and ultrasonography

	A	B	C	D	E	F
1				Test method		
2		**Default values**	**Oestrone sulphate**	**Rectal palpation**	**Ultrasono-graphy**	**Reference code**
3	**Herd Size**	200				
4	**Cows pregnant of served (%)**	88				
5	Number of cows pregnant	=B3*B4/100	176	176	176	
6	Number of cows non-pregnant	=B3-B5	24	24	24	
7						
8	**Decision variables**					
9	Sensitivity		81.8	100.0	99.9	
10	Specificity		81.0	91.4	90.9	
11	Direct test cost / cow		2.00	2.11	2.00	
12	Total test cost / herd	=B3*B11 (+mileage)	400.00	444.20	400.00	
13	Time / cow (sec)		0	60	27	
14	Total time (min)	=B3*B13/60+45	0	245	135	
15	Farmlabour cost / hr	10.00	10.00	10.00	10.00	
16	Total farmlabour cost	=B14/60*B15*2	0.00	81.67	45.00	
17	Daily autumn milk production / cow (kg MS)	1.1	1.1	1.1	1.1	1
18	Milk loss	0.05	0	0.05	0.05	
19	Price / kg Milksolids	3.20	3.20	3.20	3.20	2
20	Milk profit loss / cow	=B17*B18*B19	0.00	0.176	0.176	
21	Milk profit loss / herd	=B20*B3	0.00	35.20	35.20	
22	**Direct cost to PD herd**	=B12+B16+B21	**400.00**	**561.07**	**480.20**	
23	Cull value autumn	220 kgDW @ 1.10/kg	242.00	242.00	242.00	3
24	Cull value spring	195 kgDW @ 1.38/kg	269.10	269.10	269.10	3
25	Cost of replacement	600.00	600.00	600.00	600.00	4
26	Average lactation milkyield cow (kg MS)	300	300	300	300	5
27	Lost profit milkyield for heifer	=B26*0.25*B19	240.00	240.00	240.00	
28	Cow: Calf value	70.00	70.00	70.00	70.00	6
29	Heifer: Calf value	20.00	20.00	20.00	20.00	6
30	Lost value calf	=B28-B29	50.00	50.00	50.00	
31	Dry period (days)	142	142	142	142	7
32	Feed cost / day (7 kgDM @ 15c/kgDM)	1.05	1.05	1.05	1.05	8
33	Total feed costs	=B31*B32	149.10	149.10	149.10	
34	Direct cost of incorrect non-pregnant diagnosis	=B25+B27+B30-B23	648.00	648.00	648.00	
35	Direct cost of incorrect pregnant diagnosis	=B33	149.10	149.10	149.10	
36	Lost opportunity per incorrect pregnant diagnosis	=(B26*B19)+B28-B24	760.90	760.90	760.90	
37	No. cows diagnosed incorrectly non-pregnant	=(1-B9/100)*B5	0.00	0.00	0.18	
38	No. cows diagnosed incorrectly pregnant	=(1-B10/100)*B6	4.56	2.06	2.18	
39	No. cows diagnosed non-pregnant by OS	=(1-C9/100)*C5+C10/100*C6	51			
40	**Total cost of PD per cow (incl. cost for retesting)**	=(B22+B34*B37+B35*B38)/B3	**6.54**	**4.34**	**4.60**	
41	Cost of lost opportunity	=B36*B38	19.37	7.85	8.31	
42	**Total cost of PD per cow incl. lost opportunity**	=B40+B41	**25.91**	**12.20**	**12.91**	

* Assuming that cows diagnosed as non-pregnant are re-examined by rectal palpation. Column used to calculate cost of re-examination not shown.

Figure 4.2 Spreadsheet used to calculate cost of pregnancy diagnosis by oestrone sulphate and farmers' observation*

	A	B	C	D	E	F	G
1			**Test method**				
2		**Default values**	**Oestrone sulphate**	**Farmers' observation**	**Oestrone sulphate incl. retest**	**Farmers' observation incl. retest**	**Reference code**
3	**Herd Size**	200					
4	**Cows pregnant of served (%)**	88					
5	Number of cows pregnant	=B3*B4/100	176	176	176	176	
6	Number of cows non-pregnant	=B3-B5	24	24	24	24	
7							
8	**Decision variables**						
9	Sensitivity		85.4	98.6	85.4	98.6	
10	Specificity		80.4	66.7	80.4	66.7	
11	Direct test cost / cow		2.00	0.00	2.00	0.00	
12	**Direct cost to PD herd**	=B3*B11	400.00	0.00	400.00	0.00	
13	Price / kg Milksolids	3.20	3.20	3.20	3.20	3.20	2
14	Cull value autumn	220 kgDW @ 1.10/kg	242.00	242.00	242.00	242.00	3
15	Cull value spring	195 kgDW @ 1.38/kg	269.10	269.10	269.10	269.10	3
16	Cost of replacement	600.00	600.00	600.00	600.00	600.00	4
17	Average lactation milkyield cow (kg MS)	300	300	300	300	300	5
18	Lost profit milkyield for heifer	=B17*0.25*B13	240.00	240.00	240.00	240.00	
19	Cow: Calf value	70.00	70.00	70.00	70.00	70.00	6
20	Heifer: Calf value	20.00	20.00	20.00	20.00	20.00	6
21	Reduced value calf	=B19-B20	50.00	50.00	50.00	50.00	
22	Dry period (days)	142	142	142	142	142	7
23	Feed cost / day (7 kgDM @ 15c/kgDM)	1.05	1.05	1.05	1.05	1.05	8
24	Total feed costs	=B22*B23	149.10	149.10	149.10	149.10	
25	Direct cost of incorrect non-pregnant diagnosis	=B16+B18+B21-B14	648.00	648.00	648.00	648.00	
26	Direct cost of incorrect pregnant diagnosis	=B24	149.10	149.10	149.10	149.10	
27	Lost opportunity per incorrect pregnant diagnosis	=(B17*B13)+B19-B15	760.90	760.90	760.90	760.90	
28	No. cows diagnosed incorrectly non-pregnant	=(1-B9/100)*B5	26	2	0	0	
29	No. cows diagnosed incorrectly pregnant	=(1-B10/100)*B6	5	8	5	8	
30	No. cows diagnosed non-pregnant	=(1-B9/100)*B5+B10/100*B6	45	18			
31	**Total cost of PD per cow (incl. cost for retesting)**	=(B12+B25*B28+B26*B29)/B3	**88.76**	**13.94**	**6.63**	**6.53**	
32	Cost of lost opportunity	=(B27*B29)/B3	17.90	30.41	19.66	31.13	
33	**Total cost of PD per cow incl. lost opportunity**	=B31+B32	**106.66**	**44.35**	**26.29**	**37.66**	

Reference code for Figures 4.1 and 4.2

1. Livestock Improvement Corporation (1997a): Average daily milk production in March 1996/97
2. Anonymous (1997): New Zealand Dairy Board pay-out for 1996/97 season
3. Anonymous (1996) and Anonymous (1997): Prices for cull cows, M-grade, 196-220kg deadweight. Calculations based on average liveweight of Friesian-cross cow of 440 kg (Livestock Improvement Corporation, 1997a) and 50% slaughter percentage. For cull cow value in Spring, cows are assumed to have lost two body condition score points, equalling 50 kilogram liveweight, with an increase of 25% in price per kilogram deadweight over Autumn prices.
4. Graham (1997)
5. Livestock Improvement Corporation (1997a): Average total milkproduction for 1996/97 season.
6. Anonymous (1998): Assuming cow produces good quality calf and heifer produces small calf. Prices averaged between value of bull and heifer calf.
7. Livestock Improvement Corporation (1997a): Average days-in-milk in 1996/97 season.
8. McGrath (1997): Cost of pasture eaten at NZ$ 0.15/kgDM, assuming average dry-matter-intake of 7kg per day throughout dry period.

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* Columns E and F assume that cows diagnosed as non-pregnant are re-examined using rectal palpation. Column used to calculate cost of re-examination not shown.

Initial calculations

Prices and charges current at the time of the study were used. A reference code is given in the models where appropriate. Default sensitivity and specificity for all methods were based on the average values established in Chapters II and III. For farmers' observation, the sensitivity and specificity excluding suspect cows were used.

In addition, the cost of pregnancy diagnosis in each herd was calculated using the results relevant for that herd (size, percentage of cows pregnant, sensitivity and specificity of methods). Costs of oestrone sulphate, rectal palpation and ultrasonography were also calculated using the sensitivity and specificity established for cows at least 120 days pregnant or since their last service.

