DOCTOR OF PHILOSOPHY

The application of recently developed techniques to Welsh Black Cattle in a group breeding scheme.

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THE APPLICATION OF RECENTLY DEVELOPED TECHNIQUES TO WELSH BLACK CATTLE IN A GROUP BREEDING SCHEME

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BY

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SUMMARY

Methods of superovulation, embryo recovery and embryo transfer were adapted and developed for application to Welsh Black cattle in a Group Breeding scheme.

Three methods of oestrus detection in early postpartum suckling beef cows were evaluated. None was entirely reliable although a probe for the measurement of the electrical resistance of vaginal mucus was the most effective and the least stressful.

The postpartum interval to superovulation did not affect the responses to treatment.

The effect of suckling on embryo quality from superovulated Welsh Black cows was investigated. Three groups of cows (one dry and two suckling) were superovulated and their embryos recovered in two seasons; summer and autumn. Significant effects of season ($P=0.016$) and suckling ($P=0.015$) on embryo quality were detected. Season significantly affected the number of viable embryos ($P=0.004$). There were no effects on ovarian responses or on the numbers of embryos recovered.

Nutritional supplementation and the administration of hCG failed to improve embryo quality.

Examination of the data from three years of superovulation and embryo recovery revealed significant monthly variations in % viable embryos ($P=0.006$) and in the numbers of viable embryos ($P=0.02$) recovered.

Embryo recovery was not affected by the catheter used or by the side of uterus being flushed. The number of parities of the

donor affected the ovulation rate (P=0.009) and the numbers of embryos recovered (P=0.028) but not the embryo recovery rate (%).

No differences were detected in the superovulatory responses of cows bred by 3 methods nor from the method of oestrus synchronisation.

Embryo transfers were performed by surgical and nonsurgical methods. Fresh and frozen embryos were successfully transferred by the surgical technique but no embryos transferred nonsurgically continued development.

A measurement of cervical diameter was successful in identifying animals which were unsuitable for non-surgical embryo transfer and recovery procedures.

n-Alkanes as nutritional markers for intake estimation were applied to cattle in a performance test and to cows at pasture. The results obtained indicate that the method may be useful in the future development of selection methodologies for beef cattle.

The findings of both series of investigations are discussed in relation to beef cattle improvement through Group Breeding schemes.

PUBLICATIONS

BROWN, C.M., THOMAS, J.B., WILLIAMS, G., OWEN,J.M., AXFORD, R.F.E., WILSON, I.B.H. and OWEN, J.B. (1988). Effect of suckling on embryo quality from superovulated Welsh Black cows. In: New Techniques in Cattle Production (Ed. Phillips, C.J.C.) pp 239-240, Butterworths, London.

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BROWN, C.M., LOFTS, R.C., WILLIAMS, G., WILSON, I.B.H., AXFORD, R.F.E. and OWEN, J.B. (1989). The effect of season and suckling on the quality of embryos from superovulated Welsh Black cows. Proc. Sixth Welsh Agric. R+D Conf. Cardiff 1989 (in press).

BROWN, C.M., AXFORD, R.F.E., WILLIAMS, G., WILSON, I.B.H. & OWEN, J.B. Experience of MOET with Welsh Black Cattle in a Group Breeding Scheme. Theriogenology. (in press).

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
AI	Artificial insemination
C_X	n-Alkane carbon chain length
coata	Coata-count assay, Diagnostic Products (hormone assay)
Ch	Charriere gauge
CL	Corpora lutea
E2	plasma 17-beta oestradiol concentration
EIA	Enzyme immuno assay
ELISA	Enzyme linked immunosorbant assay
FCE	Feed conversion efficiency
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin releasing hormone
hCG	human chorionic gonadotrophin
i/v	intravenous
i/m	intramuscular
LH	Luteinising hormone
lvpl	Liverpool University, Leahurst (hormone assay)
MADF	Modified acid detergent fibre
MLW	Metabolic liveweight
MOET	Multiple ovulation and embryo transfer
ME	Metabolisable energy
NDF	Neutral detergent fibre
ns	not significant (at the 10% level)
P4	plasma progesterone concentration
PG	Prostaglandin F2 alpha
PMSG	Pregnant mare's serum gonadotrophin

PRID	Progesterone releasing intravaginal device
RIA	Radio-immuno assay
SEM	Standard error of the mean

LIST OF SUPPLIERS

EMBRYO RECOVERY EQUIPMENT

2 and 3 Lumen catheters

Franklin Medical plc.,
Cressex Ind. Estate,
High Wycombe,
Bucks.

2 Lumen (Rusch) catheters & non-surgical Embryo Transfer equipment

Medizintechnik Worrlein GmbH,
Ruglander Str. 3,
D-8800 Ansbach
West Germany.

Alpha Labs.,
40, Parham Drive,
East Leigh,
Hants. SO5 4NM

FLUSHING MEDIUM

Imperial Labs. (Europe),
20, Tonbridge Rd.,
Maidstone,
Kent.

Flow Labs.,
Woodcock Hill Ind. Estate,
Harefield Rd.,
Rickmansworth,
Herts. WD3 1PQ

FREEZING EQUIPMENT

I.M.V.,
10, Rue Clemenceau,
61300 L'Aigle,
France.

Planer Products plc.,
Windmill Rd.,
Sunbury-on-Thames,
Middlesex. TW16 7AD

SUPPLIERS cont'd

PHARMACEUTICALS, CHEMICALS AND ASSAY KITS

Willington Medicals plc.,
Lancaster Rd.,
Shrewsbury.

Sigma Chemicals,
Fancy Rd.,
Poole,
Dorset.

Diagnostic Products,
5700 West 96 St.,
Los Angeles,
CA 90045.

CHAPTER 1

INTRODUCTION

1. WELSH BLACK CATTLE AND THE HAULFRYN GROUP BREEDING SCHEME

The Welsh Black breed of cattle has been developed as a suckler animal from dual-purpose origins (Edmunds, 1981). The breed has a long history, the Welsh Black Cattle Society being formed in 1874.

In 1978 a pioneer Group Breeding Scheme, the first such scheme for cattle in the U.K., was established with the objective of improving the weaner calf production of the cows through the co-operative effort of a group of breeders (Owen, 1986).

The seven members of the scheme possessed (in 1986) 317 breeding cows in herds ranging in size from 14 to 97 (mean = 45.3). The nucleus herd was established by the loan of breeding cows, selected using the M.L.C. index for calf 200 day weight, from the member herds. These foundation animals were withdrawn as their progeny replaced them, forming the nucleus herd, Haulfryn, of 50 cows.

With in the nucleus herd, the poorest cows, rated on the index, are replaced each year by fifteen replacement heifers. The herd is mated to between 4 to 6 bulls annually. Two of these are drawn from the 25 bull calves bred each year in the nucleus. They are selected on their dam calf rearing index and their own performance on test to 400 days. The other bulls are the best 2-4 bulls available nationally, usually through artifical insemination (AI).

A multiple ovulation and embryo transfer scheme was started in 1985 with the aim of facilitating the exchange of genetic material between the base herds and the nucleus (Owen, 1986).

An on-farm performance test of bulls from the nucleus and the base herds is carried out to aid the identification of superior bulls.

The present study had three objectives;

1) to adopt and establish practical on-farm methods for superovulation, embryo collection and embryo transfer in Welsh Black cattle.

2) to assess the applicability of these methods for practical breed improvement.

3) to examine the practicality of estimating food intake of cattle as an additional assessment of their merit.

These objectives were to be assessed for their application to Welsh Black cattle in a Group Breeding Scheme. The practical problems encountered in the use of these methods are be evaluated in relation to the normal and continued operation of the Group Breeding Scheme.

CHAPTER 2

REVIEW OF LITERATURE

2.1 WELSH BLACK CATTLE

The Welsh Black Breed of cattle originated from the cattle used in the transhumance system of farming practiced in Wales before the enclosure acts. The Welsh Black Cattle Society was formed in 1874 and reformed into its present state, after the abandonment of separate North Wales and Pembroke Herdbooks, in 1905 (Edmunds, 1981). A description of the breed can be found in Keary (1848):

> 'The North Wales cattle are generally considered to be the best and most sought after by the English grazier. Those called Anglesea are black with long horns, turning upwards and outwards, short on the leg, wide carcasses and hips and good hair and handling in short possessing in many points a strong resemblence to the West Highland Scot.'

In recent years the breed has, in common with other hill breeds, declined in numbers. This has been due to its substitution by crossbred cows, or by more profitable sheep, and because of afforestation of upland areas (Allen, 1985).

2.2 SELECTION AND GENETIC IMPROVEMENT OF BEEF CATTLE

2.2.1 INTRODUCTION

Selection in a beef enterprise should have the objective of improving the biological efficiency of meat production (Dickerson and Wilhelm, 1983). Increasing biological efficiency may include a decrease in the maintenance requirements of the mature animal or an improvement in food conversion efficiency (Thompson and Barlow, 1986).

2.2.2 GROUP BREEDING SCHEMES

Genetic improvement of beef cattle breeds in the U.K. has been hindered by the small size of pedigree herds (Table 2.1) and

the infrequent use of AI, in comparison to dairy herds, in these herds.

Table 2.1: Size of herds in MLC Pedigree testing scheme

Breed	no. of herds	av. no. of cows/herd 1982	1986	1988
Aberdeen Angus	39	26	27	28
Belgian Blue	15	-	-	11
Charolais	197	10	15	16
Hereford	69	29	28	33
Limousin	215	9	14	15
Simmental	338	8	10	11
Welsh Black	12	48	54	44

(MLC, 1988)

The size of the recorded population constrains the possible selection intensity, small populations have increased levels of inbreeding and a reduced cumulative response to selection (Franklin, 1982). One solution to this problem is the establishment of Group Breeding schemes.

A Group Breeding Scheme is an hierarchical breeding scheme in which a nucleus herd is established to breed replacement sires for itself and an associated base population (James, 1977). This structure can be adopted in a single herd; however, the majority of Group Breeding Schemes involve co-operating breeders and are essentially multiherd breeding structures (Parnell et al., 1986).

Group Breeding schemes which originated in New Zealand in the late 1960's, possess three main features (Steane et al.,

undated);

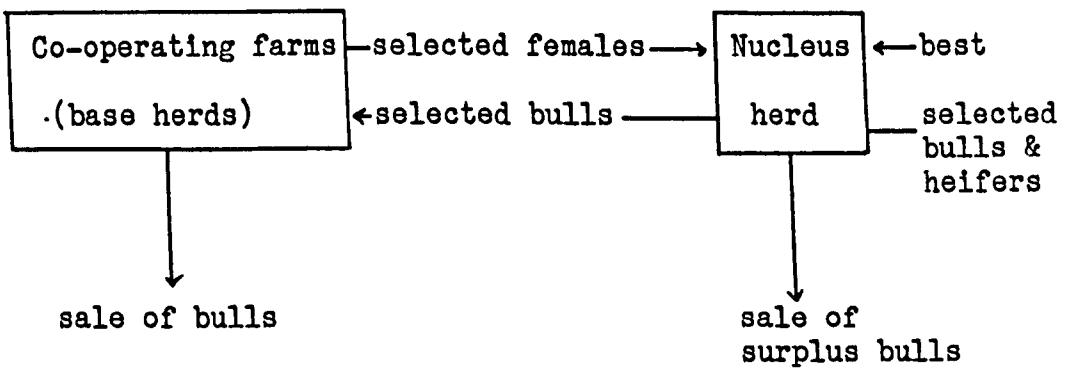
1) Co-operation among breeders in managing a jointly owned breeding herd, the nucleus, to produce replacement bulls, and to a lesser extent females, for the group.

2) The transfer of selected bulls and heifers from the nucleus to the base and of females, and possibly bulls, from the base to the nucleus.

3) Selection among animals based on performance records.

Such schemes (Figure 1) can be categorised into either open or closed nucleus schemes. An open nucleus permits the transfer of genetic material between the nucleus and the base herds. A closed nucleus breeds all its own replacements.

Figure 1: The general form of a group breeding scheme



(Steane et al., undated)

Localised breeding schemes allow for local adaptations in the selection of breeding animals, which may be important if genotype x environment interactions have to be considered as part of the selection process (Notter, 1986).

2.2.3 PERFORMANCE TESTING

Performance testing is the measurement of a trait or traits in the live animal. It is usually understood to relate to the evaluation of beef bulls reared on special farms or stations on the basis of their growth rate, feed conversion efficiency and/or conformation. However the term can also be applied to the assessment of females (Parnell et al., 1986)

Performance testing enables earlier evaluation of an animal to be made than can be achieved through progeny testing. Consequently generation intervals can be reduced. The process involves a reduction in accuracy but allows much greater screening and higher selection intensity than progeny testing. Performance testing is unsuitable for traits with low heritabilities or which can only be measured in the carcass (Preston and Willis, 1974).

The report of the working group of the Commission on Cattle production (Bech Andersen et al., 1981) proposed the following potential traits for performance testing and selection of bulls for AI: 1) growth rate; 2) feed conversion efficiency; 3) appetite; 4) quantitative carcass properties; 5) functional traits.

Methods of growth rate and quantitative carcass assessment have been evaluated (Simm, 1983). However, difficulties in the measurement of feed intake have prevented the development of selection methods using feed conversion efficiency and appetite as selection criteria.

In beef production systems the feed conversion efficiency

(FCE) of the animals is as important as the absolute production level. It is known that the production characteristics of beef breeds vary widely (Mason, 1971). However the calculation of FCE, which requires measurement of feed intake, is labour and capital intensive and usually restricted to central testing facilities. Predictors of FCE have been proposed to circumvent these problems eg. body weight, weight gain and weight gain per unit of body weight. None of these has been considered to be effective (Theissen and Taylor, 1986). Future selection criteria may consider appetite in relation to growth rate (Theissen et al., 1985). There is a paucity of information on the fundamental components of cattle production. With increased use of production records in selection more sophisticated testing procedures will be required. Two systems of performance testing have been developed; central and on-farm (Preston and Willis, 1974).

To overcome the influences of different herd management systems on the performance of individual bulls, animals can be collected and evaluated at a central location. Central test stations, managed by the MLC in the UK, were used to provide standard management. In the U.K. test criteria included growth rates, weight for age, feed conversion efficiency, back fat depth, withers height and type classification. Complete diets were fed ad lib to provide accurate measurement of feed intake. The bulls were tested from 6 months of age for 30 weeks (Lewis and Allen, 1974). Central testing ceased in G B in 1986 (MLC, 1987).

Beef bull evaluation in the U.K. is based on on-farm performance testing. Such tests rely on rearing animals of

similar type together and measuring important production characters. On-farm performance tests have been used by different breed societies to compare bull calves (Table 2.2).

Table 2.2: On-farm performance test results 1987-88

Breed	no. tested	no. of groups
Aberdeen Angus	95	7
Belgian Blue	23	3
Charolais	136	17
Hereford	118	12
Limousin	381	42
Simmental	176	23
Welsh Black	48	4

(MLC, 1988)

The MLC on-farm test requires at least eight bulls, born within a two month period, to be tested together. The bulls are housed and managed alike and fed to allow equal opportunity to each animal, although the choice of feeds is made by the farmers of the test group. The test must begin before the bulls reach 7 months of age and the pretest management should be uniform. The test period must last at least six months so the bulls are 400 days old by the end of the test. The bulls are weighed monthly. Withers height measurement, muscle scores and ultrasonic measurement of the back fat depth are carried out at 1 year of age. The bulls are evaluated using an index, and comparisons are made with the other bulls in the same test group. The selection index is calculated to rate most highly those cattle which have

increased liveweight and reduced fat levels (MLC, 1988). Such tests do not take into consideration the feed intake of animals as few, if any, farms have the facilities for the estimation of individual intakes.

In 1987/8 a total of 133 on-farm tests were carried out, of which 30 involved co-operation between two or more breeders, involving 1,212 bulls in the United Kingdom. In addition, there were 4 tests involving 28 heifers (MLC, 1988).