Herd structure

Initial calculations were based on a herd of 200 cows with a 'Cows pregnant of served' rate of 88% to reflect an average seasonal herd in New Zealand (Morris et al., 1994; Hayes, 1997; Livestock Improvement Corporation, 1997a).

Cost of each test

At the time of the study, the cost of the commercially available oestrone sulphate enzyme-immunoassay was NZ$ 2.00 per cow. When applied as routine diagnostic test, milk collection would be combined with a herd improvement test. Therefore, no further costs for milk collection or labour were added. All cows diagnosed as non-pregnant by oestrone sulphate were assumed to undergo re-examination by rectal palpation. The costs of re-examination, including costs arising from misdiagnosis by rectal palpation, were added to the total costs of oestrone sulphate. Since the accuracy of rectal palpation influences the cost of re-examination, the default values for rectal palpation were used when comparing oestrone sulphate to ultrasonography.

No direct costs were attributed to farmers' observation. None of the farms participating in this part of the study spent time purely on oestrus observation. To enable comparison, all cows diagnosed as non-pregnant were assumed to undergo re-examination by rectal palpation.

For pregnancy diagnosis by rectal palpation, the author used the average fees charged by veterinary practices of NZ$ 2.11 per cow and NZ$ 0.74 per kilometre, as established by the 1997 survey of the New Zealand Veterinary Association (NZVA, 1998). Mileage calculations were based on a one-way charge of 30 kilometres for each farm.

For ultrasonography, the fees charged by the operator participating in the trial were used. At the time these were NZ$ 2.00 per cow for >200 cows examined, NZ$ 2.20 per cow for 101 to 200 examined, and NZ$ 2.60 per cow for <100 examined (subject to a minimum fee of NZ$ 50). No mileage or set-up fee was charged. Purchase and depreciation costs for the ultrasound equipment were ignored for the purpose of this analysis.

Farm labour costs

For rectal palpation and ultrasonography, farm staff was employed in drafting cows. To establish time spent by farm labour, the speed of examination by rectal palpation and ultrasonography in each herd was recorded. A record was kept of special circumstances, like request for confirmation of service date or attendance by veterinary undergraduate students. The average time across all herds for each method was used for the initial calculations and it was assumed that two members of farm staff were assisting. In addition, one-and-a-half hours of farm staff time were allowed per visit for collecting cows from, and returning them to, the pasture. An hourly rate of NZ$ 10.00 was used for farm labour.

Effect of yarding on milk production

To establish the effect of confining cows for several hours without food on milk production, total herd milkvolume and milksolids production were recorded (based on the milk tanker meter-reading) for the milk collection immediately after pregnancy diagnosis, and two milk collections each before and after the day of examination. In two herds, milk production records of the four months January to April were used to establish behaviour of herd milk production over time. The SAS System was used for statistical analysis. An analysis of variance (ANOVA) was used to estimate the variation in each herd and construct confidence intervals for the periods before and after the examination.

It was then established whether the production on the day of examination fell within those confidence intervals. The milk production data from January to April was subjected to a time series analysis looking at the effect of 'disturbance' by the pregnancy diagnosis. Disturbance was estimated for the day of the examination and the day after the examination, i.e. the lagged effect. The estimated disturbance was translated into a percentage.

Cost of misdiagnosis

The cost of an incorrect non-pregnant diagnosis was assumed to be the sum of the cost of a replacement animal, the difference in milk production between a first lactation and an older cow[10], and the lower value of a calf born to a heifer[11], minus the cull value of the misdiagnosed cow. It was assumed that all cows diagnosed as non-pregnant were culled.

The loss arising from an incorrect diagnosis of pregnant was assumed to be the costs of feeding that cow over the dry period. It was assumed that no cows would be culled before the end of the season and all cows would be dried off on the same day. Incorrect diagnoses of pregnant were also assigned a lost opportunity cost. This was calculated as the sum of lost milk production in the following season, and lost calf value, minus the cull value of the cow in spring.

Threshold values

Sensitivity and specificity of either one or all methods were changed until a breakeven point was reached. The percentage of cows pregnant was altered. Direct test costs were gradually decreased to an endpoint of NZ$ 0.10 per cow for oestrone sulphate, and increased to a maximum of NZ$ 4.00 per cow for rectal palpation and ultrasonography. The influence of a change in the payout for milk, the price of a replacement animal, or the feeding costs over the dry period was established.

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[10] Heifers produce 73 to 76% of mature yield, depending on breed (New Zealand Dairy Board, 1981)
[11] Heifers generally produce smaller calves, resulting in beef cross calves having a lower market value and purebred dairy calves often being regarded as unsuitable dairy replacements.

Decision Tree

A decision tree was created to compare oestrone sulphate with rectal palpation and ultrasonography. The first decision node was the method of pregnancy diagnosis, followed by one of two possible diagnosis results (pregnant vs. non-pregnant), followed by a third decision node for the true pregnancy status of the animal (pregnant vs. non-pregnant; Figure 4.3, page 107). Probabilities of events were based on the average sensitivity, specificity, and positive and negative values for the three methods as established in Chapter II. The percentage of cows pregnant was assumed to be 88%. It was assumed that all cows diagnosed as non-pregnant by oestrone sulphate would undergo re-examination by rectal palpation. The cost entered for cows diagnosed as non-pregnant by oestrone sulphate was the sum of the direct test charges for these two methods. The cost for a correct diagnosis was entered as direct test charge.

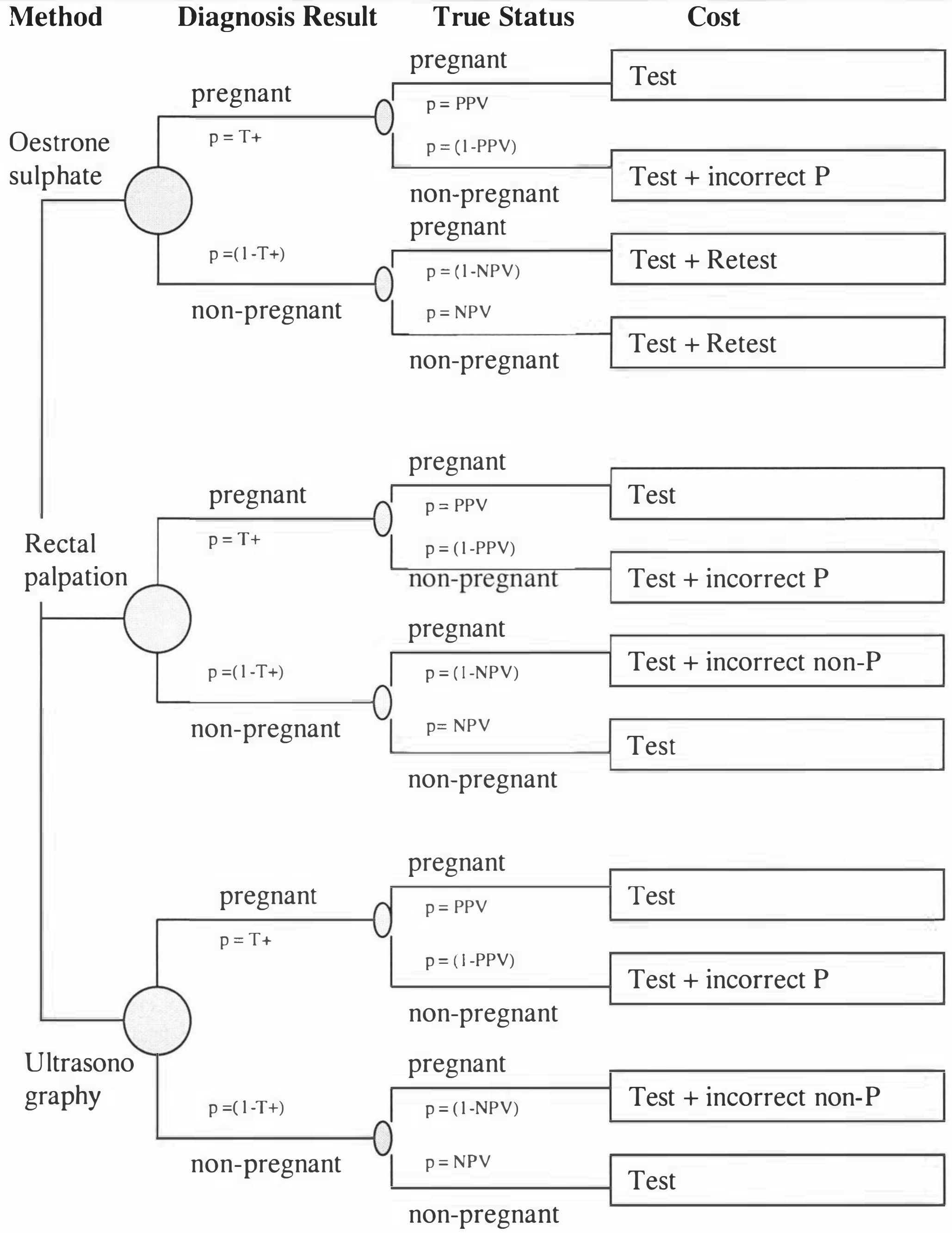
The costs of incorrect pregnant and incorrect non-pregnant diagnoses were calculated as for the partial farm budget. An expected monetary loss (EML) was calculated for each decision node. The EML is the sum of probabilities of an outcome multiplied by the costs associated with that outcome (Mohammed et al., 1990).