2.2.4 DETERMINATION OF FEED INTAKE FOR USE IN PERFORMANCE TESTING BEEF CATTLE

The methods available for the measurement of feed intake of ruminants can be categorised into two groups: herbage based or animal based measurements. This subject has been extensively reviewed by Meijs (1981) and Cordova and others (1978), and only the aspects which pertain to performance testing will be examined.

2.2.4.1 PASTURE BASED MEASUREMENTS

Pasture or sward based measurements of feed intake can be divided into those that involve cutting of the sward or the those that use non-destructive measures. Both methods apply only to groups of animals and can only give mean intakes, and are consequently of little use for selection purposes. In brief, the quantity of herbage present in a chosen area at the start of a grazing period is estimated from the product of the field area and herbage dry matter per unit area. At the end of the grazing period residual herbage is estimated and the quantity consumed is taken to be the difference between the two figures adjusted for

the growth of herbage during grazing. Herbage mass per unit area can be determined by cutting from sample sites of known area or by non-destructive methods such as pasture capacitance. Growth of herbage has been estimated through the use of exclosure cages.

Tissue flux has been suggested as a method of intake determination. This method is based on identifying a number of randomly chosen tillers in the pasture under study and allowing animals to graze this area. The amount of material removed per tiller is determined by the product of the amount of material removed per tiller and the tiller population per unit area (Clark, 1985). This method provides detailed information on the consistuents of the animals diet (leaves, stem etc.) but the measurements are time consuming to perform and prone to errors from tiller sampling.

In contrast, the feed intake measurements used in central testing stations involved feeding a weighed amount of a complete pelleted diet through individual feeding stations (MLC, 1987) allowing the estimation of each animals intake. The adoption of this system is restricted by the cost of the individual feeders and of the diet and can be criticised in that it precludes grazing under field conditions.

2.2.4.2 ANIMAL BASED MEASUREMENTS

Animal based estimates of forage intake from pasture can be categorised into four main methods: animal weight change, water turnover, faecal output / diet digestibility and eating behaviour.

1) Estimation of intake by animal weight change is achieved

by recording the weight of the subject before and after grazing (Allden and Young, 1959). This technique is based on measuring the weights of animals prior to (W1) and post (W2) grazing and recording the weights of faeces (F) and urine (U) produced over a period of time. The measurement of faecal and urinary output is achieved by harness mounted apparatus. The method must allow also for insensible weight loss (IWL), which is determined by preventing a group of animals from grazing and recording their weight loss. Additionally, an estimate of water intake is required (L). From the above information intake can be calculated:

$$\text{Intake} = (W2 + F + U + IWL) - W1 - L$$

This technique has several failings. The calculation of IWL is underestimated as the animals are prevented from displaying normal grazing activity (Greenhalgh, 1982). Penning and Hooper (1985) used this method in comparison with chromic oxide dosing and found satisfactory agreement. However, this technique is only suitable for small groups of sheep as the difficulties involved in collecting urine andfaeces make it expensive and labour intensive.

2) Intake can be estimated from measuring the water turnover in an animals body by injecting tritiated water and measuring the decline in radioactivity. The water intake from the herbage can then be calculated, assuming all water consumed is of dietary origin. Benjamin et al (1977) modified this method to allow for free water consumption in an indoor trial. Rogers et al (1985) used deuterium oxide (D_2O) turnover to establish a formula for determining dry matter intake in lactating dairy cows.

$$\text{DM intake (kg/d)} = \frac{\%\ \text{forage DM} \times (\text{water turnover rate} - \text{grain water} - \text{drinking water} - \text{faecal water})}{\%\ \text{forage water}}$$

However, this was an indoor experiment and the intake of water may not be easily controlled in a grazing situation where factors such as selective grazing and dew water consumption may reduce the accuracy of the estimate.

3) Eating behaviour measurements can be used to estimate intake. The method is based on measuring the means of the rate of bite per minute (RB), mean intake per bite (IB) and the time spent grazing (TG). Intake is calculated:

$$\text{Intake} = RB \times IB \times TG$$

(Hodgson, 1982)

The numbers of bites may be obtained from automatic recorders which count the number of bites taken during grazing and rumination (Stobbs and Cowper, 1972). The mean bite size can be obtained using oesphageal fistulated animals (Stobbs, 1973) and the time spent grazing can be estimated from interval sampling or from continuous monitoring of activity (Gary et al., 1970). This method is relatively expensive and subject to errors in the estimation of the 3 factors. Further the use of equipment may influence the animals behaviour (Hodgson, 1982).

4) Daily faecal output can be determined by its total collection using a harness or by using a marker in conjunction with faecal sampling. The ratio of ingested and excreted concentrations of the marker are used to calculate the faecal ouput of the animal. An estimate of the feed digestibility is

then used to calculate the feed intake.

The first three methods of intake measurement and the use of harnesses for total faecal collection can only be applied to small numbers of docile animals and require labour and materials which precludes their adoption for farm scale or performance test use. Markers have however, been employed in the feed estimation of large numbers of ruminants (Kotb and Luckey, 1972; Omed, 1986).

Intake markers can be internal markers which occur naturally in the diet or external markers which are added to the diet or given orally.

The level of food intake can be estimated relative to the concentration of a selected component in the faeces, frequently nitrogen, through a regression equation. This use of faecal index estimation is limited to situations where large differences in intake or digestibility exist (Cordova et al., 1978).

External markers, of which chromic oxide (Cr_2O_3) is the most commonly used, are used so that total faecal collections are unnecessary. Single or several mixed faeces samples are collected, analysed for chromium and the amount of faeces that would contain the total daily dose of marker is calculated and used as the best estimate of total daily faecal output. Intake is then calculated using an estimate of the digestibility of the feed. However chromic oxide excretion is subject to large diurnal and day to day variation in its concentration in the faeces even when dosed regularly in precise amounts so that individual intake calculations are associated with large errors (Hopper et al., 1978). Attempts have been made to improve the potential of

chromic oxide as a faecal marker by more frequent dosing or by the incorporation of the marker in different carriers to ensure an even distribution in the faeces (Chamberlain and Thomas, 1983). The properties, evaluation and application of dietary markers have been reviewed (Kotb and Luckey, 1972; Omed, 1986).

It has been considered that most estimates of intake by grazing livestock vary more with the techniques used than with the forages or environmental conditions tested (Cordova et al., 1978). Different techniques may be superior to others in some respects but many appear to produce results which may be seriously biased (Cordova et al., 1978). Extrapolation of results obtained from penned animals are of little value in approximating the intake of grazing animals because the effects of forage availability and the energy expended in foraging and grazing are not evaluated (Cordova et al., 1978).

One recent proposal is the use of plant components of low digestibility in conjunction with dosed related compounds. Long-chain fatty acids (C19-C32) have been proposed as dietary markers as they occur in plant material and are indigestible (Grace and Body, 1981). However, the difficulties of analysing these led to the evaluation of n-alkanes as alternatives (Mayes and Lamb, 1984). n-Alkanes, which are constituents of plant cuticular wax (Tulloch, 1981), increase in their faecal recovery as their carbon chain length increases. The recoveries of C_{31} and C_{35} have been reported as 0.854 and 0.931 respectively (Mayes et al., 1986). Recoveries in the faeces of herbage and of dosed alkanes allow the estimation of herbage intake and herbage digestibility

respectively to be estimated in individual animals (Mayes et al., 1986).

2.3 REPRODUCTION AND GENETIC IMPROVEMENT OF CATTLE

2.3.1 INTRODUCTION

The implications of developments in reproductive biology in the genetic improvement of cattle have been examined (Nicholas, 1985). The development of artificial insemination (AI), (reviewed by Parkes, 1957), led to the proposal that breeding schemes take advantage of this technique (Robertson and Rendel, 1950). The dairy cattle industry has adopted AI as the principle pathway for genetic improvement worldwide (Nicholas, 1985). Improvement in beef cattle has relied on conventional breeding. Richard (1971) demonstrated that the use of performance tested bulls, through AI, on commercial herds would reduce the time lag in transferring genetic merit from elite herds to commercial herds from 15 years, when using conventional breeding, to 3 years.

Artificial insemination has however not been widely adopted for beef breeding because of the extensive nature of the production systems, which precludes the day to day supervision of females which is essential for oestrus detection and effective AI (Nicholas, 1985).

2.3.2 THE APPLICATION OF MUTIPLE OVULATION AND EMBRYO TRANSFER (MOET)

The first registered calf to be born from embryo recovery and transfer procedures was registered in 1964 (Aitichison, 1982). It has been estimated that in the U.S.A. and Canada one in every five hundred calves born is the result of embryo transfer (Seidel and Seidel, 1989). The users of this technology can be subdivided; 50-60% of the commercial activity was by farmers or hobby farmers to increase the reproductive rate of donors, either to improve the herd or to sell breeding stock; 15-25% was by investors or venture capitalists who have others to manage the cattle, often as tax shelters; and 10-25% was by embryo transfer companies for direct sale (Seidel and Seidel, 1989). It is probable similar ratios hold in the U.K.

2.3.3 APPLICATION OF MOET TO BEEF BREEDING SCHEMES

The applications of multiple ovulation, embryo recovery and embryo transfer in breed improvement schemes are referred to as MOET schemes. The attributes of such schemes can be categorised as follows:

2.3.3.1 RAPID BREED EXPANSION.

The use of MOET technologies enables females to produce many more offspring than would be possible naturally. The mean number of pregnancies that result from a single superovulation and embryo collection has been estimated to be 3.5 (Seidel, 1981). The same author calculated that 43% of donors would produce 3-12

pregnancies per treatment. This ability to rapidly multiply numbers of offspring from a dam, with the associated development of frozen embryo storage, has enabled scarce, often exotic, cattle breeds to increase rapidly in numbers. Embryo transfer is utilized for three purposes; to obtain relatively small numbers of embryos from high quality donor cows fertilised by superior sires; to obtain large numbers of good quality embryos to provide a base herd with new genetic material; and to obtain large numbers of embryos to upgrade rapidly a population of cows where the use of AI would be too long term. The introduction of European beef breeds to the U.K. and the U.S.A. provided the stimulus for the development of MOET methods (Anderson, 1983). The use of MOET to increase breed numbers has been widely adopted, even to the extent of herd substitution (Newcomb et al., 1978).

2.3.3.2 LIVESTOCK EXPORT AND IMPORT

Embryo transfer, primarily of frozen embryos has facilitated the transfer of genetic material between and within countries. The import of frozen embryos enables the acquisition of high genetic merit livestock, both cow and bull families, at reasonable cost (McGuirk, 1989).

The cost of embryo transfer compares favourably with alternative methods of genetic procurement (Mahon and Rawle, 1987) and allows access to material which would not otherwise be available. The international trade in cattle embryos (eg. Munar and Hasler, 1989) has important animal health considerations. The

disease risks from the transfer of embryos are considered to be low (Singh, 1987). The very low risk of pathogen transfer from the transfer of frozen embryos has been demonstrated by Thibier and Nibart (1987) who found no evidence of disease transfer via embryos from herds of differing herd health status and by Bowen and others (1983) who found no evidence of transmission of bluetongue virus when embryos were transferred from infected dams to uninfected recipients. Treatments to reduce further the risk of infection from imported embryos have been investigated (Thomson et al., 1989).

2.3.3.3 GENETIC IMPROVEMENT

Current methods of livestock reproduction use far fewer males than females whilst both sexes contribute equally to the genetic composition of the following generation. With approximately equal numbers of each sex available as parents, selection among males can be greater than that of females.

Embryo transfer increases the rate of genetic improvement through increasing the reproductive rates of the female and placing greater emphasis on selection among female candidates than is possible with traditional methods. MOET can be used in two breeding cow pathways:

Firstly, the dam to breeding bull pathway; to increase the number of bull calves available for testing, thus increasing the selection intensity on the dams (Bradford and Kennedy, 1980).

Secondly, the dam to breeding cow pathway; to reduce the current high proportion of dams being used to breed replacement females.

The high costs of MOET have prevented the general application of these methods (Wilmut and Hulme, 1978; McDaniel and Cassel, 1981). Ruane (1988) reviewed the use of MOET schemes for improvement of dairy cattle, and concluded that there was little benefit to be obtained from applying embryo transfer to efficient progeny testing schemes. However, little information is available on the benefits of using embryo transfer in beef cattle.

The theoretical benefits from the application of MOET in a variety of cattle breeding schemes have been evaluated (Land and Hill, 1975; Nicholas and Smith, 1983; Tanaka et al., 1983; Smith, 1988; Wooliams and Wilmut, 1989). Land and Hill (1975) proposed the use of an elite herd, a nucleus, for beef cattle improvement. This herd, with a fixed number of cows would use nonselected cows as recipients for the embryos from selected donors. Thus selection intensity would be dependent on the number of embryos transferred per donor and on the total herd size. In their analysis, this scheme theoretically would result in an increase in the annual response to selection of 400 day weight of 16 kg compared to the response of 9 kg achieved through conventional breeding methods. The application of MOET in a nucleus herd has been evaluated for dairy cattle and McGuirk (1989) described the implementation of such a scheme but not for beef breeds where the selection traits can be measured in both sexes.

The usefulness of theoretical calculations is limited by the accuracy of the assumptions made to produce them. A major

difficulty in the application of MOET breeding schemes is the operational problem of achieving the performance standards assumed in theoretical calculations. Juga and Maka-tamila (1987) identified three potential areas of concern: where donors or sires were selected for use in more than one year so reducing the genetic base; the logistic problems of transferring embryos in a short period of time; the variation in superovulation response. In particular, the variation in response to superovulation could result in a choice having to be made between either producing equal family sizes from the repeated flushing of donors or accepting the embryo yield from a fixed number of flushes. The decision would determine either the number of progeny or the generation interval. McGuirk (1989) considered these factors to have minimal effect on the overall rate of progress and considered the consequences of inbreeding and genetic drift to be of greater significance.

2.4 THE DEVELOPMENT OF EMBRYO RECOVERY AND TRANSFER METHODS

Embryo transfer is the process of removal and transfer of fertilized ova from the reproductive tract of one female to that of another where the pregnancy may continue (Anderson, 1983). The first transfers of mammalian embryos were described by Heape in 1891, using rabbits, but the method was not applied to economically important species until 1951 when the first successful embryo transfer in the bovine was reported (Willett and others, 1951). At that time the technique was valued only as a research tool. However, the institution, by the Agricultural

Research Council, of a Unit of Animal Reproduction at Cambridge, U.K. led to the development of methods for cattle, which by the 1970's were applied outside the laboratory (Betteridge, 1977). These techniques were and are used to multiply breeds of cattle, often exotic, which are in short supply and hence expensive. These developments, occurring on both sides of the Atlantic, provided the stimulus for research to develop more consistently successful systems for embryo recovery and transfer which could be utilized at the farm level (Wooliams and Wilmut, 1989).

2.5 SUPEROVULATION OF THE COW

Superovulation is the production of three or more ova in mono-ovulating species (Gordon, 1982) and is thought to involve a decrease in the numbers of antral follicles undergoing atresia present at the initiation of the treatment (Moor and others, 1984). Superovulation in the bovine was first achieved by Casida (1940) (cited by Gordon, 1983) using Follicle Stimulating Hormone-like substances to produce 10-20 mature preovulatory follicles.

2.5.1 SUPEROVULATORY AGENTS

Cole and Hart (1930) detected a gonadotrophin in the serum of pregnant mares during early pregnancy. This glycoprotein, pregnant mare's serum gonadotrophin, PMSG, possesses alpha and beta subunits similar to LH and FSH. The molecule has a high carbohydrate, especially sialic acid, content. The molecular weight is approximately 60000 daltons (Reeves, 1987). PMSG is secreted by specialized trophoblast cells which invade the

maternal endometrium between 36 and 40 days of gestation (Gordon, 1983). It is now commonly used in superovulatory protocols (Sreenan, 1983).

Pituitary extracts of Follicle Stimulating Hormone (FSH), of porcine (Beckers, 1987), equine and ovine origins, have been used to superovulate cattle (Sreenan, 1983). The origin of the FSH preparation does not affect the resulting superovulatory responses in cows (Donaldson, 1988). FSH preparations have a short half-life and therefore need to be administered repeatedly in order to superovulate successfully. Several comparisons have been made between PMSG and FSH but no consistent superiority for either has been reported (Lubbadeh et al., 1980; Broadbent and Hutchinson, 1989). The variable responses achieved using FSH preparations have been attributed to variations in the batch activity (Donaldson, 1990).