Results

Time taken for examination

Table 4.1, page 108, shows time taken to examine each cow by rectal palpation and ultrasonography, throughput of cows per hour, facilities, and special circumstances, like confirmation of service dates. The average time to carry out pregnancy diagnosis by rectal palpation was 60.2 seconds per cow (range 46.7 to 89.0), leading to an average of 60 cows examined per hour. Pregnancy diagnosis by ultrasonography took on average 26.9 seconds per cow (range 17.3 to 35.4), resulting in an average of 134 cows examined per hour.

Figure 4.3 Decision tree for comparing different methods of pregnancy diagnosis



p = probability of that event
T+ = probability that diagnosis result is 'pregnant'
= P_{preg} x Sensitivity + (1-P_{preg}) x (1-Specificity)
where P_{preg} = probability of being pregnant based on percentage of 'Cows pregnant of served'
PPV = positive predictive value
NPV = negative predictive value
incorrect P = cost of misdiagnosis as pregnant
incorrect non-P = cost of misdiagnosis as non-pregnant

Table 4.1 Time taken for pregnancy diagnosis by rectal palpation (RP) and ultrasonography (US) in each herd

Herd	Method	No. cows examined	Cows / hour	Seconds / cow	Facilities	Special circumstances
1	RP	388	71	51	Rotary	Service confirmed
	US	381	208	17	Rotary	Late service confirmed
	US	359	154	23	Herringbone	Late service confirmed
2	RP	268	70	51	Herringbone	Late service confirmed
	US	272	121	30	Herringbone	-
3	RP	236	40	89	Rotary	Service confirmed & students attended
	US	213	160	23	Rotary	-
	US	218	109	33	Rotary	-
4	RP	239	51	70	Herringbone	Service confirmed
	US	237	102	35	Herringbone	-
5	RP	270	77	47	Herringbone	Service confirmed & students attended
	US	270	154	23	Herringbone	-
6	RP	215	59	61	Herringbone	Service confirmed
	US	350	105	34	Herringbone	-

Rotary / Herringbone = Parlour type

Average speed of rectal palpation appeared to be greater in herringbone parlours (63 cows per hour) compared to rotary parlours (55 cows per hour). The reverse was true for ultrasonography, with an average of 124 cows per hour examined in herringbone parlours, compared to 157 cows per hour in rotary parlours.

Effect of yarding on milk production

Milk volume on the day of examination was significantly lower ($p<0.05$) than the volume on the previous two days in only one herd each for both rectal palpation (herd 2) and ultrasonography (herd 5). Production of milksolids on the day of examination was significantly lower ($p<0.05$) on three occasions: for both methods in herd 5, when compared to production on the days preceding the examination. And for ultrasonography in herd 6, when compared to production after the pregnancy diagnosis (see Table 4.2, page 110). With only two data points available for the 'Before' and 'After' period, the confidence intervals were wide and the model was not robust enough to fit a trend line.

A trend line could be established, however, for the two herds with milk production data from four months. Both farms showed a gradual decline in milk yield over time. There was also an interesting correlation between day-to-day yields. A day with above average production tended to be followed by a day with below average production and vice versa.

The effect of the disturbance caused by the pregnancy diagnosis was not significant at the 5% level. In one herd, there was a marginally significant reduction immediately after the rectal palpation ($p=0.098$), but production appeared to have increased in the lag period after the examination ($p=0.164$). There were suggestions of an increase in milk production both immediately after ($p=0.17$) and in the lag period ($p=0.116$) for ultrasonography in this herd. Similar observations were made in the second herd, with rectal palpation showing a hint of reduced production immediately after the day of examination ($p=0.125$) and a marginal decrease during the lag period ($p=0.096$).

Table 4.2 Comparison of herd milk production on Day of Test with days before and after test

Herd	Phase	Method	Milkvolume (l) Mean	SD	95% CI Lower Limit	95% CI Upper Limit	Production on Day of Test	Comparison	Milksolids (kg) Mean	SD	95% CI Lower Limit	95% CI Upper Limit	Production on Day of Test	Comparison
2	Before	RP	5223.0[a]	432.7	4669.9	5776.1	4549	S	451.0[a]	33.9	392.8	509.2	408	NS
	After	RP	4099.5[a]	251.0	3546.4	4652.6	4549	NS	362.5[a]	31.8	304.3	420.7	408	NS
	Before	US	4099.5	251.0	3546.4	4652.6	4131	NS	362.5	31.8	304.3	420.7	366	NS
	After	US	4352.0	65.1	3798.9	4905.1	4131	NS	383.0	18.4	324.8	441.2	366	NS
3	Before	RP	5442.0	0.0	2783.0	8101.0	5461 / 5908[c]	NS	448.5	3.5	235.0	662.0	468 / 480[c]	NS
	After	RP	3993.0	188.8	1334.0	6652.0	5461 / 5908[c]	NS	326.5	11.3	113.0	540.0	468 / 480[c]	NS
	Before	US	5874.0	41.0	3215.0	8533.0	5495 / 5359[c]	NS	486.0	7.1	272.5	699.5	480 / 432[c]	NS
	After	US	5102.0	33.9	2443.0	7761.0	5495 / 5359[c]	NS	406.0	11.3	192.5	619.5	480 / 432[c]	NS
4	Before	RP	5327.5	106.8	4961.0	5694.0	5068	NS	474.0	4.2	437.6	510.4	465	NS
	After	RP	5063.0	137.2	4696.5	5429.5	5068	NS	456.0	8.5	419.6	492.4	465	NS
	Before	US	5745.0	24.0	5378.5	6111.5	5796	NS	499.0	5.7	462.6	535.4	501	NS
	After	US	5636.0	329.5	5269.5	6002.5	5796	NS	502.0	35.4	465.6	538.4	501	NS
5	Before	RP	3308.5[b]	10.6	3009.8	3607.2	3087	NS	292.5[a]	4.9	277.6	307.4	275	S
	After	RP	2951.5[b]	143.5	2652.8	3250.2	3087	NS	271.0[a]	4.2	256.1	285.9	275	NS
	Before	US	3505.0	96.2	3206.3	3803.7	3150	S	300.5	4.9	285.6	315.4	284	S
	After	US	3318.0	250.3	3019.3	3616.7	3150	NS	289.0	12.7	274.1	303.9	284	NS
6	Before	RP							680.5	29.0	635.4	725.6	703 / 704[c]	NS
	After	RP							695.5	30.4	650.4	740.6	703 / 704[c]	NS
	Before	US							669.5	3.5	624.4	714.6	646	NS
	After	US							705.0	18.4	659.9	750.1	646	S

[a] = Significant difference between 'Before' and 'After' phase ($p<0.05$) [b] = Marginal difference between 'Before' and 'After' phase ($p<0.1$) [c] = Test carried out on two days
SD = Standard deviation CI = Confidence interval RP = Rectal palpation US = Ultrasonography S = Significant ($p<0.05$) NS = Not significant ($p>0.05$)

Ultrasonography caused a slight increase in production immediately after examination (p=0.182) and a significant increase during the lag period (p=0.013) in this second herd. Estimates of the disturbance caused ranged from –5.8% to +6.4%. The default value for lost milk production due to yarding was set at five percent in the partial farm budget.

Cost of pregnancy diagnosis

Oestrone sulphate vs. rectal palpation and ultrasonography

Based on default values

Using the average sensitivity and specificity established for each method in Chapter II, oestrone sulphate was the most expensive method at NZ$ 6.54 per cow and rectal palpation the least expensive at NZ$ 4.34 per cow, with ultrasonography costing NZ$ 4.60 per cow. Including opportunity costs for incorrect diagnoses of pregnant increased the cost of pregnancy diagnosis by oestrone sulphate almost four-fold to NZ$ 25.91. The cost of rectal palpation and ultrasonography increased just under three-fold to NZ$ 12.20 and NZ$ 12.91, respectively. Based on the sensitivity and specificity for cows at least 120 days pregnant or since their last service, the costs reduced to NZ$ 4.81, NZ$ 3.91 and NZ$ 3.53, respectively, making ultrasonography the least expensive method. Total costs including lost opportunity were NZ$ 16.98, NZ$ 9.58, and NZ$ 6.36 for oestrone sulphate, rectal palpation and ultrasonography, respectively.

Based on factors in each herd

Cost of pregnancy diagnosis per cow using oestrone sulphate, rectal palpation or ultrasonography ranged from NZ$ 3.24 to 10.43, NZ$ 3.21 to 5.27, and NZ$ 3.55 to 6.99, respectively, in the six trial herds. Inclusion of opportunity costs for incorrect pregnant diagnoses increased the costs by between NZ$ 3.41 to 39.97, NZ$ 2.39 to 13.06, and NZ$ 5.97 to 11.15, for oestrone sulphate, rectal palpation and ultrasonography, respectively (Table 4.3, page 112). Oestrone sulphate was the least expensive method in one herd, rectal palpation in two herds, and ultrasonography in three herds.

Table 4.3 Cost (NZ$) of pregnancy diagnosis by oestrone sulphate (OS), rectal palpation (RP) and ultrasonography (US) in each herd

	Cost per cow			Cost per cow including Opportunity costs			Percent pregnant
Herd	**OS**	**RP**	**US**	**OS**	**RP**	**US**	
1	5.35	3.92	3.60	19.48	10.29	9.97	89.4
2	5.07	3.93	3.55	13.94	9.90	9.52	84.0
3	10.43	5.27	6.23	50.40	18.33	17.38	86.8
4	3.24	3.52	6.99	7.39	7.17	14.30	97.6
5	7.28	4.69	3.67	33.53	14.46	10.17	95.3
6	3.82	3.21	3.80	7.23	5.60	11.09	85.7

Threshold values

A change in sensitivity had a much larger impact on the cost of pregnancy diagnosis than a change in specificity (see Figure 4.4, page 113). The apparent lower increase in costs when sensitivity of oestrone sulphate decreased was due to the re-examination of cows diagnosed as non-pregnant by rectal palpation. Cows initially diagnosed incorrectly are largely diagnosed correctly on re-examination. Costs for ultrasonography follow those for rectal palpation closely.

Even when increasing the sensitivity of oestrone sulphate to 100%, while keeping the specificity of all three methods, and the sensitivity of rectal palpation and ultrasonography, at their default values, did oestrone sulphate still cost more at NZ$ 5.88. Keeping sensitivity and specificity of oestrone sulphate at their default values, the breakeven point was reached when decreasing sensitivity of rectal palpation and ultrasonography to 99.5%.

Figure 4.5, page 114, shows the change in costs when only the specificity of rectal palpation and ultrasonography was changed, with all other parameters kept at default values. The change in the cost of oestrone sulphate, despite values for sensitivity and specificity not being changed for this method, was caused by the re-examination of cows diagnosed as non-pregnant. The cost of these re-examinations altered depending on the accuracy entered for rectal palpation. Breakeven points were reached at 74.8% specificity for rectal palpation, and 80% for ultrasonography.

Figure 4.4 Change in costs depending on increase in either specificity or sensitivity. All other parameters kept at their default value. (OS = Oestrone sulphate, RP = Rectal palpation)

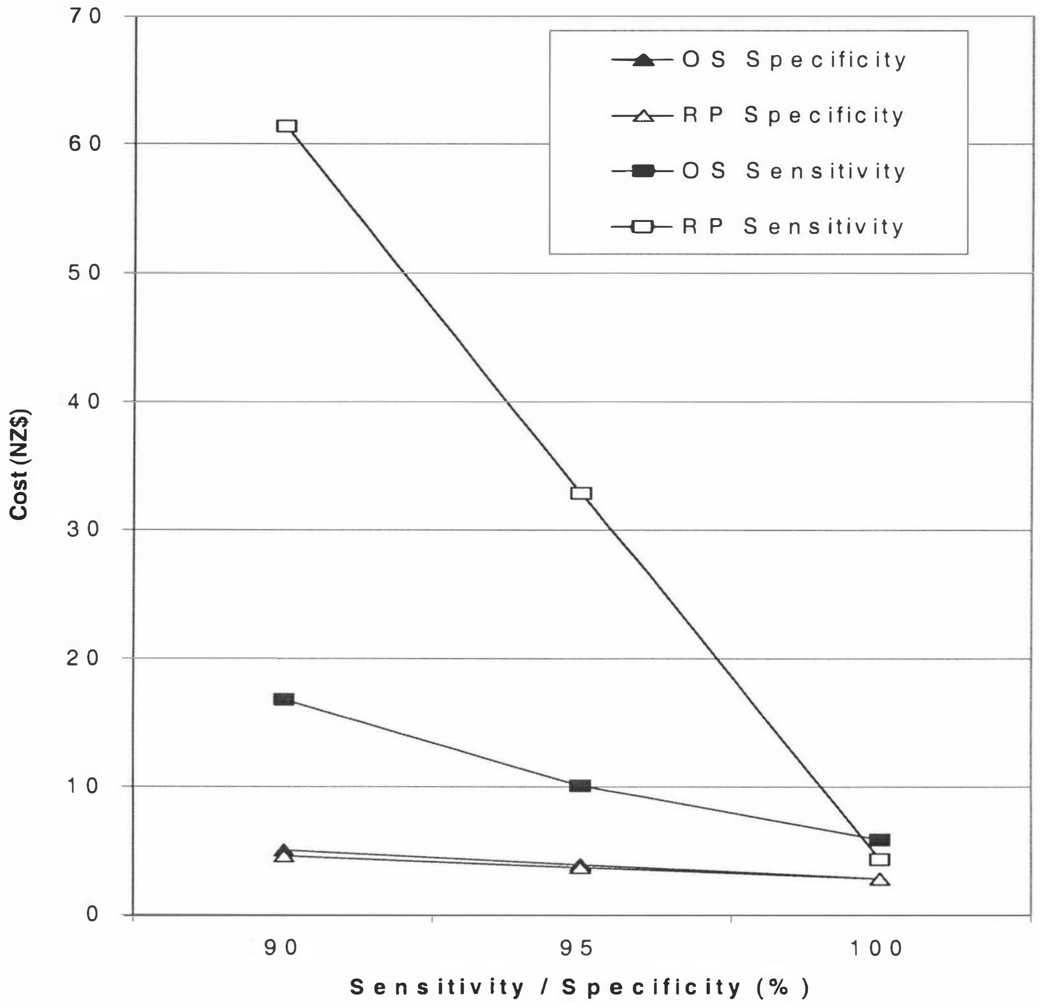
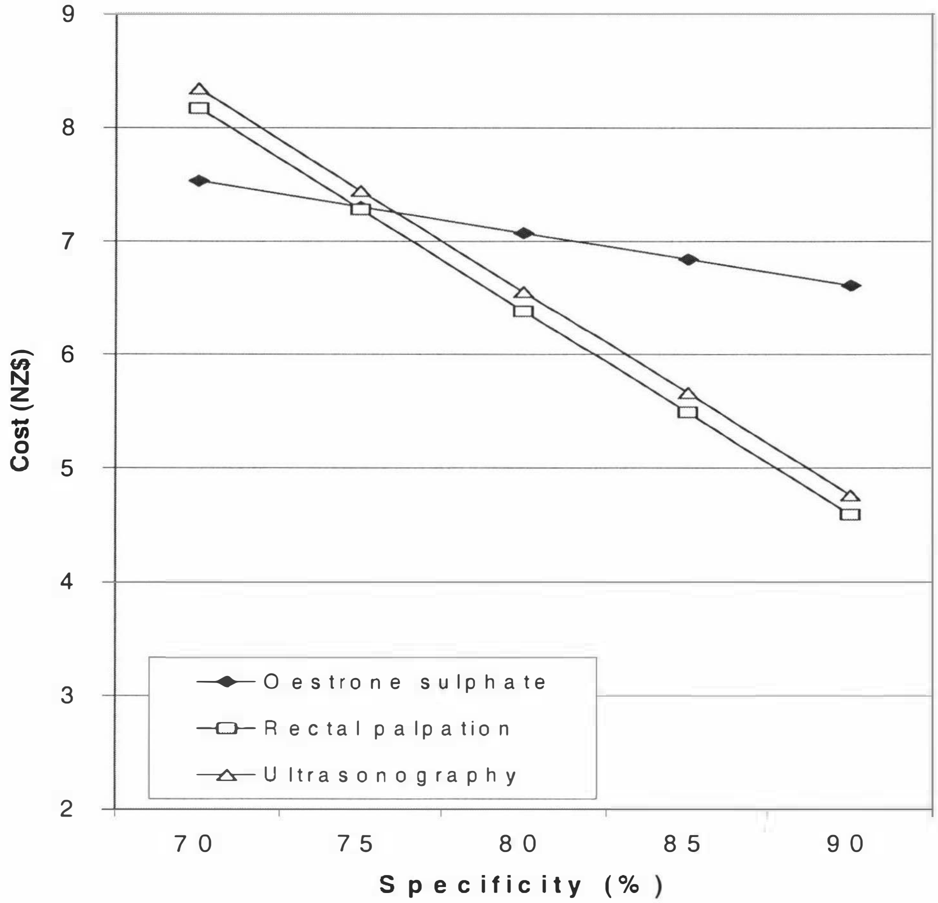