Other gonadotrophins have been evaluated for use in superovulation treatments. An equine anterior pituitary extract (HAP) was employed by Moore (1975) and compared with PMSG. No significant difference in ovulation rate was detected and the limited availability of this preparation has prevented its wider adoption. Human Menopausal Gonadotrophin (hMG) has been utilized in bovine embryo transfer systems (McGowan et al., 1985). Newcomb (1980) used hMG as a superovulator in a comparison with PMSG. No differences between the treatments were observed but the use of hMG in animals which did not respond to PMSG was proposed.

2.5.2 GONADOTROPHIN ADMINISTRATION

The timing of the administration of gonadotrophin in the oestrous cycle is a major factor in the successful superovulation protocol. Superovulation was achieved when the gonadotrophins were adminstered during the follicular phase from Day 16 onwards (oestrus = Day 0). Sreenan and Gosling (1977) showed the timing of PMSG injection to be critical. Lower ovulatory responses were observed when the animals were treated in the early luteal phase (Days 3-8) than in the mid luteal phase (Days 9-12). These findings demonstrate the value of accurate oestrus detection for superovulation, and as will be shown later, embryo recovery and transfer.

2.5.2.1 OESTRUS DETECTION

Oestrus in the cow is characterised by its willingness to stand and be mounted by a bull. This reflex is the indication that an animal is in oestrus (Kiddy, 1976). Esslemont (1973) concluded that the standing-to-be-mounted reflex was closely related with oestrus and, since it was rarely observed at other times, was an important indication of oestrus. This was in agreement with the findings of Hackett and McAllister (1984).

The onset of oestrus appeared to be evenly distributed during the 24 hours of a day (Hackett and McAllister, 1984). O'Farrel (1978) observed that 50-55% of heats occurred between midnight and 10 am and 20% between 6pm and midnight.

Mounting activity has a diurnal pattern with increased activity during the late evening and early morning (Esslemont and

Bryant, 1974; Hackett and McAllister, 1984) and is influenced by factors such as lactation number, supplementary feeding and weather (Kilgour et al., 1977).

2.5.2.2 **PHYSIOLOGICAL CHANGES DURING OESTRUS**

The oestrous cycle is regulated by hormones and changes in blood hormone concentrations reflect this (Chenault et al., 1975).

The variation in plasma progesterone concentration during the oestrous cycle has been described by many authors (eg. Smith et al., 1975). The concentration of progesterone is low (0.1-0.4 ng/ml) during the first 3 days of the cycle and increases to a maximium (6-9 ng/ml) at Days 11-18. The concentration falls rapidly 72-96 hours before the onset of oestrus. This pattern is reflected in the milk progesterone concentration (Dobson et al., 1975).

17 beta-Oestradiol concentration rises as progesterone concentration falls. This rise is associated with the exhibition of the behavioural patterns at oestrus and is also a trigger for the secretion of LH (Chenault et al., 1975).

Luteinising hormone is secreted in pulses which increase in frequency and amplitude up to ovulation when a rapid and brief increase in concentration causes ovulation (Peters and Lamming, 1984).

Cervical mucus exhibits cyclical changes in both its physical and chemical properties in response to the hormonal status of the animal. Ferning, spinnbarkeit and elasticity of mucus are increased during oestrus whilst viscosity and tack are

decreased (Linford, 1974). The dry matter content of mucus is lowest at oestrus and highest mid-cycle, the protein content of the mucus follows a similar cycle whilst the glucose and chloride concentrations display opposite cyclical changes (Linford, 1974). Mucus secretion has been observed to be stimulated by oestrogen and inhibited by progesterone (Abrams et al., 1975).

2.5.2.3 METHODS OF OESTRUS DETECTION

The efficiency of oestrus detection has been acknowledged as a crucial factor in the reproductive performance of dairy herds (Schofield, 1988). Observation is the major component in oestrus detection and recognition.

The main criterion for cattle to be recognised in oestrus is standing to be mounted. However, the intensity and length of time during which this is expressed can vary from 6 to 20 hours. Thus the frequency and duration of the observation period are important. O'Farrel (1978) calculated that 3 observations per day would detect 75% of heats whereas 5 observations would detect 90%. This was contradicted by Donaldson (1968) who used 2 observations and detected 90% of oestri.

Since continuous observation is impractical, methods to aid oestrus detection have been developed. These have been reviewed by Schofield (1988). The main on-farm methods are examined below.

External markers are used to indicate whether a cow has been mounted. These include paint on the point of the pelvic ridge which is removed by mounting, or pigmented grease which is applied to a teaser animal to mark any mounted animals (Mills et al., 1969).

The development of rapid assays for the estimation of milk progesterone concentration (Stanley et al., 1986) has allowed the monitoring of a cows endocrine status (Britt and Holt, 1988). Kits have been developed to allow milk samples to be analysed through simple colour changes without recourse to laboratory facilities.

The cyclic variation in the properties of cervical mucus have also been used to detect oestrus. The variation is accompanied by changes in electrical resistance. Resistance is found to fall at oestrus (Aboul Ela et al., 1983) correlating with the pattern of progesterone secretion (Foote et al., 1979). The pregnancy rate achieved after performing insemination on the basis of electrical resistance of cervical mucus has been reported as 52% for animals which were not otherwise examined for signs of oestrus. This compares with a pregnancy rate of 49% for cows inseminated when oestrus was detected visually only (Foote et al, 1979).

2.5.2.4 SYNCHRONISATION OF OESTRUS

The detection of oestrus is facilitated when the time of oestrus is controlled and can be predicted. This is especially important in superovulation treatments where a reference oestrus is used as the basis for all subsequent operations (Sreenan, 1983).

Two methods are currently available for manipulating the bovine oestrous cycle to induce oestrus at a selected time. The first is to regress the corpus luteum so that the animal enters

he follicular phase and returns to oestrus. The luteolytic properties of commercially available analogues of prostaglandin F2 alpha are utilised to regress a functioning CL prematurely, and so induce oestrus approximately 72 h after the prostaglandin injection (Cooper, 1974). The second is to administer progesterone or an analogue continually to simulate the function of the corpus luteum and hence suppress follicular development. The progesterone administration prevents gonadotrophin release. The treatment does not affect the lifespan of the natural CL. Consequently the progesterone has to be administered in conjunction with a luteolytic agent to eliminate the natural CL. Once the progesterone treatment is ceased, after 12 days, the peripheral plasma progesterone concentration falls and the animal enters the follicular phase and hence into oestrus, approximately 48 h after the withdrawal of the progesterone. The control of oestrus in cattle has been reviewed by Roche (1979).

2.5.3 SPLIT DOSES OF GONADOTROPHIN

Experiments designed to increase the follicular population of the ovaries by using a low priming dose of gonadotrophin early in the oestrous cycle (cir. Day 2) prior to the normal dose have been equivocal. Rieger and others (1988) found that pretreatment with FSH had no effect on either the total superovulatory response or the number of transferrable embryos recovered per donor. Ware and others (1987) however, obtained an increased ovulation rate, and recovered more fertilised ova per donor when heifers received a priming dose. Priming doses of gonadotrophins

could be useful in populations of cattle where the superovulatory responses are low (Rieger et al., 1988).

2.5.4 GONADOTROPHIN AND PROSTAGLANDIN

Cows treated on Day 16 of their natural cycle show considerable variations in the interval from PMSG administration to oestrus (Betteridge, 1977). The production of prostaglandin F2 alpha (PG) and several analogues (eg. Cooper, 1974) has allowed the accurate control of oestrus in cycling cows. Scanlon and others (1968) showed that, the interval between gonadotrophin administration and ovulation can markedly affect superovulation. In their study, an increase in ovulation rate was observed as the interval between PMSG administration and oestrus increased from 2 to 5 days. The superovulation regimen currently utilised when PMSG is used is to inject the PG forty-eight hours after the gonadotrophin (Nancarrow and Miller, 1976; Betteridge, 1977). Luteolysis of the corpus luteum is induced by PG and oestrus commences approximately forty eight hours later. This interval is less than that observed in unsuperovulated animals which take 72 h compared to 48 h (Gordon, 1982). The use of PG in superovulatory protocols was recommended by Church and Shea (1977) who found superior ovulation rates and a reduced incidence of missed oestri.

Comparisons have been made between the various prostaglandin analogues available (Guay and others, 1988), and the effect of using PG in split doses on ovarian response (Perry and Donaldson, 1984). No treatment differences were observed in either trial.

The use of twice the normal pharmaceutical dosage of PG has been found to more effective than the normal dose for regressing CLs from superovulated cattle (Garcia et al., 1983), as has intramuscular administration of PG compared to intravenous injections (Maurer and others, 1989).

2.5.5 ADDITIONAL SUPEROVULATION TREATMENTS

Progestagens have been used as alternatives to prostaglandins to control oestrus in superovulated cattle. The protocol requires the gonadotrophins to be given prior to progestagen withdrawal. Sreenan and Beehan (1976a) compared prostaglandin injection, Norgestomet implants and progesterone impregnated intravaginal sponge pessaries for oestrus control in superovulated cattle. PMSG was used in all treatments, being given either one day prior to progestagen withdrawal or two days post PG injection. The PG treatment produced a highly sychronous oestrus and high numbers of fertilised embryos compared to the responses of the animals treated with progestagens, 84 % of PG treated cows displayed oestrus at 2 days post injection whilst 35.9 of the progesterone treated and 34.3 of the Norgestomet treated animals were in oestrus at this time. The PG treated animals also had higher ovulation rates than the other treatment animals.

The long biological half life of PMSG, which enables single injections to be used, may adversely affect the shed ova (Lammond, 1970). The use of anti-PMSG antibodies to prevent secondary effects have been extensively investigated (Alfuraiji

et al., 1988; Callesen et al., 1988a) but only in the report of Dieleman and others (1989) has any significant improvement in embryo quality been demonstrated.

Newcomb (1980) investigated the use of human Chorionic Gonadotrophin (hCG) in 32 superovulated lactating British Friesian cows at the onset of oestrus. Although the embryo recovery rate was increased by hCG administration (74.5 cf 50.2%) no improvement in ovarian response was observed. This data agreed with that reported by Moore (1975) who did not find any benefit from the use of hCG.

Gonadotrophin releasing Hormone (GnRH), which plays an important role in regulating the release of gonadotrophins (Peters, Pimentel and Lamming, 1985) and analogues have been used in superovulatory treatments with the aim of improving the embryo recovery rate and the quality of embryos. GnRH has been shown to improve the reproductive efficiency in the early post partum cow (Cavestany and Foote, 1985a) and several studies have reported the application of GnRH to superovulated animals. Voss and others (1988) treated 58 dry Holstein cows and observed that a sharp LH peak was induced at 36 h when GnRH was given 36 h after PG. At later administration the induced LH peak was either a major or a minor peak depending on whether a spontaneous LH peak had already occurred. No benefit was found from GnRH administration on embryo recovery. Foote and others (1988) treated 33 lactating Holstein cows with GnRH at differing times in the superovulatory protocol; either 48 h post PG; 54 h post PG; or 24 h after the onset of standing oestrus. The mean number of embryos recovered was unaffected. There were no significant differences in

fertilisation rate between the groups but there was an increase in the fertilization rate between the treated cows and the controls at the ten percent level. The above studies were performed on FSH superovulated cows. However, Newcomb (1980) found similar results when GnRH was administered to PMSG treated cows.

2.5.6 REPEATED SUPEROVULATION

To increase the numbers of embryos collected from a cow, repeated superovulation treatments have been employed. Christie and others (1979) superovulated fourteen heifers of mixed breeding, using 2000 i.u. PMSG and PG, up to ten times with a mean interval between superovulations of 42.1 (sem 0.97) days. There was a significant fall in mean ovulation rate from the first treatment (14.25 CL) to the second (10.9 CL). However subsequent responses were not significantly different from the initial response. Four heifers were treated ten times, these animals continued to respond with multiple ovulations (mean = 8 CL) and no macroscopic uterine damage was observed from the repeated flushes. Thirteen of the 14 heifers were subsequently inseminated, 12 conceived, requiring 1.67 services per pregnancy. Ali Dinar and others (1987) repeated superovulation in 23 Friesian cows up to twelve times, using 3000 iu PMSG, at an interval of 50 days with a slight improvement in response from the first treatment to the second but with no significant differences thereafter. Their study produced an overall aggregate of 65.0 ovulations per donor for the 8 superovulations. Hasler

and others (1983) found that repeated superovulation did not affect the numbers of ova recovered but that the fertilization rate and embryo numbers decreased.

It is possible that the interval between superovulations is critical. Lubbadeh and others (1980) repeatedly superovulated 16 lactating dairy cows, with either PMSG or FSH. The cows were treated four times; the first three times at 16 day intervals and the fourth after one oestrous cycle. The responses to the same gonadotrophin were similar in the first and final treatments for the number of ovulations (FSH; 17.9 cf 21.5, PMSG; 13.9 cf 16.4 CLs.) and the numbers of ova recovered (FSH; 6.7 cf 16.5, PMSG; 6.9 cf 8.9). The second treatment showed significantly reduced responses; the mean ovulation rates for both gonadotrophins were, in chronological order: 15.9; 8.8; 9.4; 16.4. The corresponding mean number of embryos recovered were: 6.8; 1.5; 1.9; 12.2.

The influence of superovulation on the subsequent performance of the cow has been investigated. Bak et al (1988 a,b) found no effects on reproductive performance (mean 1.9 services to conception; interval embryo collection to conception = 60.6 days) or milk yield. Cowen and Susnik (1987) observed that superovulation increased the number of days open (194 cf 100) and increased the number of services to conception (2.53 cf 1.7). The effects on milk yield could be attributed to the intervention in the normal breeding cycle.

2.5.7 FACTORS AFFECTING SUPEROVULATION

2.5.7.1 DOSE AND BATCH OF GONADOTROPHIN

Initial studies with PMSG indicated that an optimal response was obtained with 3000 i.u. (Hafez et al., 1963; Scanlon et al., 1968) and dose linear responses have been reported (Lammond, 1972). High doses of gonadotrophins have been associated with increased numbers of persistant follicles (Dowling, 1949). Hafez and others (1963) superovulated 80 Hereford heifers with either 3000 or 5000 iu PMSG. Those treated with 3000 i.u. produced a mean of 26.3 CL and 45 follicles, those receiving 5000 iu produced 28.0 CL and 63 follicles. Dowling (1949) used 3000 iu and found the mean ovulation rate to be 12 CLs. Scanlon and others (1968) treated 89 cattle, mainly heifers, with 3000 iu PMSG and observed a mean ovulation rate of 12 CLs. Sreenan and Beehan (1976a) used 3 dose rates of PMSG; 1500, 2000 and 2500 i.u. PMSG. The mean ovulation rates were 8.0, 14.7 and 9.4 respectively. The doses currently utilized range from 2000-4000 i.u., according to body weight, given as a single intramuscular or subcutaneous injection (Sreenan, 1983). The PMSG molecule is a glycoprotein possessing both LH and FSH properties (Reeves, 1987). It is possible that the variability observed following PMSG induced superovulation could be due to the ratios of these components varying between batches . Newcomb and others (1979) examined the effect of dose level (1000 vs 2000 i.u.) on ovarian response using three different commercial batches of PMSG. Forty two heifers were used and treatments were repeated twice. A

significant effect of PMSG dose was detected but no batch differences were found. Radioreceptor assays of 5 commercial batches of PMSG were performed by Stewart and others (1976). The FSH:LH ratios ranged from 0.87 to 1.3 and these batches were used to superovulate a total of 99 heifers. The response varied from 3 to 45 CLs but no significant differences were detected between the mean response of the groups treated with the different batches of PMSG. Significant differences, however have been found in the FSH and LH ratios in batches of hMG (Cooke et al., 1988) and it is possible similar variation may apply to PMSG preparations.

2.5.7.2 BREED AND AGE OF COW

Several workers have suggested that the variable responses achieved in superovulating cows were genetically based (Sreenan and Beehan, 1976a; Monniaux and others, 1983;). Church and Shea (1976) examined the responses to superovulation of Simmental, Maine-Anjou, Limousin and Chianina cattle (mainly heifers). The respective mean ovulation rates were: 15.2; 9.9; 13.6; 12.4. Similarly Gordon (1982) found that 16 Friesian cows produced a mean of 5 CLs when treated with 2000 i.u. PMSG. Fourteen Charolais cows produced a mean of 10.4 CLs. The more prolific breeds of cattle are generally more sensitive to superovulation than the less prolific breeds (Bindon et al., 1986).

The effect of age on cow fertility has been documented. The rate of follicular atresia increases with age and this would be expected to result in a decline in ovarian response (Gordon,

1982). Experimental data confirms this hypothesis; Moore (1975) reported a greater response to PMSG with heifers (6.7 CLs) than with cows (2.9 CLs). Newcomb and others (1978) also observed a higher ovulation rate with heifers than with cows.

2.5.7.3 **SEASON AND NUTRITION**

Hasler and others (1983) found significant seasonal differences in the numbers of embryos collected. In data collected from approximately 1000 cows and heifers the numbers of embryos recovered were higher in winter and spring than in summer and autumn. This study also showed the highest fertilization rate was obtained in summer with a trough in winter.

The effect of season on superovulation could be due to nutritional stress associated with the seasons. Church and Shea (1977) reported clear seasonal variation in the superovulatory responses at a Canadian unit. The least satisfactory responses were recorded in January, February and March. Pregnancy rates were lowest in Oct-Dec (45%) but highest in Apr-May (53%). The authors postulated that the oestrogen content of the forage could be a significant factor. Other workers have recorded superior superovulatory responses in the summer and spring (Crister et al., 1979; Lerner et al., 1986). However conflicting data has also been published where no influence of season or month could be detected on the responses to superovulation (Massey and Oden, 1984; Shea et al., 1984).

The effect of stress on superovulation has been evaluated. Fasting animals for 72 h before PMSG administration was found to

be detrimental, the mean ovulation rate declined from 2.5 to 0.5 CLs (Lammond, 1972). Transporting donors every 12 hours for the 4 days of a FSH treatment caused increased cortisol concentrations in the blood and reduced ovulation rate (20.4 cf 15.4 CLs) (Edwards et al., 1987). Putney and others (1988) exposed donors to elevated ambient temperatures of 30°C for 16 h and 42°C for 8 h after insemination. The controls were maintained at 20°C. The stressed donors produced an increased incidence of abnormal or retarded embryos; 20.7% normal embryos were collected from the stressed animals, 51.8% from the controls.

2.5.8 PREDICTING THE RESPONSE TO SUPEROVULATION

Blood hormone concentrations are routinely monitored in human fertility treatment (Iritani, 1989) in an attempt to predict the quality and quantity of ova. A similar approach has been investigated in the bovine. Donaldson (1985a) studied the blood patterns of LH and FSH during superovulation. Both hormones showed preovulatory surges of normal concentrations but in many cases the timing of the surge was abnormal. Cows were grouped according to the timing of their gonadotrophin peaks where: FSH and LH surges occurred coincidentally; LH surge occurred within 4 h of the onset of oestrus; LH surge occurred before the onset of oestrus; the LH surge occurred more than 4 h after the onset of oestrus; FSH surge occurred within 4 h of the onset of oestrus; FSH surge occurred before the onset of oestrus; the FSH surge occurred more than 4 h after the onset of oestrus. The respective

mean numbers of embryos recovered were: 10; 5.5; 6.7; 9.8; 12.0; 8.4 and 6.3. Transferrable embryo production appeared to be lower in cows in which LH and FSH surges were either early or late with reference to the time of oestrus. The respective mean percentages of transferrable embryos were: 35; 64; 40; 16; 23; 17 and 43%. The occurrence of a normal FSH surge appeared to be primarily responsible for the embryo number and LH for their quality (Donaldson, 1985a). In a further study (Donaldson, 1985b) related the superovulatory response in 9 cows to ovary size, numbers of corpora lutea (palpated per rectum), CL weight and plasma progesterone concentrations. Corpora lutea (CL) from superovulated ovaries were found to be smaller than from non-superovulated cows. Plasma progesterone concentrations at embryo recovery were positively correlated with the numbers (r=0.9) and weight of CLs (r=0.95). However none of these parameters were correlated to the numbers of embryos produced.

Callesden and others (1988b) defined criteria for normal and deviant profiles for progesterone and LH at four sampling times: at gonadotrophin injection and 0,24 and 48 h post PG, to provide a basis for the assessment of the profiles shown by donors. Donors with endocrine profiles which differed from those observed in normal i.e. unsuperovulated cows, were significantly poorer producers of quality embryos. Bevers and Dieleman (1987) reported that 16.7% of superovulated heifers did not display a LH surge. In the animals which produced an LH surge, the mean interval from PG injection to LH surge was 43.9 h (SEM=1.5), this interval was negatively correlated with the number of preovulatory follicles (r=-0.483) and the mean interval between

the onset of oestrus and the maximum of the LH surge was 1.96h (SEM=0.54). The concentration of progesterone at the time of gonadotrophin administration is not a reliable predictor of superovulatory response (Sreenan and Gosling, 1977; Bevers and Dieleman 1987).

2.5.9 BREEDING THE SUPEROVULATED COW

Successful superovulation produces a highly synchronized oestrus with at least 95% of cows exhibiting oestrus in a 24-36 hour period (Sreenan, 1983). The factors affecting the fertilization of the resulting ova have been investigated. Initial studies used natural service (Scanlon and others, 1968) but the greater versatility of AI combined with the increased access to superior sires by artificial insemination (AI) has led to its general adoption. The use of AI required the development of appropriate AI schedules to achieve high fertilization rates. Gordon (1982) recommended that two straws (20 million live sperm/straw) be given at the onset of oestrus with an additional one or two straws after a further 12-24 hours. Schiewe and others (1983) considered two straws given after the onset of oestrus to be effective. The trend to reducing semen volume and improving the timeliness of insemination was continued by Donaldson (1985c) who concluded that a single insemination twelve hours after the onset of oestrus was sufficient. One, two or three inseminations with 1 straw of semen at each time produced the same mean percentage of fertilised embryos; 74, 68 and 74% ; and the same mean number of fertilised embryos; 8.2, 6.3 and 9.1. His

conclusion was supported by Lopez Gatius and others (1988) who found a half straw of semen deposited into the cranial half of each horn was as efficacious as multiple insemination. The quality of the semen used is critical. Newcomb (1980) found that more ova were unfertilized (10.5) when semen from a bull with below average fertility was used than when a bull of above average fertility was used (7.4). In the same study more unfertilized ova were recovered when the animals were inseminated on the day of oestrus than when insemination was performed after the end of oestrus. The author attributed this to the requirement to maintain viable spermatozoa in the reproductive tract which allowed for the variation in the timing of ovulation between animals.

2.6 EMBRYO RECOVERY FROM CATTLE

2.6.1 RECOVERY AT SLAUGHTER

Initial investigations with embryo transfer used embryos collected from the donors at slaughter (eg. Rowson and Moor, 1966). Methods of embryo recovery were later developed which did not require post mortem collection. Rowson and others (1969) showed that embryos collected from live donors were superior to those recovered at slaughter. The pregnancy rate achieved with embryos recovered at slaughter was 33% whilst that of embryos surgically recovered was 91% (Rowson et al., 1969). The time interval between slaughter and embryo recovery was sufficently large to effect the viability of the embryos, and its reduction should improve embryo quality to an acceptable level (Newcomb,

1982).

2.6.2 SURGICAL RECOVERY

Several approaches for surgical embryo recovery have been employed; mid-ventral laparotomy, transvaginal recovery and intravaginal recovery.

The Cambridge group pioneered the mid-ventral laparotomic method. This required the anaesthetized cow to be placed on her back and a 15 cm incison was made in the mid line just anterior to the udder. The uterus was exteriorized and retained by a ligature. Fluid was flushed through the uterus, and collected via a fine cannula inserted in the ovarian end of the oviduct (Newcomb and Rowson, 1975). Methods employing the vaginal route, both trans- and intra- , were assessed (Newcomb, 1982) but required too great a technical input and surgical skill. Baker and Jillela (1980) fixed catheters surgically in the uterine horns in an attempt to collect embryos repeatedly. This technique failed to collect any ova.

2.6.2.1 PROBLEMS WITH SURGICAL RECOVERY

The results achieved with surgical recovery were encouraging but the various techniques all had the same limitations. The type of surgery required facilities not normally available on farms; the operation risked the impairment of the donors future fertility; and the formation of adhesions imposes a limit on the number of times a donor can be flushed (Newcomb, 1982). These

factors combined to stimulate the development of less traumatic non-surgical methods of embryo recovery.

2.6.3 NON-SURGICAL RECOVERY

The first attempt to recover bovine embryos without recourse to surgery was described by Rowson and Dowling (1949).

Non-surgical flushing systems can be classified into two categories; circulation systems and to and fro systems.

Circulation systems use 3-lumen catheters which possess separate fluid inlets and outlets between which the flushing fluid flows. These catheters require manipulation almost up to the uterotubal junction of the uterine horn before the cuff is inflated occluding the uterine lumen. Fluid is introduced from a syringe through the catheter into the sealed portion of the uterine horn and runs freely out into a collecting vessel. Recovery of the fluid is assisted by per rectum manipulation (Newcomb et al., 1978).

To and fro systems rely on the ebb and flow movement of fluid introduced and withdrawn from a single inlet to recover the embryos present. The method was originally described by Elsden and others (1976). A Foley, 2 lumen, catheter was inserted into the base of the uterine horn to be flushed and the cuff inflated to seal the lumen, isolating the horn. The outlet aperture was clamped and fluid was run into the horn from a reservoir. Once the horn was filled the inlet was clamped and the outlet released. The fluid was collected in test tubes and the process repeated. The second horn was flushed using the same technique

(Newcomb, 1982).

2.6.4 SUCCESS RATES WITH EMBRYO RECOVERY

The choice of method of embryo collection is dependent on the time of embryo collection. Surgical recovery is still necessary to retrieve early or late development stage embryos.

The development of nonsurgical embryo recovery systems has allowed the on-farm application of these methods. The discovery that standing sedated animals could be flushed nonsurgically as successfully as fully anaesthetized ones increased the practical uses of the technique (Newcomb and others, 1978). Newcomb (1980) found the circulation system, using 3 lumen catheters, of embryo recovery to be significantly more efficient than the to and fro, 2 lumen catheter, at day 7 post oestrus. The to and fro method was more successful at day 8. Newcomb and others (1978) found that the proximity of the catheter tip to the tip of the uterine horn had a significant effect on embryo recovery when using circulation flushing systems. The most successful recoveries were achieved when the tip of the catheter was positioned within 5 cm of the utero-tubal junction.

The differences between the two types of flushing system can be summarized; circulation systems use small volumes of flushing fluid and may recover embryos more rapidly as the flow is continuous; to and fro systems are easier to position in the uterus but require double the volume of fluid necessary with circulation systems (c. 500 ml cf 250 ml) (Newcomb, 1980).

2.6.5 FACTORS AFFECTING EMBRYO RECOVERY

2.6.5.1 LOCATION OF THE OVA

Newcomb and others (1976) examined the entry of ova into the uteri of PMSG treated beef heifers. There was a rapid decrease in the proportion of ova recovered from the oviduct after Days 3-5 post oestrus (Table 2.3).

Table 2.3: Effect of time on the position and development stage of recovered ova

Days post oestrus	3	5	6	7	8
No of ova	146	354	281	357	163
% ova in oviduct	96	17	14	8	7
Development stage	8 cell	16-32	32-64	blastocyst	hatched blasto.

(Newcomb et al., 1976)

In the same study ligation was used to show that from Days 6-8 post oestrus (oestrus = Day 0) 73% of all ova recovered came from the anterior 10 cm of the tip of the uterine horn. These findings emphasized the need to flush the uterine horn fully to the tip. There was an increase in the proportion of degenerate ova forms with increasing time from oestrus to egg recovery with a dramatic increase in embryo mortality post Day 8. The uterine environment of the superovulated cow may be suboptimal for embryo survival (Booth and others, 1975; Newcomb and others, 1978). The high concentrations of oestrogens which occur after ovulation in

the superovulated cow could modify the motility of the oviduct and uterus causing premature entry of embryos into the uterus (Booth and others, 1975). Bovine embryos do not form intimate attachments to the uterus until Day 18 and could be recovered nonsurgically up to this time despite the drastic lessening in trauma resistance post Day 14 (Seidel, 1981). The best yield of viable embryos was achieved by recovery on Days 6 to 8 post oestrus (Newcomb, 1980; Seidel, 1981; Gordon, 1982).

2.6.5.2 AGE OF DONOR

Donor age has a large effect on embryo recovery rate. Recovery rates are lower in younger than older cows (Newcomb, 1980), possibly due to uterine size, older cows possessing larger uteri due to the numbers of parities. Newcomb and others (1978) observed the embryo recovery rate of heifers to be 60% compared with 80% achieved with cows.

Calves can be superovulated but low embryo recovery rates are achieved with surgical recovery and the recovered embryos have high rates of mortality (Seidel, 1981).

2.7 EMBRYO TRANSFER TO RECIPIENTS

2.7.1 SURGICAL EMBRYO TRANSFER

2.7.1.1 MID-VENTRAL LAPAROTOMY

This method resembled that for embryo recovery in that the recipient was anaesthetised and restrained in dorsal recumbancy. Less manipulation was required however as only the uterine horn ipsilateral to the ovary bearing the corpus luteum was exteriorized. The horn was penetrated by a blunted 18 gauge needle, a pipette passed into the uterine lumen and the embryo expelled towards the utero-tubal junction (Newcomb, 1982). This system was both intensive in equipment and labour and was not applicable at the farm level.

2.7.1.2 FLANK TRANSFER

Flank transfer is the method of choice for the surgical transfer of Day 7 bovine embryos (Seidel, 1981). The side of the recipient ipsilateral to the CL was determined by rectal examination. Differing methods of restraint have been applied: local anaesthesia with or without a crush crate. An incision was made 12-15 cm long in the flank below the tubae coxae. The muscle layers were separated by dissection and the tip of the horn exteriorized. A blunted needle was used to penetrate the horn and a flexible cannula, a modified tom-cat (4 Ch.) catheter, was introduced into the lumen. The embryo bearing fluid was expelled through it towards the utero-tubal junction (Newcomb, 1979; Seidel, 1981).

Hasler and others (1987) reported a pregnancy rate of 71.3% using surgical transfer in approximately 1000 recipients.

2.7.1.3 TRANSVAGINAL TRANSFER

Various methods have been proposed to circumvent the major surgery of the methods described above. Sugie (1965) described a device which transfixed a needle through the dorsal vaginal and uterine walls. The careful manipulation necessary was facilitated by the insufflation of CO_2 gas into the uterine lumen. This method needed special expertise and has been discarded in favour of flank and nonsurgical transfer techniques.

2.7.2 NON-SURGICAL TRANSFER

Development of a non-surgical transfer method has allowed the application of embryo recovery and transfer techniques at the farm level. Early experiments revealed that early embryos failed to develop following transfer due to expulsion of the embryos. Transferring Day 6-9 embryos rather than Day 3-5 was more successful (Lawson and others, 1975) as the myometrial activity had by then decreased, reducing the risk of expulsion (Harper et al., 1961). Earlier stages of embryo development are unable to survive in the uterus (Betteridge, 1977). Initial investigations into nonsurgical transfer methods used insufflation of the uterus with CO_2 gas prior to transfer (Vincent et al., 1965; Rowson and Moor, 1966) but this technique was discarded. Currently insemination guns are used to deposit the transferred embryo in

the uterine horn (Sreenan, 1975). Newcomb (1979) found success rate differences between surgical and nonsurgical techniques to be not significant although Sreenan and Diskin (1987) observed pregnancy rates to be 10-30% higher from surgical transfer compared to non-surgical transfer.