Figure 4.5 Indifference curve for cost of pregnancy diagnosis according to change in specificity for rectal palpation and ultrasonography. Sensitivity for all three methods is kept at default values, as is specificity of oestrone sulphate.



Specificity of oestrone sulphate had to increase to 93.6% to become less expensive than the other two methods, when the accuracy of rectal palpation and ultrasonography was kept at the default values.

The percentage of cows pregnant in the herd had to reach 98% for oestrone sulphate to become comparable in costs to the other two methods. When using sensitivity and specificity for cows at least 120 days pregnant or since their last service, the breakeven point lied at 95% cows pregnant.

Table 4.4, page 115, shows the maximum direct test costs that could be charged for oestrone sulphate to keep this diagnostic method competitive, depending on the direct cost charged for pregnancy diagnosis by rectal palpation or ultrasonography.

At the prices applicable at the time of the study, the charge for oestrone sulphate would have had to be reduced to less than NZ$ 0.10 for this method to become cheaper.

A change in milk price had hardly any effect on the cost of pregnancy diagnosis, regardless of method used. The cost for oestrone sulphate, for example, would have been NZ$ 6.53, 6.54, and 6.56 for a price per kilogram milksolids of NZ$ 2.00, 3.00, and 4.00, respectively. With a default sensitivity value of 100% for rectal palpation, which produces equal accuracy for oestrone sulphate because of the re-examination of cows diagnosed as non-pregnant, a change in the price of replacement animals or the value of cull cows only affected the cost of ultrasonography. But again, the influence was small. Changing the price of a replacement animal from NZ$ 200.00 to 1000.00 increased the cost of ultrasonography from NZ$ 4.25 to 4.95. Assuming either a quarter of or twice the default value for cull cows changed the cost of pregnancy diagnosis from NZ$ 4.76 to 4.39. The cost of feeding a cow through the dry period had a larger impact on the cost of pregnancy diagnosis. With feeding costs of NZ$ 100.00, 200.00 or 300.00, the cost of pregnancy diagnosis by oestrone sulphate would have been NZ$ 5.29, 7.84, or 10.39, respectively. Similar changes were seen for rectal palpation and ultrasonography, with costs of NZ$ 3.84 and 4.06, 4.87 and 5.16, and 5.90 and 6.25, respectively.

Table 4.4 Breakeven points for the direct charge per examination per cow for oestrone sulphate (OS) depending on charge per cow for rectal palpation (RP) or ultrasonography (US). Column A is based on average sensitivity and specificity for each method, and column B is based on sensitivity and specificity for cows at least 120 days pregnant or since their last service as established in Chapter II.

	Breakeven direct charge for OS (NZ$)			
	A		B	
Direct test charge for RP and US (NZ$)	**RP**	**US**	**RP**	**US**
2.00	< 0.10	0.10	1.00	0.75
2.50	0.10	0.50	1.45	1.15
3.00	0.50	0.80	1.85	1.60
3.50	0.80	1.20	2.30	2.05
4.00	1.20	1.60	2.75	2.50

Oestrone sulphate vs. farmers' observation

Based on default values

Farmers' observation was less expensive than oestrone sulphate (NZ$ 6.53 vs. 6.63) when using the average sensitivity and specificity established in Chapter III for both methods. There was a substantial difference between the methods when calculating costs under the assumption that no re-examination of cows diagnosed as non-pregnant would be carried out. Pregnancy diagnosis by oestrone sulphate would have come to NZ$ 88.76 per cow, versus 13.94 for farmers' observation. Using the sensitivity and specificity established for cows at least 120 days pregnant or since their last service, oestrone sulphate was less expensive than farmers' observation (NZ$ 6.44 vs. 7.58). However, without re-examination of cows diagnosed as non-pregnant, the cost of oestrone sulphate was more than twice that of farmers' observation (NZ$ 32.49 vs. 15.59) for this group of cows. Including opportunity costs for an incorrect diagnosis of pregnant increased the cost of pregnancy diagnosis by oestrone sulphate about four-fold to NZ$ 26.29, and by farmers' observation about five-and-a-half-fold to NZ$ 37.66.

Based on factors in each herd

Cost of pregnancy diagnosis using oestrone sulphate or farmers' observation ranged from NZ$ 2.63 to 8.86, and NZ$ 0.42 to 14.47, respectively, per cow in the seven trial herds. Including lost opportunity costs for incorrect pregnant diagnoses increased the costs by between NZ$ 0.00 to 33.05, and NZ$ 0.00 to 72.38 for the two methods, respectively (Table 4.5, page 117). Oestrone sulphate was the least expensive method in two of the seven herds.

Threshold values

A change in sensitivity did not have a large impact, because all cows diagnosed as non-pregnant were assumed to have undergone re-examination by rectal palpation. With a sensitivity of 100% for rectal palpation (as established in Chapter II), most cows were diagnosed correctly on re-examination. The minor changes seen were a reduction in cost from NZ$ 6.46 to 6.09 for an increase in sensitivity of oestrone sulphate from 90% to 100%. Costs for farmers' observation changed to a similar degree (NZ$ 6.84 to 6.48).

An increase in specificity from 70% to 100% decreased the costs from NZ$ 8.44 to 3.22, and NZ$ 5.95 to 0.74, for oestrone sulphate and farmers' observation, respectively.

Table 4.5 Cost (NZ$) of pregnancy diagnosis by oestrone sulphate (OS) or farmers' observation (FO) in each herd

	Cost per cow		Cost per cow including lost opportunity		Percent pregnant
Herd	**OS**	**FO**	**OS**	**FO**	
7	2.63	0.42	2.63	0.42	100
8	5.47	1.76	18.20	7.97	89.3
9	4.88	0.88	9.32	1.78	89.2
10	4.71	6.32	15.16	36.00	88.0
11	7.96	14.47	35.80	86.85	87.9
12	3.91	0.83	6.15	1.37	90.9
13	8.86	5.84	41.91	33.54	89.3

The breakeven point for an increase in the sensitivity of oestrone sulphate only was reached at 88%. For cows at least 120 days pregnant, a sensitivity for oestrone sulphate of 64% was sufficient to make this method less expensive than farmers' observation. The sensitivity of farmers' observation could be reduced to 95.7%, before oestrone sulphate became cheaper, when default values were used for the latter method. The threshold value for specificity of oestrone sulphate was 81% and for farmers' observation 66.1%.

A percentage above 87.5% of cows pregnant made farmers' observation the less expensive method. Using sensitivity and specificity of cows at least 120 days calved or since their last service, a percentage of cows pregnant of 92.3% or below favoured oestrone sulphate.

Assuming no direct cost for farmers' observation, a charge of up to NZ$ 1.90 made oestrone sulphate the less expensive method. Using the sensitivity and specificity for cows at least 120 days pregnant or since their last service, the charge for oestrone sulphate could rise to NZ$ 3.15.

Assuming specific time is spent on oestrus observation with a cost of NZ$ 5.60 per cow[12], oestrone sulphate would remain competitive at a charge of NZ$ 7.50 (or 8.75 for cows in advanced pregnancy).

Farmers' observation remained the less expensive method if feeding costs over the dry period stayed below NZ$ 157.00. With feeding costs of NZ$ 50.00, 100.00 or 300.00, the cost of pregnancy diagnosis by oestrone sulphate or farmers' observation would have been NZ$ 4.07 or 2.57, NZ$ 5.36 or 4.57, and NZ$ 10.53 or 12.56, respectively.