2.7.3 FACTORS AFFECTING THE SUCCESS OF EMBRYO TRANSFER

2.7.3.1 EMBRYO VIABILITY

Embryo quality is assessed by the morphological microscopic appearance of the embryo (Shea, 1981). In the absence of any reliable direct test of embryo viability or quality this technique of assessment with grading has been widely adopted. Various biochemical parameters have been proposed as assessors for identifying viable embryos but none are in current application (Mohr and Trounson, 1980 and review; Butler and Biggers, 1988).

Embryos recovered on day 5 post insemination are comprised of 16-32 cells and are described as early morulae. Exact cell numbers are difficult to estimate without fixing and staining in order to count nuclei. The cells, blastomeres, secrete fluid which forms the blastocoel. This is visible by Day 7 in the bovine. By Day 8 the blastocoel expands forming a group of cells on the perimeter, the trophoblast. Up to Day 9 the embryo is contained within the zona pellucida. Shea (1981) graded embryos on their appearence: an above average embryo, graded 4, possessed symmetry, even granulation, no deformation in the zona pellucida

and no extruded blastomeres. A below average embryo, graded 2, was characterized by uneven blastomere size, extensive blastomere extrusion and evidence of membrane rupture. Shea used this grading system with 809 nonsurgical transfers and found the higher the grade of embryo the better the pregnancy rate of the recipient; 71% grade 4 embryos survived compared to 44% survival for grade 2 embryos. Donaldson (1985d), used a similar system and observed that there was a decline in survival rate of transferred embryos of 23.6% between embryos graded 1 and 4. The data obtained from field experiments tends to be confused as the best quality embryos tend to be transferred to the most synchronous recipients and the poorer embryos are transferred to the least synchronous (Sreenan and Diskin, 1987). Embryos transferred after being stored frozen suffer a 10-20% decline in viability irrespective of grade (Nelson and Nelson, 1988).

2.7.3.2 SYNCHRONY OF OESTRUS BETWEEN DONOR AND RECIPIENT

The importance of synchrony between donor and recipient oestrus has been stressed in several reports. Rowson and others (1969) demonstrated that exact synchrony between donor and recipient produced the best pregnancy rates but a variation of +/- 2 days could be tolerated. The degree of synchrony necessary was dependent on the age of the ovum (Newcomb and Rowson, 1975) and the quality of the embryo (Donaldson, 1985d). The pregnancy rate was found to be highest with exact synchrony using early blastocysts. Early morulae were able to continue development in

recipients which were in oestrus after the donor. Grade 3 and 4 embryos were able to tolerate asynchrony better than grades 1 and 2. Donaldson (1985d) suggested that exact synchrony was necessary for the embryo development stages from late morula to collapsed blastocyst. Embryo stages before and after these stages, respectively early morula and late blastcyst, would develop in recipients in oestrus 12 hours before the donor oestrus.

Other studies have confirmed these observations. Seidel (1981) suggested there was a tolerance of 36 h in either direction and a possible benefit if the recipient was in oestrus in advance of the donor. Hasler and others (1987) confirmed the tolerance of embryos to 36 h asynchrony. Repeated treatment of asynchronous recipients with GnRH has proved successful in improving the pregnancy rate of the transferred embryos and may be useful in improving the survival of more synchronous embryos (Drost et al., 1988).

2.7.3.3 AGE OF THE EMBRYO

The age of an embryo at transfer has a major effect on the resulting pregnancy rate. The transfer of Day 3 embryos was significantly less successful than that of 4,5,6 and 7 Day embryos (Newcomb and Rowson, 1975). The Day 3 embryo pregnancy rate of 10% compares with the rate of 82% achieved with Day 8 embryos. Further studies confirmed this age effect (Hahn and Hahn, 1976; Lawson and others, 1975). The failure of early embryos to continue development after transfer was explained as being due to their location in the uterus. Newcomb (1979)

reported no differences in the pregnancy rates of transferred Day 7,8 or 10 embryos. Hasler et al (1987) observed lower pregnancy rates with Day 9 or older embryos compared with younger embryos and that early or mid-stage blastocysts were the most successfully transferred.

2.7.3.4 EMBRYO PLACEMENT

The embryo should be placed in the uterine horn ipsilateral to the ovary bearing the corpus luteum (Sreenan and Beehan, 1976). Ideally the placement should be at the tip of the horn (Newcomb and Rowson, 1980). Embryos transferred to the contralateral horns of recipients are much less likely to continue development (Sreenan and Beehan, 1976b; Del Campo and Gunther, 1980). Deep penetration of the uterus is prevented by the curvature of the horns. At Day 7 the embryo is in the horn at a greater depth than a cassou gun can penetrate (Newcomb et al., 1975). Surgical transfer allows high placement in the horn without the endometrial damage associated with the cassou gun system and has to be used for the transfer of early embryos.

2.8 STORAGE OF EMBRYOS

2.8.1 SHORT TERM STORAGE

Storage of bovine embryos for less than 24 h can be easily achieved by maintaining the embryos in simple buffered salt solutions supplemented with protein (reviews; Maurer, 1976; Kane, 1987). Such media are used for flushing and the maintenance of recovered embryos during evaluation.

2.8.2 LONG TERM STORAGE

The long term storage of bovine embryos by deep freezing in liquid nitrogen (LN_2) is an established technique (review; Leibo, 1989). The first reported use of LN_2 for storing bovine embryos was by Wilmut and Rowson (1973). Bovine early embryos are slowly cooled (-1°C/min.) to a temperature of -30 to -40°C, suspended in molar concentrations of one or more of several low molecular weight solutes which are capable of permeating the embryo at room temperature. Slow cooling allows the embryo to become more fully dehydrated prior to transfer to LN_2. More rapid freezing leads to greater water retention and possibly to injury (Leibo, 1989). The rate of freezing determines the rate of thawing with slowly frozen embryos requiring rapid thawing and vice versa. After thawing the cryoprotectant must be removed to avoid damaging the embryo through osmotic swelling due to the rapid rehydration. The cryoprotectant can be removed in three ways: small step dilution; the use of an osmotic buffer; or by elevated temperature. A technique using sucrose as an osmotic buffer is applied widely as it allows one-step thawing and transfer systems to be adopted with little reduction in efficiency (Prather et al., 1987).

A method of freezing mammalian ova using very high concentrations of cryoprotectants to prevent ice formation, a process described as vitrification (Rall and Fahy, 1985), has been applied to bovine blastocysts (Van der Zwalmen et al., 1989) but is not yet in general use.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter details the materials and methods which were common to the investigations into MOET in Welsh Black cows. Variation from these is recorded in the text where appropriate.

3.2 MATERIALS

Catheters: a) Foley (Franklin & Rusch)

b) Gibbon (Franklin)

c) 3-lumen, fixed distance (Franklin)

Flushing fluid: Ovum culture medium (Flow Labs.& Imperial Labs.)

Glucose Saline,polythene pack (Boots, Willington Medical)

Antibiotics: Streptomycin sulphate (Evans Medical, Willington Medical)

Crystalline Penicillin (Glaxovet, Willington Medical)

Speculum, Introducers & Cannulae: Franklin

PMSG: Intervet, Willington Medical

PGF2 alpha:Estrumate,Coopers plc & Lutalyse,Upjohn plc, Willington Medical

PRID: Sanofi Ltd., Willington Medical

Anaesthetic agent: 2% Lignocaine, Norbrook Labs., Willington Medical

Sedatives: Xylazine, Bayer & Acetyl promazine, C-vet, Willington Medical

3.2.1 ANIMALS

The cows used in this study were drawn from the Haulfryn nucleus herd of Welsh Black cattle. The herd consisted of 55 breeding cows and approximately 20 followers.

The herd grazed the ffridd area of University farm in summer, which was 250 m above sea level and had an annual

rainfall of 1424 mm (mean 1968-1977). The cows were wintered indoors from January to April and fed ad lib. silage. The herd was spring calving with the majority of cows calving between March and April. The calves were suckled throughout the summer and autumn until weaning in January and in general cows had their calves at foot during the conduct of experiments. The cows performance was assessed from the 200 day weight of the calf. Replacement heifers were selected on their dams index for adjusted 200 day weight.

3.3 METHODS

3.3.1 SYNCHRONISATION OF OESTRUS.

The control of the oestrous cycle to provide reference oestri in the donor and recipient animals was usually achieved by the insertion and withdrawal of a progesterone releasing intravaginal device. Constraints of time and materials occasionally required different methods to be applied. The methods used are outlined below.

a) Progesterone releasing intravaginal device (PRID). This consisted of a steel coil covered with progesterone impregnated silastic rubber and an attached gelatine capsule containing 200mg oestradiol benzoate (Roche, 1979). After thorough cleansing of the perineum the PRID was inserted into the animals vagina with a speculum. Withdrawal, after 9-13 days, resulted in oestrus approximately forty-eight hours later.

b) Prostaglandin (PG)- PGF2 alpha analogue (eg. Cooper,

1974). Two injections, of 2ml Estrumate or 4ml of Lutalyse, were given intramuscularly 11 days apart. 11-12 days after the initial injection the majority of the animals would be in the luteal phase of the oestrous cycle and would respond to the second injection by exhibiting oestrus within 56-72 h (Roche, 1979).

c) Combined PRID insertion and withdrawal with PG injection. In situations where 11 days were not available for synchronization a PRID was inserted for 7 days and an injection of PG i/m administered the day prior to PRID withdrawal (Short et al., 1987).

The cows were observed for standing-to-be-mounted oestrus three times a day; at 8.00, 12.00 and 16.00, and the day of standing oestrus was recorded as the reference for subsequent procedures.

3.3.2 INDUCTION OF MULTIPLE OVULATIONS AND FERTILIZATION OF OVA

Superovulation was induced by the method of Christie and others (1979). Pregnant mares serum gonadotrophin (PMSG) was given in a single intra-muscular injection between Days 10-13 of the synchronised oestrous cycle (oestrus = Day 0). The dose of PMSG used varied between 2000-3000 i.u. in 10 ml of phosphate buffered saline. To induce luteolysis, PG was given 48 h later by intramuscular injection at double the normal pharmaceutical dose (Garcia et al., 1983). Standing oestrus normally started forty-eight hours after the PG injection. The animals were usually bred by AI but natural service was used where indicated. Natural service required constant observation so that the bull

could be removed after each service and returned after 1 h. This prevented the bull exhausting himself on any one donor. The AI protocol consisted of the insemination of two straws of semen, 20 million sperm per straw, approximately 12 h after the onset of oestrus followed a further 12 h later by a single straw of semen. After the final insemination a prophylatic dose of pencillin and streptomycin (15 ml, Streptopen, Glaxovet) was given i/m.

3.3.3 RECOVERY OF EMBRYOS

All embryo recoveries were performed non-surgically seven days after the onset of oestrus (oestrus = Day 0). The cows were placed in a crush and sedated with either acetyl promazine (0.6 ml ACP, C-vet) or xylazine (0.6 ml Rompun, Bayer). Following manual evacuation of faeces from the rectum and palpation of the reproductive tract, the tail head was clipped and swabbed (70% alcohol) and 5 ml of 2% lignocaine adminstered to induce low epidural anaesthesia. The vulva and perineum were thoroughly cleansed with aqueous solutions of Hibitane or Savlon and then 70% alcohol. Occasionally, persistent gas in the rectum had to be removed by a vacuum pump. All apparatus was disinfected with aqueous and tincture solutions of Hibitane or Savlon, all traces of which were removed by rinsing in distilled water. Metal equipment was sterilized by immersion in boiling water for 20 minutes. New catheters were supplied sterile. Reused catheters were washed in detergent, rinsed in distilled water, dried and then sterilized using ethylene oxide. Before use all catheters were flushed with flushing fluid to remove any traces of

sterilant. All internal equipment was lubricated (K-Y jelly, Johnson & Johnson).

The flushing fluid used was usually Dulbecco's phosphate buffered saline supplemented with bovine serum albumin (Whittingham, 1971). For the final experiments polythene packed physiological saline with 5% glucose, supplemented immediately before use, with 1% heat treated (30 min at 56° C) oestrus cow serum was used. The fluid was introduced into the catheter through a 2-way valve (I.M.V. or Alpha Labs.) on a 50 ml syringe with another 2-way valve for fluid return.

The method of flushing used varied according to the characteristics, principally the length, of the reproductive tract to be flushed (Newcomb, 1979).

A) Long catheters: Three-lumen Fixed distance and Two-lumen (Gibbon) catheters.

The catheters were primed with flushing fluid. Spigots (Southern Syringe Co.) were inserted into the outlet and inlet apertures to retain the fluid. This added rigidity to the catheter facilitating its detection and manipulation in the uterus. The cuff was checked for patency.

A sterile metal speculum was passed into the vagina to the cervix. The core of the speculum was removed and a metal introducer passed through the speculum to the os cervix. The introducer consisted of an outer cannula and a central insert with a bullet shaped anterior end to aid its passage through the cervix. Once through the cervix the introducer was manipulated into a uterine horn and the insert removed. This was achieved by rectal manipulation, with the cannula being held in position

whilst an assistant withdrew the insert. The catheter was then passed through the cannula into the horn and advanced as far up the horn as possible. To aid the passage of the catheter beyond the curvatures of the horn, and to avoid any kinking or doubling back of the catheter, the uterine horn was straightened by rectal manipulation whilst an assistant was introducing the catheter.

Once positioned correctly, about 5 cm from the utero-tublar junction for the 3-way and 15 cm for the 2-way catheters, the cuff was inflated with flushing fluid pressurized with 2 ml of air. Cuff inflation ceased when corrugations were felt on the uterine wall or it was considered that the lumen was fully occluded and the catheter firmly fixed. Incomplete occlusion was indicated by fluid seepage around the side of the catheter. Fluid was introduced and recovered in 50 ml aliquots until a final volume of approximately 250 ml had been introduced and recovered. On completion of the flush the cuff was deflated, the catheter withdrawn and the introducer insert replaced. The introducer was positioned in the second horn and the procedure repeated.

B) Short catheters: 2-lumen Foley catheters.

The method of flushing differed slightly from that described above when Foley type catheters were used. These being shorter could not be placed as deeply in the uterus. No introducer was used. The catheter was stiffened by an internal stylet to aid its passage through the cervix and into the uterine horn. Once positioned in the horn as deeply as possible, approximately 15 cm from the utero-tublar junction, the cuff was partially inflated and whilst the catheter was held by the hand in the rectum the

stylet was withdrawn by an assistant. The cuff was fully inflated with fluid pressurized by 2 ml of air. Fluid was instilled into the horn in aliquots of 50-60 ml, these volumes, on average, filled the occluded portion of the lumen. However, large uterine horns could require over 100 ml to fill the occluded portion. In these cases proportionally greater total volumes were required. A total of approximately 250 ml of fluid was used to flush each horn. The horn being flushed was manipulated per rectum to facilitate fluid recovery. Once the catheter was positioned correctly and fluid was being successfully introduced and recovered, rapid surges of 10 ml would be instilled to dislodge any captive embryos. After flushing, the cuff was deflated and the stylet replaced. The catheter was positioned in the second horn and the method repeated.

For all catheter types the recovered fluid was allowed to return by gravity into either polystyrene insulated 250 ml test-tubes or, in the final experiment, embryo filter cups (50 ml, Em-Tran, U.S.A.).

When using the 2-lumen catheters it was necessary to 'milk' the horn and thereby eliminate any pools of flushing fluid which might have formed in the curvatures of the uterus. Occasionally the horn was partially filled and mixing movements were performed to ensure thorough washing.

After both uterine horns had been flushed the catheter was withdrawn into the body of the uterus. One million units of crystalline pencillin (Crystapen, Glaxovet) and 1 g of streptomycin (Evans Medical) were each dissolved in 5 ml of

flushing fluid, and infused together through the catheter pressurized by air. Any animals considered to have been traumatized were given further antibiotics (15 ml Streptopen i/m). Donors subsequently required for breeding were observed for oestrus and given PG 17 days post flushing if they had not previously been confirmed in oestrus.

3.3.4 EMBRYO ASSESSMENT

Fluid recovered into test-tubes was allowed to stand for 10 minutes in a warm (25°C) laboratory. The upper aliquot from each tube was removed leaving 10 ml to be searched for embryos. When the embryo filter was used excess fluid was drained off leaving a minimum of fluid to be searched. The fluid for examination was transferred to concave petri dishes and searched for embryos under a stereo-microscope. Recovered embryos were transferred in ovum culture medium to petri dishes and assessed under higher magnification. The suitability of an embryo for freezing or transfer was assessed according to its morphology, the vitality of appearence, symmetry and the presence of any defects (Shea, 1981). The single most reliable indicator of an embryos viability is the extent of its development (Shea, 1981). Thus for embryos recovered on Day 7, morula and blastocyst stages should be observed. Embryos of normal development were evaluated according to the scheme of Shea (1981) (Table 3.1). An average appearing embryo was rated 3. An above average embryo, symmetrical in appearence; even granulation; no deformities in the zona pellucida; no extruded blastomeres; was rated 4. Below average in

appearence embryos were characterised as possessing uneven sized blastomeres, extensive blastomere extrusion and evidence of membrane rupture. Embryos of grade 2 and above were considered viable and were used for freezing and storage of for fresh transfer to recipients.

Table 3.1: Embryo characterisation and grade

Grade	Appearance
4	symmetrical, even granulation, no deformities in the zona pellucida, no extruded blastomeres
3	possessing 1-2 deviations from the above eg. extruded blastomere or uneven granulation
2	uneven blastomere size, blastomere extrusion, evidence of membrane rupture
1	extensive membrane rupture, delayed development, evidence of fragmentation, possibily unfertilised

3.3.5 EMBRYO STORAGE

Embryos were maintained for short periods (2-4 h) in an incubator at 25°C in ovum culture medium. For long term storage they were frozen and held in liquid nitrogen (-196°C) (Iritani, 1988). The cryoprotectant used in the freezing procedure was glycerol (Analar grade, BDH) at a final concentration of 1.4M in supplemented phosphate buffered saline (sPBS). A 3-step concentration gradient was employed; 0.3M for 10 min, 0.8M for 10 min and 1.4M for 10 min. The embryos were loaded into 0.25 ml AI straws (IMV). The AI straws were sealed with either polyvinyl chloride powder or plastic plugs (Planer). All these operations

were performed in a warm laboratory (25°C) to avoid temperature fluctuations. The straws were placed in a programmable freezer, Planer model R204. The freezing curve used was that described by Iritani (1988). The chamber was cooled at -1°C/min to a temperature of -7°C when seeding, the formation of ice crystals, was induced by touching the outside of the straw with forceps which had been cooled in liquid nitrogen for 5 min. The seeding temperature was held for 6 minutes and then the frozen embryos were cooled at -0.3°C/min to -33°C before being transferred to liquid nitrogen (plunged).

Embryos required for transfer were thawed rapidly in a water bath for 15 seconds at 35°C. Removal of the cryoprotectant was achieved by transferring the embryo in a minimum of freezing solution to a 1.0M solution of sucrose in sPBS for 10 min. The embryo was washed in 2 changes of sPBS before being assessed using the same criteria as for fresh embryos.

3.3.6 TRANSFER OF EMBRYOS

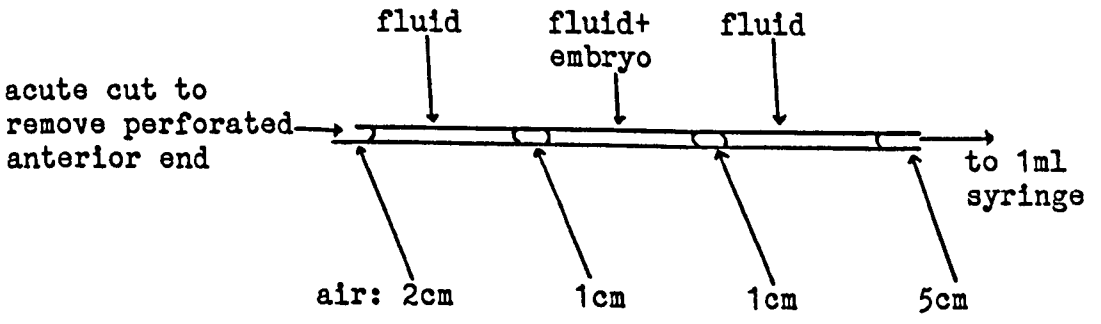
All transfers were performed on Day 7 (oestrus = Day 0) of the synchronized cycle. The recipients were placed in a crush and examined for the presence of a functional corpus luteum. Suitable animals were sedated (0.5 ml ACP i/v).

3.3.6.1 SURGICAL TRANSFER

Recipients to receive surgically transferred embryos had their flanks ipsilateral to the corpus luteum clipped. The surgical area was disinfected (aqueous Hibitane solution) and a

paravertebral block applied; 30 ml of 2% Lignocaine each at lumbar vertebrae L1 and L2. The operation area was also desensitised with anaesthetic (30 ml, Lignocaine) infiltrated in an inverted 'L', so as to avoid injecting into the actual incision site. The area was sterilized with 70% alcohol. A 15 cm incision was made, excising skin and muscle layers and peritoneum, downward and forward below the tubor coxae. The uterine horn was located and drawn to the incision. A blunted 18 gauge needle filled with epoxy resin was used to produce a conduit into the lumen on the outer curvature of the horn. The embryos for transfer had concurrently been transferred into modified tomcat catheters (4 Ch., length 30.5 cm, o.d. 1.3mm)(figure 2). The tip of the catheter was inserted through this puncture and fed on into the lumen. The medium containing the embryo was expelled from the catheter towards the anterior end of the horn. The exteriorising and subsequent manipulation of the uterus was performed with care and speed to avoid trauma which would be detrimental to embryonic survival. After the infusion of 1g streptomycin (Evans Medical) the incision was closed with 2 layers of sutures (No. 5 coated vinyl, Ethicon). Systemic antibiotic (Streptopen, 15 ml) was given i/m.

Figure 2:4 Ch. catheter modified for surgical transfer of embryos



3.3.6.2 NON-SURGICAL TRANSFER

Recipients to be implanted non-surgically received low epidural anaesthesia (5 ml Lignocaine) after sedation. The rectum was manually evacuated, the vulva and perineum were cleansed with disinfectant (Savlon or Hibitane) and 70% alcohol swabs. The embryo was loaded into a 0.25 ml straw, shortened to fit the cassou gun. This straw was then placed in the cassou gun (I.M.V.). The gun was placed in a sterile polythene sleeve and passed up the vagina to the cervix, where it was forced through the sleeve and manipulated through the cervix into the horn ipsilateral to the corpus luteum. An attempt was made to minimise handling of the uterus while achieving maximum penetration up the uterine horn. The aim was to pass the gun as far as it would travel on the first or second attempt. At this point the embryo was expelled into the lumen.

All recipients in this study were given 10 ml of GnRH (Receptal, Hoechst) i/m five days after transfer (Day 13 of the oestrous cycle) in an attempt to improve pregnancy rates (MacMillan et al., 1982).

3.4 STATISTICAL ANALYSIS

All statistical analyses were performed using Minitab, Statpack and Genstat statistical packages on the University College of North Wales Vax cluster. A variety of statistical methods were used, depending on the nature of the data, to determine treatment means and the statistical significance of differences between means.

Because the number of observations was in many cases small, caused by the need to control the cost of experiments, and because characteristics such as ovulation rate were very variable, it proved necessary to use non-parametric methods since the nature of the underlying variance could not be accurately assessed for treatment groups. In other cases data were tested for normality of distribution at the 5 % level and where this was found to be so, analysed using General Linear Model methods. Such an approach can be criticised for data relating to counts of CLs, embryos etc. as the variables are not continuous but in common with others (Herrler et al., 1990) it is assumed that there is an underlying continuous causal influence which can only be recognised in count form and that normal errors can be assumed. Percentage data were analysed after arcsine transformation. Differences in means were tested at the 5% level using Scheffe's test (Whitaker, pers. comm.). Standard errors of means for unbalanced data were calculated from the error mean square, from the analysis of variance table, and the harmonic mean of the number of replicates per treatment (Snedecor and Cochran, 1980)

Some cows in the study yielded much larger numbers of embryos than others. To avoid such animals having a disproportionate influence on group percentage data, embryo recovery and embryo viability, these were calculated individually for each cow for each collection. The group mean was derived from the percentages for each cow rather than from the total data. For example, if three cows produced 18, 2 and 2 embryos and of these

18, 0 and 0 were viable then the individual proportions of viable embryos would be 100, 0 and 0%. The mean of these values is 33.3%. This is the statistic used in the present study. If total data is analysed, then the proportion of viable embryos from the total number of recovered embryos is 18/22 or 81.8%.

Embryo viability was calculated as the percentage of embryos, categorised as good enough for freezing or transfer, obtained from the total number of embryos collected. Embryo recovery was assessed by the mean percentage of embryos, of all qualities, which were obtained from the number ovulated, estimated per rectum.

CHAPTER 4

PRELIMINARY ATTEMPTS AT SUPEROVULATION AND EMBRYO RECOVERY

4.1 SUPEROVULATION AND EMBRYO RECOVERY FROM FRIESIAN AND HEREFORD X FRIESIAN HEIFERS

4.1.1 INTRODUCTION

Before attempts were made to superovulate and to recover embryos from Welsh Black cows at the University farm it seemed necessary to establish methods for the recovery of embryos using less valuable cattle. Consequently, experiments were performed to develop the procedures necessary for embryo recovery and to examine the superovulatory responses of animals drawn from the College's dairy herd replacements and from the beef herd, these animals being more readily available. Heifers rather than cows were chosen as it was expected such animals would respond better to superovulation (Moore, 1975; Newcomb et al., 1978).

4.1.2 METHODS

Two groups of four heifers, Friesian and Hereford x Friesian, were induced to superovulate using one of three dosages of PMSG: 2000, 2250 or 2500 i.u. All Friesians received 2500 i.u. PMSG. The beef animals were divided into 2 groups receiving either 2000 or 2250 i.u. PMSG. Insemination was by AI for the Friesians and by natural service for the beef cattle. Non-surgical recovery of embryos was attempted seven days after oestrus.

4.1.3 RESULTS

The results from the two sets of embryo recoveries are summarized in table 4.1. Great difficulty was found in passing the non-surgical equipment through the cervices of the donors and in 5 cases the cervix was impassable. Four viable embryos were recovered from one Friesian donor but none were obtained from any of the other Friesians or from the beef cattle. Only empty zonae pellucidae were recovered from AD26 and AC154.

No estimate of ovulation rate was possible for the beef heifers as the ovaries were extremely follicular and consequently, embryos were not recovered from these animals.

4.1.4 DISCUSSION

No explanation can be offered for the collection of empty zonae pellucidae. It is possible that the ovaries of the treated animals had been stimulated by the LH content of the PMSG and this had interfered with the processes of ovulation causing premature ovulation (Callesden et al., 1987).

The experience obtained in these trials was to have been used as the basis for the application of embryo transfer techniques in Welsh Blacks but the poor responses obtained did little to indicate that the appropriate techniques were being applied and occasioned a reappraisal of the value of such work to the general aims of this study. It was decided that as the Welsh Black cows differed fundamentally from the trial heifers; the cows being of a different breed, multiparous, larger and purely beef in type, trial investigations would be more usefully

employed directly on Welsh Black cattle.

Table 4.1: Ovulation rate and embryo recovery from superovulated dairy heifers.

Breed	Heifer	PMSG dose	CL	Embryos	Good Embryos
	AD49	2500	12	6	4
Friesian	AD26	2500	12	0	-
	AC154	2500	10	0	-
	AD69	2500	6	cervix impassable to introducer	

4.2 REPEATED SUPEROVULATION AND EMBRYO RECOVERY FROM WELSH BLACK COWS

4.2.1 INTRODUCTION

No published information was available on the responses of Welsh Black cows to superovulation and subsequent embryo collection. An investigation was performed, using selected cows from the Welsh Black nucleus herd, of their responses to repeated superovulation, with increasing doses of PMSG, and of the non-surgical recovery of embryos from treated cows.

4.2.2 METHOD

A total of four Welsh Black cows were selected. These were the four highest index scored (MLC) cows from the nucleus herd, which conformed to breed type, were selected as embryo donors. The donors were synchronised in oestrus using 2 injections of PG, given 11 days apart. The animals were each repeatedly superovulated with 3 different dose levels of PMSG; 2000, 2500 and 3000 i.u., administered 11 days after the reference oestrus, on 3 occasions in September, November and January. The PMSG dose was increased from September to January. The cows were bred by AI. Embryos were recovered non-surgically 7 days after insemination.

4.2.3 RESULTS

The ovulation rate and details of the embryos recovered are summarized in table 4.2. The animals responded to superovulation with an estimated mean of 5.6 CLs. A mean of 2.5 embryos were recovered per collection but only an average of 1.2 embryos of transferrable quality were obtained. The overall recovery rate of embryos was 36.7%. The uteri of these multiparous donors were extremely long and flaccid, which made the accurate siting of the flushing catheters difficult at the ovarian end of the uterine horn and probably accounts for the low recovery of embryos observed.

No differences in ovarian response or embryo production were detected between cows or between the dose of PMSG.

4.2.4 DISCUSSION

The response of Welsh Black cows to superovulation in this investigation (mean = 5.6 CLs per superovulation) were within published ranges (Sreenan, 1983). Sreenan and Beehan (1976a) found 2000 i.u. PMSG produced the highest ovarian response with mixed breeding cattle. Hafez and others (1963) and Scanlon and others (1968) considered the optimum dose of PMSG to be 3000 i.u. for the superovulation of cattle. However, the genetic variation found in superovulation responses (Gordon, 1982) necessitate assessment of individual breed requirements. These data do not reveal any advantage for any of the doses of PMSG used and the median dose of 2500 i.u. was adopted for use in further

experiments.

The published data on the effects of repeated superovulation and embryo recovery are conflicting. Christie and others (1979) superovulated heifers on up to ten occasions without incurring any macroscopic uterine damage or any reduction in subsequent fertility. The same study showed a decline in ovulation rate from the first superovulation to the second. However, Ali Dinar and others (1987) reported conflicting data where the response to superovulation was improved in the second treatment compared to the intial superovulation. The embryo recovery rate and the numbers of good embryos collected per donor in this investigation were lower than expected. It was considered that the donor's age (mean = 10 yrs.) may have affected their fertility. Although the donors had no records of infertility, significant detrimental effects of age on the responses of cattle to superovulation and embryo recovery have been reported (Moore, 1975; Garcia-Winder et al., 1988). Newcomb and others (1978) also observed that within breed, a higher ovulation rate with heifers compared to cows was achieved with PMSG/PG induced superovulation. Consequently it was decided that younger donors should be used for future attempts at superovulation and embryo collection in order to increase the numbers of progeny possible at a single superovulation.

Table 4.2: Mean ovulation rate and mean embryo recovery from repeatedly superovulated Welsh Black cows.

Dose of PMSG	n	Mean (SEM) CL	Embryos	Viable embryos	% Viable embryos	Embryo recovery(%)
2000	3	6.0 (5.0)	1.7 (1.7)	0 -	0 -	15.6 (15.6)
2500	4	5.0 (1.7)	2.8 (1.6)	1.3 (1.3)	41.7 (41.7)	54.6 (29.2)
3000	4	6.0 (2.7)	3.0 (1.7)	2.3 (1.9)	66.7 (33.3)	34.2 (17.8)
overall mean		5.6	2.5	1.2	36.7	36.7
Kruskal Wallis H= for 2 degrees of freedom		0.33	0.58	2.82	1.84	1.52
	P=	ns	ns	ns	ns	ns

CHAPTER 5

THE FACTORS AFFECTING SUPEROVULATION IN A BREEDING HERD OF WELSH BLACK COWS

5.1 ATTEMPTS TO IDENTIFY THE FIRST OESTRUS POST PARTUM USING A) MILK PROGESTERONE ASSAY AND B) PEDOMETERS.

5.1.1 INTRODUCTION

Suckling beef cows are known to have prolonged anoestrus post-partum and a high incidence of silent or low intensity oestri (Tervit et al., 1977). Dunn and Kaltenbach (1980) stated that the post-partum interval to oestrus varied from 46 to 168 days in suckling beef cattle. Mulvehill and Sreenan (1977) observed that, for suckling beef cows, the post-partum interval to the resumption of ovarian activity ranged from 24.5 to 91.5 days and, that this time interval was affected by the season of calving, with cows that calved in January to June taking 85.5 days to resume ovarian activity whereas cows that calved from July to December took 26.9 days.