Decision Tree

The EMLs derived for each diagnostic method from the decision tree were slightly lower than the costs calculated using the partial farm budget. However, the figures were comparable and followed the same pattern with oestrone sulphate being the most expensive method (NZ$ 5.71), and ultrasonography (NZ$ 4.51) costing slightly more than the least expensive method, rectal palpation (NZ$ 4.14; see Figure 4.6, page 119).

Discussion

A diagnostic test with the lowest initial charge does not necessarily provide the most economical option, as demonstrated by the comparison of the four methods of pregnancy diagnosis. The direct, and obvious, charge per cow was higher for rectal palpation and ultrasonography because of required farm labour and possibly reduced milk production on the day of examination. But their superior sensitivity and specificity produced a total cost per cow of only approximately two-thirds of that for oestrone sulphate. The impact of indirect costs that arise from a misdiagnosis is also clear from the comparison of oestrone sulphate and farmers' observation.

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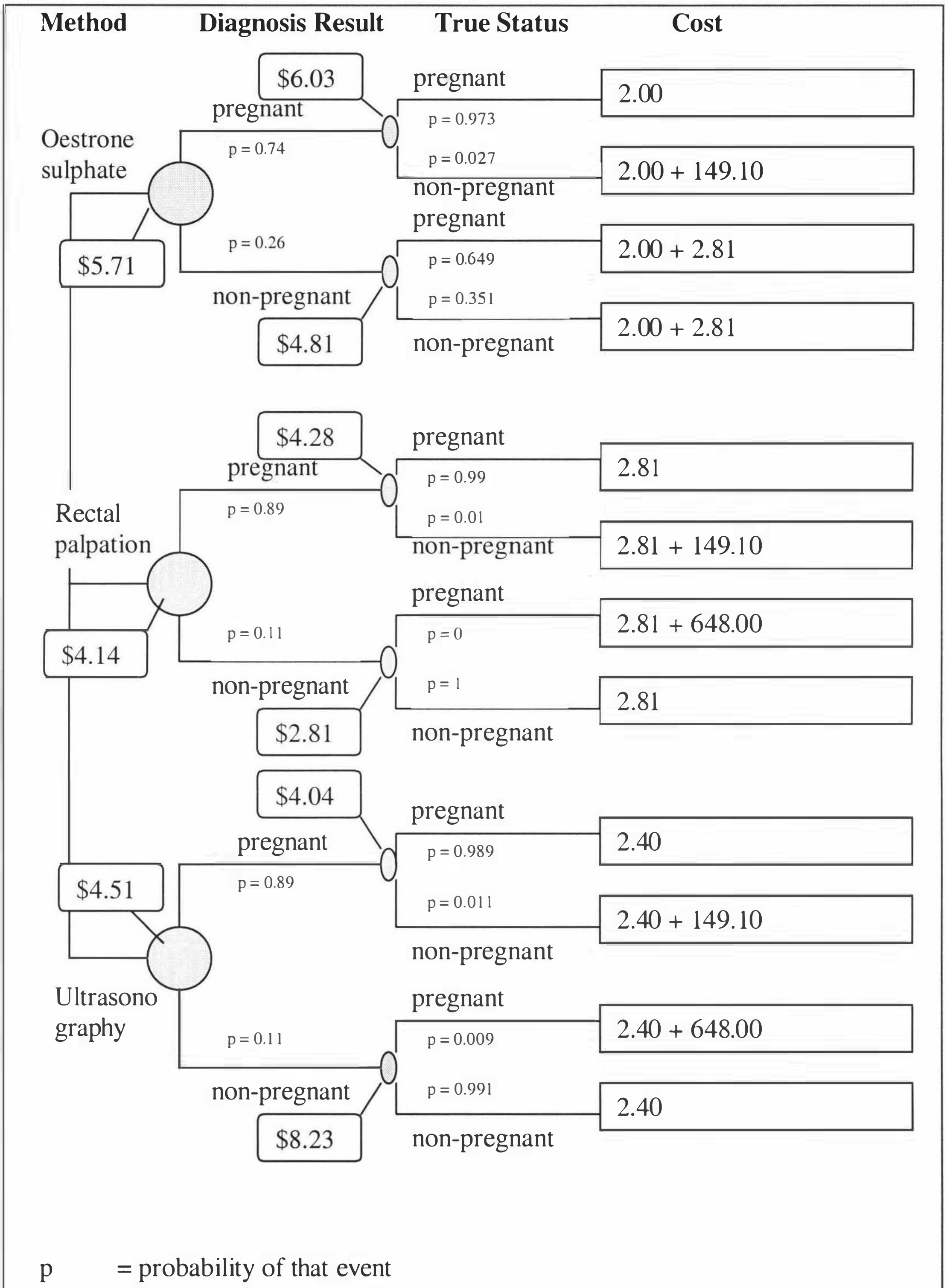
[12] Based on three 20-minute periods per day @ NZ$ 10.00 per hour labour over a ten week breeding period plus 42 days for observation of returns-to-service

Figure 4.6 Decision tree for comparing different methods of pregnancy diagnosis with probabilities and monetary values (NZ$) entered



Gives expected monetary loss (EML) expected with each diagnosis or test outcome. EML is the sum of probabilities of an outcome multiplied by the cost associated with that specific outcome (Mohammed et al., 1990).

A farmer using oestrus observation for pregnancy diagnosis would probably argue that this method does not cost anything. However the actual cost, taking the consequences of misdiagnosis into account, are substantial and accuracy of oestrone sulphate would have to be improved only slightly to be competitive with farmers' observation. These examples demonstrate that economic analysis is important to look beyond the obvious, or visible direct costs of a test.

The comparison of costs in different herds based on their situation showed the range of expenditure pregnancy diagnosis may carry. Although a change in sensitivity had a greater influence on cost in the model, the herd comparison made the influence of specificity obvious, especially when considering the opportunity costs arising from an incorrect diagnosis of pregnant. Specificity was low in herd 3, and the costs of oestrone sulphate and rectal palpation were the highest in this herd. Specificity of ultrasonography was very low in herd 4, and this was reflected in the increased cost of pregnancy diagnosis by this method in this herd.

Similarly, in the herds with a low specificity for farmers' observation (herds 10, 11, and 13) the cost of this method increased substantially, making it almost twice as expensive as oestrone sulphate in herd 11. Herd comparison also demonstrated the dependence of oestrone sulphate on time elapsed between examination and the end of the breeding season. When calculating costs based on sensitivity and specificity for cows at least 120 days pregnant or since their last service, oestrone sulphate became much more competitive to rectal palpation and ultrasonography, and less expensive than farmers' observation.

The economic model allows establishment of threshold values beyond which one method would become more cost effective than the others (Fetrow et al., 1985; Fetrow and Blanchard, 1987; Pitcher and Galligan, 1990). When developing a new diagnostic test, or a new application of an existing method as in the case of oestrone sulphate in this study, threshold values can act as goals. The development of the method can be focused on reaching those goals or a decision can be made whether those goals are achievable. For example, for oestrone sulphate to become competitive to rectal palpation or ultrasonography, its specificity would have to be improved to 93.6% or its charge reduced to NZ$ 0.10 per cow. The analysis also showed that increasing the sensitivity to 100% would not be sufficient to make this the favourable method compared to rectal palpation or ultrasonography.

Several factors that may influence a management decision are difficult to quantify in monetary terms. Erb (1984) suggests that management decisions reflect personal satisfaction or preference (such as lifestyle, pride, tradition) as well as economic considerations. For example, farmers with very accurate observation skills would probably not benefit from another method of pregnancy diagnosis. But even if they were confident of their accuracy in determining a cow's pregnancy status, they may decide to employ another method to ensure 'peace of mind'.

Likewise, a more expensive method might be chosen if it provides additional information, such as confirmation of service date, ovarian status of non-pregnant cows, or sex of the foetus. Oestrone sulphate may be preferred over invasive methods on the grounds of animal welfare.

Another factor that can be difficult to quantify is opportunity costs. These can include considerations as to the likely benefit gained if the investment for one management procedure (e.g. pregnancy diagnosis) is spent on an alternative (e.g. improved cow nutrition; Erb, 1984). In addition, they can include effects beyond the immediate future. These can be positive as well as negative consequences. For example, replacing a culled cow with a heifer may advance genetic progress. On the other hand, culling of a cow incorrectly diagnosed as non-pregnant may prevent the culling of a cow with sub-optimal production or chronic lameness. The economic implications of these positive or negative repercussions are often difficult to quantify. In the case of an incorrect diagnosis of pregnant, for example, the immediate loss arises from the cost of feeding this non-pregnant animal over the dry period. However, the farmer, expecting a return in terms of milk production and calf value from this cow, faces a shortfall in his budget when that animal fails to calve.