The practical difficulties of oestrus detection have important implications for the application of superovulation and embryo transfer techniques in Welsh Black cows. The treatment of cows for superovulation and embryo recovery incurs a time penalty. The superovulation routine adopted in this study took 37 days from commencing the synchronisation of the reference oestrus to embryo collection. An additional period, approximately 14 days, is the theoretical miminum needed for full reproductive function to be resumed after this. These procedures reduce the opportunity to keep a suckling beef cow within an annual calving block when superovulated.

In a beef breeding herd a tight calving pattern is necessary both for economic reasons and to provide accurate

contemporary comparisons. Accordingly, any delay in detecting oestrus before superovulation could compromise the calving pattern of the nucleus herd and limit the application of MOET methods in beef breeding herds.

The only current, reliable indicator of oestrus is the standing-to-be-mounted reflex (Peters and Ball, 1987). Continuous observation of extensively managed cattle is difficult to accomplish and the first postpartum oestrus may not be observed. In this study methods of oestrus detection were assessed for use in recently calved suckling Welsh Black cows in the detection of the first oestrus post-partum.

5.1.2 MATERIALS AND METHODS

Six cows due to be superovulated were transferred in June to lowland grazing to be accessible for the investigations. They had been observed daily but no oestrus activity had been seen since calving and they were amenable to handling. The reproductive tracts and ovaries of the cows were examined per rectum for abnormalities, and blood samples were taken for determination of their trace mineral status (Cu and Se). Copper and Selenium preparations (Copporal or Coprin and Deposel, Beechams and Rycovet) were administered according to manufacturers' recommendations and blood from the treated animals was resampled 4 weeks later.

After a period of two weeks at pasture they were separated from their calves for periods of up to one hour prior to hand milking at 9 am daily for 18 days. The foremilk was discarded and

a 10 ml milk sample collected. The samples were stored at -15°C until required for assay. The samples were thawed and shaken, and 100 µl aliquots analysed in duplicate for progesterone using a commercial ELISA kit (Enzygost, Hoechst) (Stanley et al., 1986). The optical density of the sample was measured at 405 nm using a plate reader (Bio-Rad plc).

Measurements of the cows' physical activity, and hence indication of oestrus, were to have been made using pedometers by the method of Schofield (1988). However, attempts to attach the pedometers to the hind leg of the animals were strongly resisted and no pedometers could be correctly fitted.

The animals were observed for oestrus activity 3 times a day; at 8.00, 12.00 and 16.00 hours over an 18 day period commencing 36 to 70 days post calving.

5.1.3 RESULTS

Standing oestri were observed in 3 of the cows during the experimental period. Their mean post partum interval to first oestrus was 66.0 days (std. dev.=15.6). One animal was observed in oestrus immediately after the end of the experimental milking period and when this data was included the mean interval was 63.5 days (std. dev.=13.7).

The milk progesterone concentrations for individual cows are shown in figures 3 to 8. The highest measured concentrations of milk progesterone were 12 and 12.5 ng/ml, in peak values (figures 3 and 8).

All the cows observed in oestrus had increases in progesterone concentration at some stage. The mean postpartum

interval to first rise in progesterone was 61.8 d (SEM = 6.3).

Two of the cows observed in oestrus had sustained increased levels of progesterone concentration followed by rapid declines to base concentrations either 3 (figure 5) or 4 (figure 7) days before oestrus. One cow exhibited a large brief increase, 12 ng/ml, in milk progesterone concentration on the day of oestrus (figure 3).

However, the cows which were not observed in oestrus also all showed elevated concentrations of progesterone. In two of these cows which had been calved 70 and 65 days before sampling commenced, progressive but fluctuating increases in progesterone concentrations were observed (figures 4 and 8). The remaining animal (figure 6) had a low concentration of milk progesterone with a small brief surge, 2.5 ng/ml, in secretion. This is the cow which was observed in oestrus after the sampling period had ended.

The plasma copper concentration increased (15.2 cf 10.3 µmol/L; P=0.014) and the concentration of the selenium containing enzyme, GSHPx, decreased (42.5 cf 88.6 iu/ml; P=0.05) over the four week period.

5.1.4 DISCUSSION

The decline in enzyme concentration cannot be explained but the animals remained in the adequate range of concentrations. The copper status of the cows was improved as indicated by the increase to adequate concentrations from concentrations considered to be marginally deficient (Pickard, 1986).

Variations in the fat content of the milk samples were obvious and this has been thought to affect the assay as progesterone is concentrated in milk fat (Claus et al., 1983). The manufacturers of the assay were unable to offer any recommendations to overcome this problem.

The mean postpartum interval to first rise in progesterone in the present study (61.8 d) was in agreement with that reported in previous work (53.5 d; Peters and Riley, 1982a). Short lived surges in progesterone, such as were observed in some of the profiles, can occur 3-4 days prior to the first oestrus (Donaldson et al., 1970; Odde et al., 1981) and have been observed in 56.1% of animals (Peters and Riley, 1982b). LaVoie and others (1981) found that peaks of progesterone of 10 times the basal concentration occurred 11 days prior to the observed oestrus in some animals. In two cows (figures 4 and 7) milk progesterone concentrations followed the pattern of progesterone secretion described by Humphrey and others (1983), where the preovulatory progesterone peaks began 4.6 days, and ended 0.6 days, prior to oestrus.

One animal exhibited a short-lived surge in milk progesterone concentration on the day she was observed in oestrus (figure 3). This observation can be attributed to either the false identification of oestrus, an aberrant assay result or it may be an unusual finding. Three cows were not observed in oestrus during the sampling period. Of these, two exhibited increases in progesterone concentration consistent with resumption of ovarian function but they never exhibited oestrus

despite the fact that every effort was made to detect it (figures 4 and 8). The remaining cow had low systemic progesterone concentrations and was not fully cycling during the sampling period but was observed in oestrus after this period. This animal had a small, 2.5 ng/ml, surge in progesterone concentration 13 days prior to oestrus (figure 6).

As the cows examined were to be retained in the spring calving pattern of the nucleus herd the trial was ceased before a confirmed oestrus was detected for each cow. The progesterone assays indicated that the cows were approaching cyclicity or were cycling and so the cows were synchronised by the insertion of a PRID for 11 days at the end of the trial in preparation for superovulation.

This investigation showed milk progesterone assays aided first oestrus detection producing results that could be confirmed by observation and suggested ovarian activity in animals not seen in oestrus. However, the milk progesterone assay required milk sampling which stressed the cows through calf separation and close human contact. The high frequency of cow observation for oestrus detection used in this trial was probably too labour intensive for use in upland beef herds. The need for an alternative method of oestrus detection is indicated by this study.

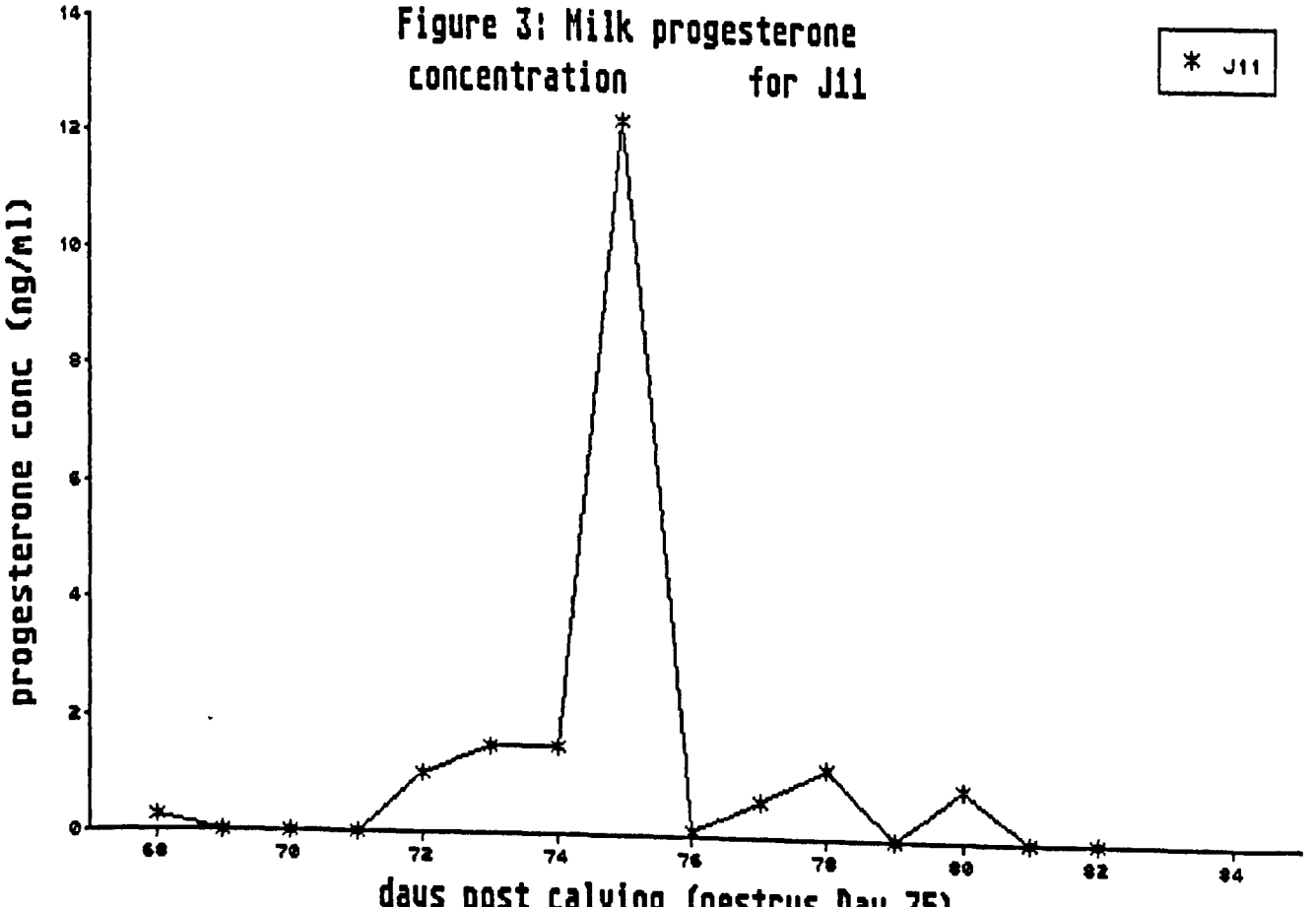


Figure 3: Milk progesterone concentration for J11
* J11
progesterone conc (ng/ml)
0
2
4
6
8
10
12
14
68
70
72
74
76
78
80
82
84
days post calving (oestrus Day 75)

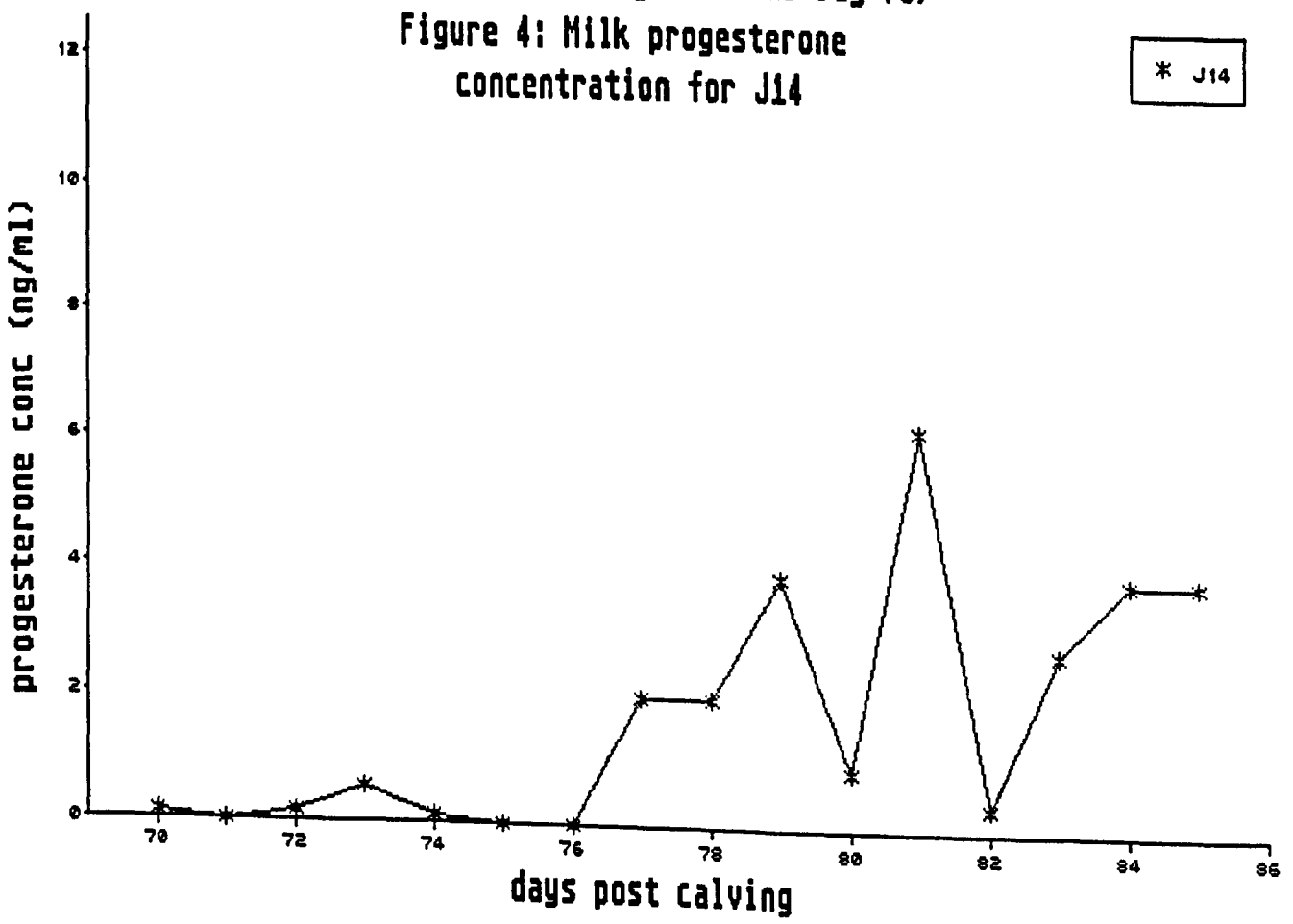


Figure 4: Milk progesterone concentration for J14
* J14
progesterone conc (ng/ml)
0
2
4
6
8
10
12
70
72
74
76
78
80
82
84
86
days post calving