A variety of models have been used to calculate the economic benefit of different methods of pregnancy diagnosis (Boneschanscher et al., 1982; Oltenacu et al., 1990; Pitcher and Galligan, 1990; Esslemont, 1995). Findings from these studies are not directly comparable to the results of the current study, as they assume interference for cows diagnosed as non-pregnant (e.g. prostaglandin administration), calculate the benefit of pregnancy diagnosis or an additional method (e.g. milk progesterone assay) vs. no intervention, or compare different treatments of non-pregnant cows. Cameron and Malmo (1993) derived at costs of A$ 22.15 (about NZ$ 26.80) for rectal palpation and A$ 36.57 (about NZ$ 44.10) for Doppler ultrasonography in a study comparing these two methods. However, sensitivity of rectal palpation was much lower in their study than in this study (95.0% vs. 100%).

McDougall (1996) used a decision tree to compare rectal palpation, real-time ultrasonography, oestrone sulphate and farmers' observation based on published or self-observed sensitivity and specificity for each method. Calculated costs associated with misdiagnoses were based on different assumptions to this study and he derived values of NZ$ 31.03, 47.07, 31.27, and 31.93 to 37.42 for the four methods, respectively, making ultrasonography the most expensive method. Sprecher et al. (1989) used a partial farm budget for a cost-benefit analysis of using no pregnancy diagnosis vs. real-time ultrasonography in sheep.

Economic analysis models have been used to establish financial implications in areas other than pregnancy diagnosis, including oestrus detection (Esslemont, 1974a; Holmann et al., 1987; Mohammed et al., 1990; Pecsok et al., 1994), induction of oestrus (Fetrow and Blanchard, 1987), fertility and herd health problems (Pelissier, 1972; MacKay, 1981; Esslemont, 1992; Wheadon, 1993), use of milk progesterone for post-service monitoring (Markusfeld et al., 1990) or preventing insemination errors (Ruiz et al., 1989), use of artificial insemination in New Zealand dairy replacement heifers (Livestock Improvement Corporation, 1997b), and costs of raising dairy replacement heifers (Gabler et al., 2000).

Time taken for examination

A true comparison of speed of examination between rectal palpation and ultrasonography was impossible due to special circumstances, like farmers requesting a confirmation of the service date or students becoming involved in the examinations. However, the data does suggest that pregnancy diagnosis by ultrasonography may be quicker. The speed of rectal palpation achieved in this study was comparable to the 58 seconds per cow stated by Cameron and Malmo (1993), who examined cows at eight or more weeks of gestation. Examination by ultrasonography in this study achieved a greater speed than McDougall (personal communication, 1998), who estimated an average throughput of 80 to 120 cows per hour (range over several herds of 76 to 143). He observed that greater speeds could be achieved in rotary compared to herringbone parlours. The same observation was made in this study for ultrasonography examinations.

Conclusion

A simple economic analysis model was successfully used to compare the four methods of pregnancy diagnosis. The analysis did not attempt to determine whether pregnancy diagnosis was more beneficial than no intervention. It was purely established which of the four methods was most cost effective under the conditions of this study.

The lower sensitivity and specificity of oestrone sulphate resulted in this method being uneconomic compared to rectal palpation and ultrasonography. It is questionable whether the oestrone sulphate enzyme-immunoassay could be improved to fulfil the threshold values required for this method to become competitive.

The analysis carried out did not take into account additional benefits of rectal palpation and ultrasonography, like capability of establishing stage of pregnancy, normality and abnormality of the reproductive tract, and foetal health. The inclusion of these factors would make oestrone sulphate even less economical.

Only minor improvements in sensitivity, specificity or test costs would be necessary to make oestrone sulphate competitive with farmers' observation. There was a wide variation between herds, though. Herds with excellent observation skills would not benefit from the extra expenditure incurred by the oestrone sulphate assay.

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Appendix I Details of abortions and premature calvings

Herd	Type	Date	CowID	Gold Standard	Oestrone Sulphate	Rectal Palpation	Ultrasono-graphy
1	Aborted?		14119971	MT	MT	P	P
	Aborted?		12447532	MT	P	P	P
	Aborted?		12284602	MT	P	P	P
	Aborted?	31-May-97	12110889	P	MT	P	P
	Aborted	05-Jun-97	12357855	P	MT	P	P
	Aborted	30-May-97	6635578	P	P	P	P
	Aborted	10-Jun-97	12284604	P	P	P	P
	Aborted	10-Jun-97	12284574	P	P	P	P
	Aborted	10-Jun-97	10574806	P	P	P	P
	Aborted	10-Jul-97	12284617	P	P	P	P
	Aborted	10-Jul-97	12279354	P	P	P	P
	Aborted	10-Jul-97	12357862	P	P	P	P
	Aborted	10-Jul-97	12404342	P	P	P	P
	Aborted	10-Jul-97	12284590	P	P	P	P
	Premature	29-Jul-97	12357827	P	P	P	P
	Premature	03-Sep-97	8988269	P	P	P	P
2	Aborted-Date?	30-Apr-97	4748081	P	MT	P	P
	Premature	28-Jul-97	8119966	P	MT	P	P
	Aborted	15-Apr-97	11642302	P	P	P	P
	Aborted	19-May-97	11583197	P	P	P	P
3	Aborted	05-Jul-97	8874319	P	P	P	P
	Aborted	14-Jul-97	11689803	P	P	P	P
	Aborted	15-Jul-97	11689787	P	P	P	P
	Aborted	09-Aug-97	11949132	P	P	P	P
	Premature	15-Aug-97	8874375	P	P	P	P
	Premature	16-Aug-97	6364729	P	P	P	P
4	Aborted	21-May-97	7510468	P	MT	P	P
	Aborted	27-May-97	10098713	P	P	P	P
	Premature	23-Jul-97	11708432	P	P	P	P
	Premature	30-Jul-97	11708436	P	P	P	P
	Aborted	01-Aug-97	7510475	P	P	P	P
6	Aborted	01-Apr-97	10531528	P	P	P	P
	Aborted	27-Apr-97	9826809	P	P	P	P
	Aborted	04-Jun-97	10006712	P	P	P	P
	Aborted	15-Jun-97	10042882	P	P	P	P

Appendix II Details of induced parturitions

HerdKey	Type	Date	CowID	Gold Standard	Oestrone Sulphate	Rectal Palpation	Ultrasonography
1	Induced	15-Aug-97	11381592	P	MT	P	P
	Induced	15-Aug-97	11381602	P	MT	P	P
	Induced	15-Aug-97	6690262	P	MT	P	P
	Induced	15-Aug-97	11413730	P	MT	P	P
	Induced	15-Aug-97	11413726	P	MT	P	P
	Induced	15-Aug-97	11413737	P	MT	P	P
	Induced	15-Aug-97	4681998	P	MT	P	P
	Induced	15-Aug-97	11413735	P	MT	P	P
	Induced	15-Aug-97	11413742	P	MT	P	P
	Induced	15-Aug-97	11381630	P	MT	P	P
	Induced	15-Aug-97	10570291	P	MT	P	P
	Induced	15-Aug-97	10574815	P	MT	P	P
	Induced	15-Aug-97	10574812	P	MT	P	P
	Induced	15-Aug-97	10574809	P	MT	P	P
	Induced	15-Aug-97	10574794	P	MT	P	P
	Induced	15-Aug-97	9740316	P	MT	P	P
	Induced	15-Aug-97	9776356	P	MT	P	P
	Induced	15-Aug-97	9818763	P	MT	P	P
	Induced	15-Aug-97	9903584	P	MT	P	P
	Induced	15-Aug-97	8449829	P	MT	P	P
	Induced	15-Aug-97	8263183	P	MT	P	P
	Induced	15-Aug-97	8263192	P	MT	P	P
	Induced	15-Aug-97	8449799	P	MT	P	P
	Induced	15-Aug-97	8263221	P	MT	P	P
	Induced	15-Aug-97	8449786	P	MT	P	P
	Induced	15-Aug-97	12284554	P	MT	P	P
	Induced	15-Aug-97	12168521	P	MT	P	P
	Induced	15-Aug-97	12168520	P	MT	P	P
	Induced	15-Aug-97	12284649	P	MT	P	P
	Induced	15-Aug-97	12168551	P	MT	P	P
	Induced	15-Aug-97	12168537	P	MT	P	P
	Induced	15-Aug-97	11815584	P	MT	P	P
	Induced	15-Aug-97	12284622	P	MT	P	P
	Induced	15-Aug-97	12284635	P	MT	P	P
	Induced	15-Aug-97	12284626	P	MT	P	P
	Induced	15-Aug-97	12284631	P	MT	P	P
	Induced	15-Aug-97	12284633	P	MT	P	P
	Induced	15-Aug-97	14119960	P	MT	P	P
	Induced	15-Aug-97	12269638	P	MT	P	P
	Induced	15-Aug-97	14119963	P	MT	P	P
	Induced	15-Aug-97	12269635	P	MT	P	P
	Induced	15-Aug-97	12357837	P	MT	P	P
	Induced	15-Aug-97	12357841	P	MT	P	P
	Induced	15-Aug-97	12003932	P	MT	P	P
	Induced	15-Aug-97	12357821	P	MT	P	P
	Induced	15-Aug-97	12001328	P	MT	P	P
	Induced	15-Aug-97	12357848	P	MT	P	P
	Induced	15-Aug-97	12028286	P	MT	P	P
	Induced	15-Aug-97	12357882	P	MT	P	P
	Induced	15-Aug-97	14119930	P	MT	P	P
	Induced	15-Aug-97	14119926	P	MT	P	P
	Induced	15-Aug-97	12526079	P	MT	P	P
	Induced	15-Aug-97	11488781	P	MT	P	P
	Induced	15-Aug-97	11488762	P	MT	P	P
	Induced	15-Aug-97	12284589	P	MT	P	P
	Induced	15-Aug-97	11488693	P	MT	P	P
	Induced	15-Aug-97	14120049	P	MT	P	P
	Induced	15-Aug-97	11476971	P	MT	P	P

HerdKey	Type	Date	CowID	Gold Standard	Oestrone Sulphate	Rectal Palpation	Ultrasono-graphy
1	Induced	24-Sep-97	12357847	P	MT	P	P
	Induced	26-Sep-97	8449784	P	MT	P	P
	Induced	26-Sep-97	12284627	P	MT	P	P
	Induced	28-Sep-97	12269628	P	MT	P	P
	Induced	30-Sep-97	11630393	P	MT	P	P
	Induced	01-Oct-97	11209877	P	MT	P	P
	Induced	01-Oct-97	8449844	P	MT	P	P
	Induced	01-Oct-97	11477020	P	MT	P	P
	Induced	01-Oct-97	11476957	P	MT	P	P
	Induced	01-Oct-97	12284576	P	MT	P	P
	Induced	10-Oct-97	11815589	P	MT	P	P
	Induced?	29-Jul-97	11381627	P	P	P	P
	Induced	07-Aug-97	14120044	P	P	P	P
	Induced	15-Aug-97	11381626	P	P	P	P
	Induced	15-Aug-97	11381600	P	P	P	P
	Induced	15-Aug-97	11381611	P	P	P	P
	Induced	15-Aug-97	11126801	P	P	P	P
	Induced	15-Aug-97	6636908	P	P	P	P
	Induced	15-Aug-97	6012944	P	P	P	P
	Induced	15-Aug-97	11381653	P	P	P	P
	Induced	15-Aug-97	11381664	P	P	P	P
	Induced	15-Aug-97	10570278	P	P	P	P
	Induced	15-Aug-97	9256341	P	P	P	P
	Induced	15-Aug-97	10570259	P	P	P	P
	Induced	15-Aug-97	8263203	P	P	P	P
	Induced	15-Aug-97	7496912	P	P	P	P
	Induced	15-Aug-97	7677706	P	P	P	P
	Induced	15-Aug-97	8263241	P	P	P	P
	Induced	15-Aug-97	8449790	P	P	P	P
	Induced	15-Aug-97	8446511	P	P	P	P
	Induced	15-Aug-97	12284643	P	P	P	P
	Induced	15-Aug-97	12284644	P	P	P	P
	Induced	15-Aug-97	12168569	P	P	P	P
	Induced	15-Aug-97	12168547	P	P	P	P
	Induced	15-Aug-97	12284619	P	P	P	P
	Induced	15-Aug-97	12357828	P	P	P	P
	Induced	15-Aug-97	12357880	P	P	P	P
	Induced	15-Aug-97	12357824	P	P	P	P
	Induced	15-Aug-97	12028276	P	P	P	P
	Induced	15-Aug-97	12028249	P	P	P	P
	Induced	15-Aug-97	14119940	P	P	P	P
	Induced	15-Aug-97	14119936	P	P	P	P
	Induced	15-Aug-97	12407734	P	P	P	P
	Induced	15-Aug-97	11681510	P	P	P	P
	Induced	23-Sep-97	5549750	P	P	P	P
	Induced	24-Sep-97	11084540	P	P	P	P
	Induced	24-Sep-97	5165932	P	P	P	P
	Induced	24-Sep-97	10244378	P	P	P	P
	Induced	01-Oct-97	12390864	P	P	P	P
	Induced	04-Oct-97	11381648	P	P	P	P
2	Induced	10-Aug-97	5540097	P	MT	P	P
	Induced	17-Aug-97	9040941	P	MT	P	P
	Induced	17-Aug-97	7997375	P	MT	P	P
	Induced	18-Aug-97	8992981	P	MT	P	P
	Induced	21-Aug-97	11973938	P	MT	P	P
	Induced	22-Aug-97	7997373	P	MT	P	P
	Induced	23-Aug-97	6089276	P	MT	P	P
	Induced	23-Aug-97	9724346	P	MT	P	P
	Induced	23-Aug-97	8190381	P	MT	P	P
	Induced	26-Aug-97	11973942	P	MT	P	P

HerdKey	Type	Date	CowID	Gold Standard	Oestrone Sulphate	Rectal Palpation	Ultrasono-graphy
3	Induced	18-Sep-97	8867238	P	MT	P	P
	Induced	18-Sep-97	10900338	P	MT	P	P
	Induced	18-Sep-97	11949164	P	MT	P	P
	Induced	18-Sep-97	11949139	P	MT	P	P
	Induced	14-Sep-97	9310240	P	P	P	P
	Induced	18-Sep-97	8874351	P	P	P	P
	Induced	18-Sep-97	8166482	P	P	P	P
	Induced	18-Sep-97	8874392	P	P	P	P
	Induced	18-Sep-97	10866054	P	P	P	P
	Induced	18-Sep-97	11689758	P	P	P	P
	Induced	20-Sep-97	10634618	P	P	P	P
	Induced	20-Sep-97	9884531	P	P	P	P
5	Induced	20-Sep-97	12032759	P	MT	P	P
	Induced	25-Sep-97	12042684	P	MT	P	P
	Induced	25-Sep-97	7703114	P	MT	P	P
	Induced	19-Sep-97	12032718	P	P	P	P
	Induced	20-Sep-97	7180894	P	P	P	P
	Induced	20-Sep-97	6193576	P	P	P	P
	Induced	20-Sep-97	12042683	P	P	P	P
	Induced	20-Sep-97	12037004	P	P	P	P
	Induced	20-Sep-97	12032768	P	P	P	P
	Induced	20-Sep-97	9256750	P	P	P	P
	Induced	20-Sep-97	12032720	P	P	P	P
	Induced	20-Sep-97	12032727	P	P	P	P
	Induced	21-Sep-97	9256759	P	P	P	P
	Induced	21-Sep-97	12032743	P	P	P	P
	Induced	21-Sep-97	11017668	P	P	P	P
	Induced	24-Sep-97	7659283	P	P	P	P
	Induced	24-Sep-97	11017689	P	P	P	P
	Induced	25-Sep-97	6436799	P	P	P?	P
	Induced	25-Sep-97	9256775	P	P	P	P
	Induced	25-Sep-97	12038229	P	P	P	P
	Induced	25-Sep-97	12032714	P	P	P	P
	Induced	21-Oct-97	7677754	P	P	P	P
	Induced	21-Oct-97	6370327	P	P	P	P
	Induced	21-Oct-97	6150858	P	P	P	P
	Induced	21-Oct-97	2964103	P	P	P	P
6	Induced	07-Aug-97	10006747	P	P	P	P
	Induced	07-Aug-97	6803879	P	P	P	P
	Induced	12-Aug-97	9197059	P	P	P	P
	Induced	17-Aug-97	10006721	P	P	P	P
	Induced	23-Aug-97	9826824	P	P	P	P
	Induced	03-Sep-97	7900139	P	P	P	P