Figure 5: Milk progesterone concentration for K31

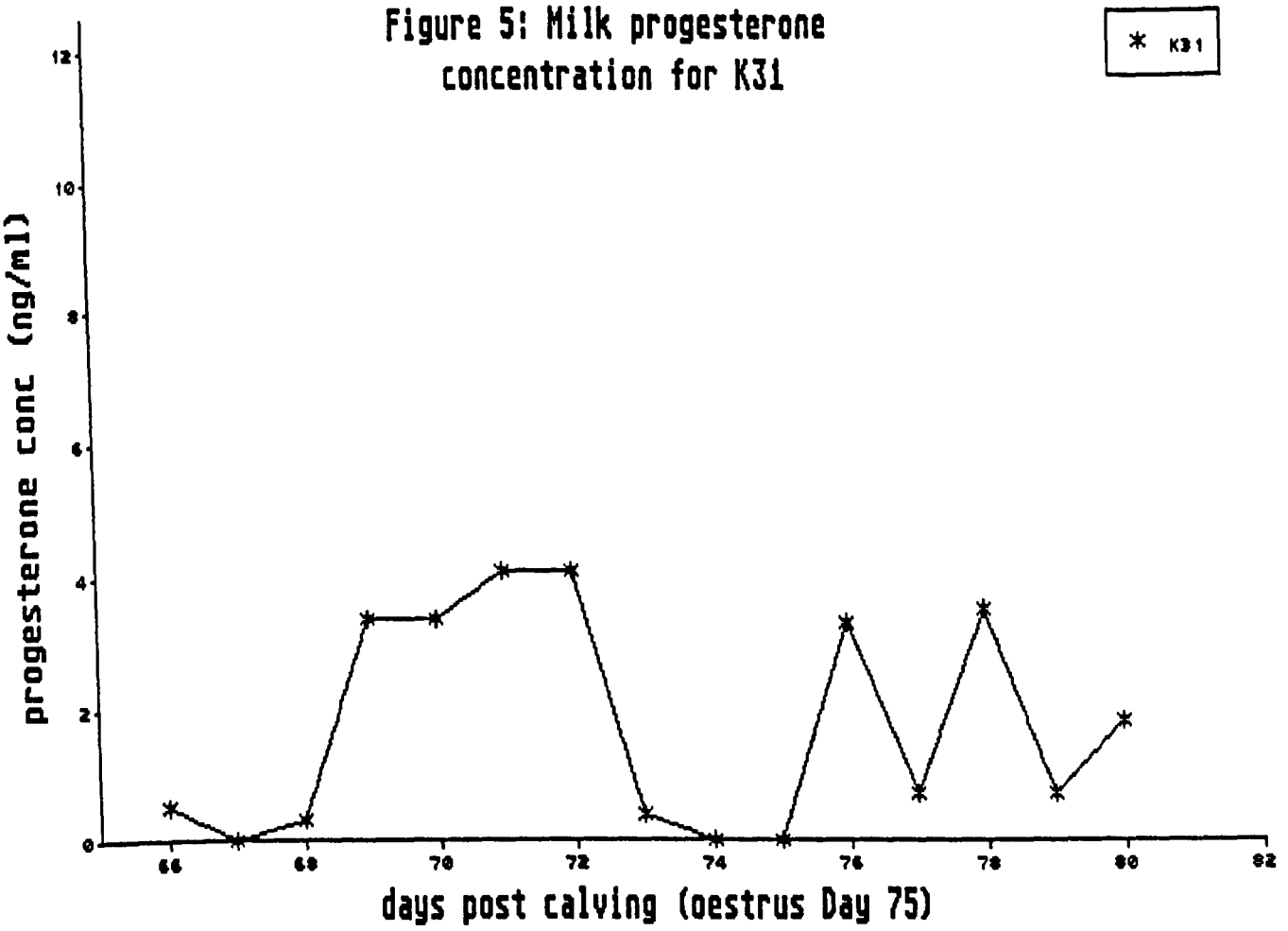
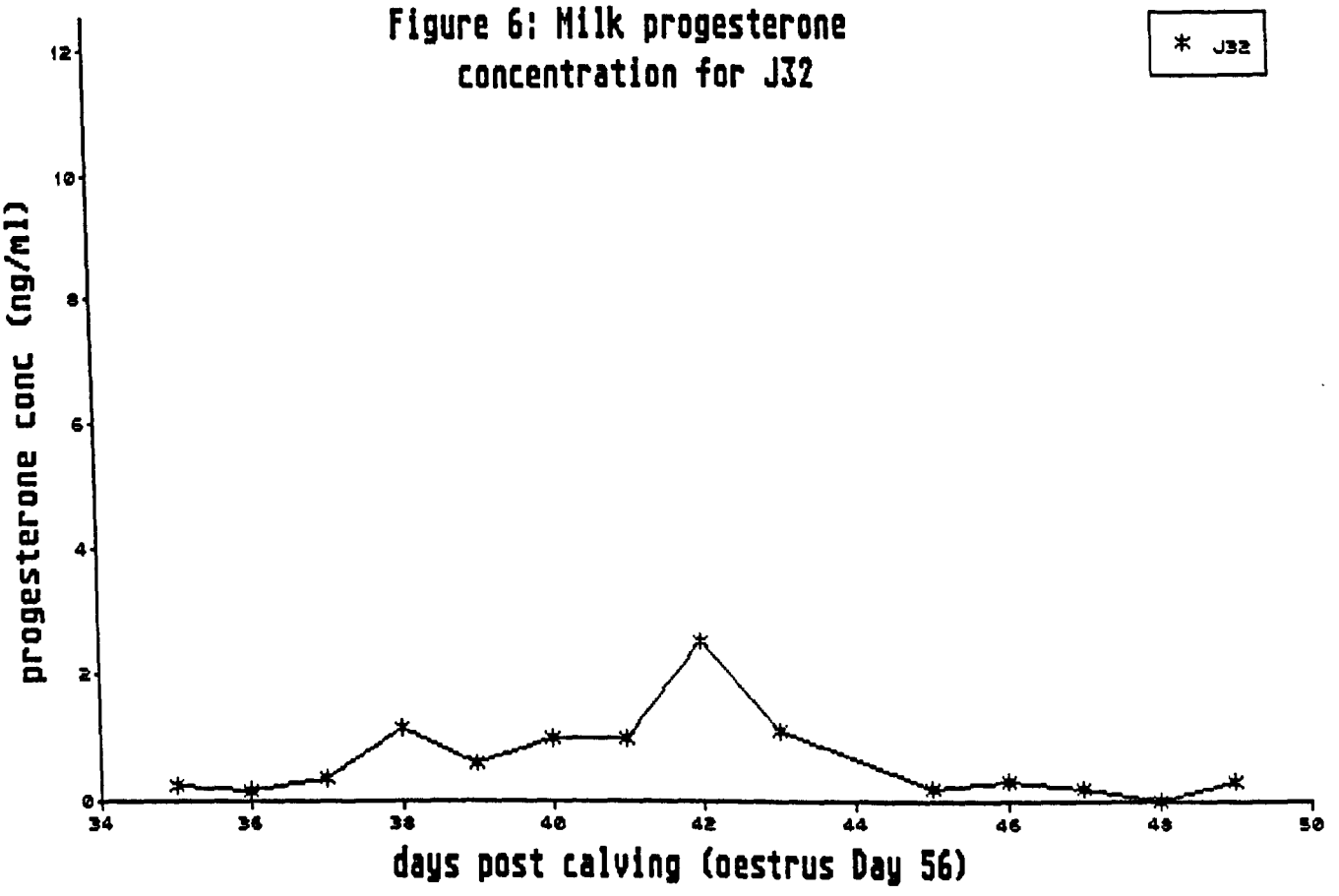


Figure 6: Milk progesterone concentration for J32



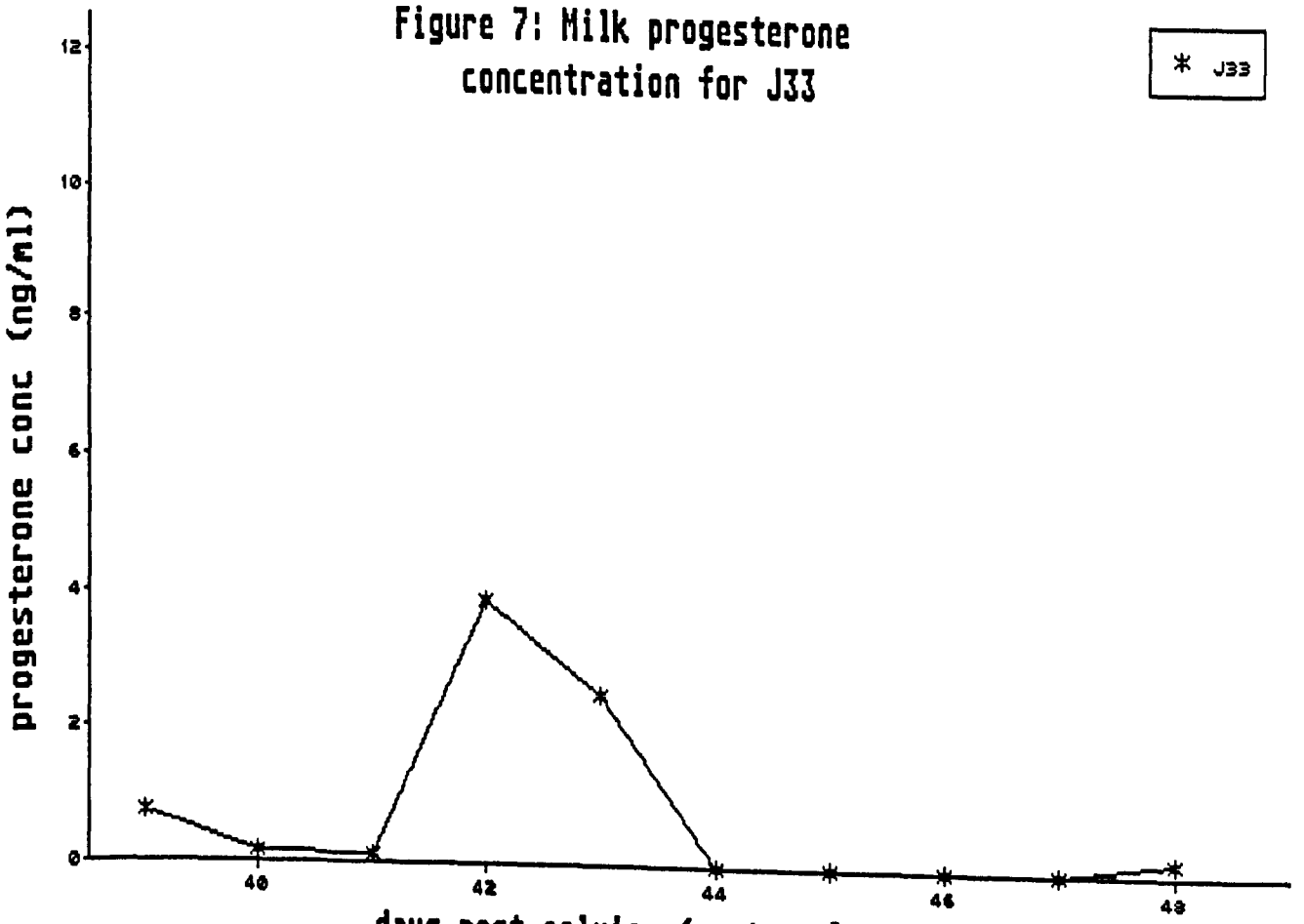


Figure 7: Milk progesterone concentration for J33
* J33
progesterone conc (ng/ml)
0
2
4
6
8
10
12
40
42
44
46
48
days post calving (oestrus Day 48)

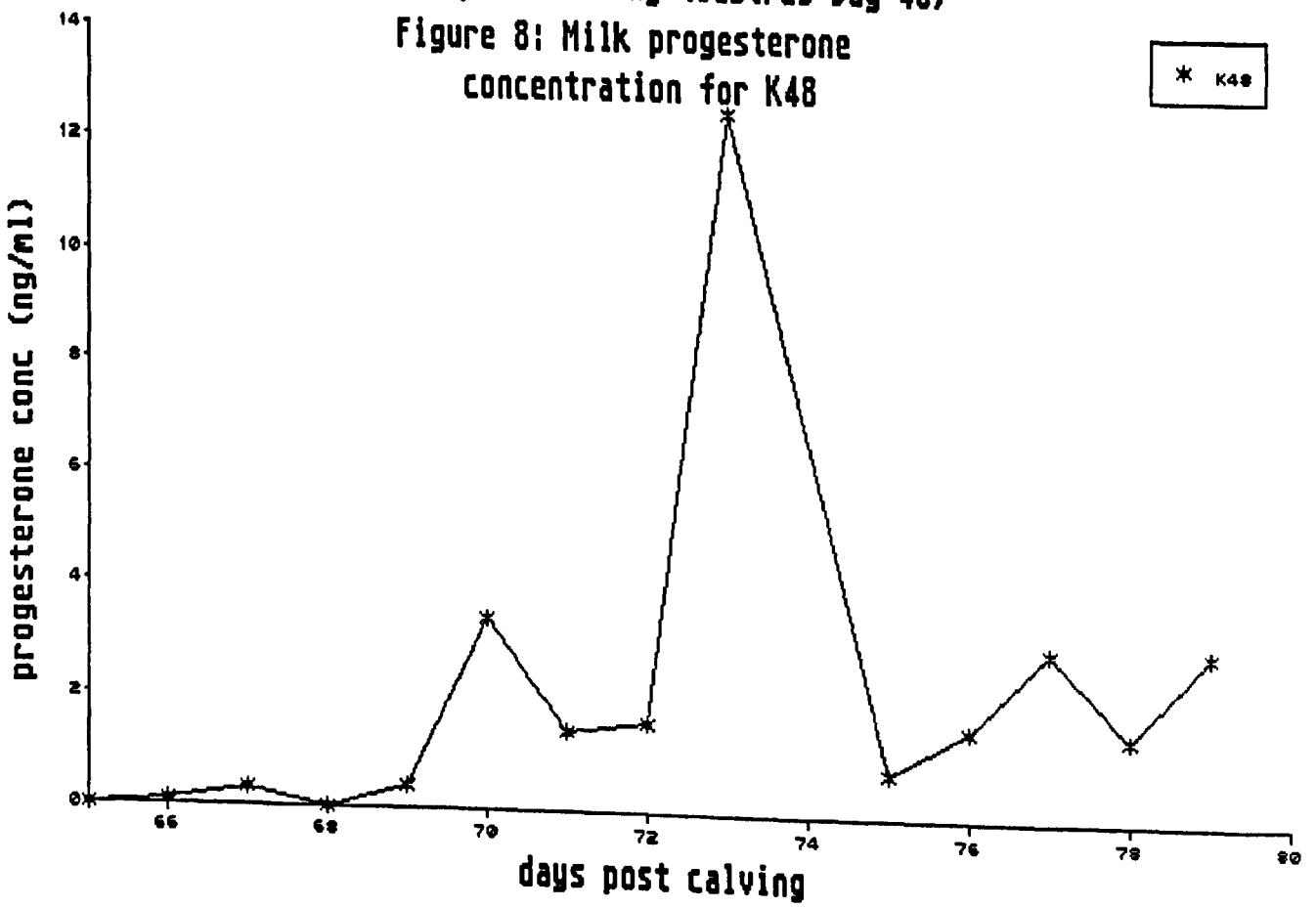


Figure 8: Milk progesterone concentration for K48
* K48
progesterone conc (ng/ml)
0
2
4
6
8
10
12
14
66
68
70
72
74
76
78
80
days post calving

5.2 MEASUREMENT OF ELECTRICAL RESISTANCE OF VAGINAL MUCUS AND MUCOSA IN ORDER TO DETECT OESTRUS IN WELSH BLACK COWS.

5.2.1 INTRODUCTION

The previous attempt to aid oestrus detection using milk progesterone assay kits had only been partially successful. An alternative method of oestrus detection was investigated which utilised a probe measuring the electrical resistance of vaginal mucus and mucosa. This method of oestrus detection offered potential advantages in terms of ease of use and rapid production of results over other methods of oestrus detection (Heckman et al., 1979; Peters, 1989). The cyclic pattern of vaginal resistance is in phase with that of progesterone secretion (Heckman et al., 1979). A vaginal resistance probe was evaluated for application to the breeding management of beef cows with respect to oestrus detection.

5.2.2 MATERIALS AND METHODS

The electrical resistance of the vaginal mucus and mucosa was measured in six cows undergoing superovulation. The resistance was measured, using a 'Wallsmeta' probe (Masterbreeders plc.) using the method described by Peters (1989), daily for up to 14 days, during a superovulation treatment. Each cow was placed in a crush and the perineum cleansed with aqueous disinfectant (Hibitane, I.C.I.) and dried with paper towels. The probe was disinfected in a separate

solution (Hibitane) and rinsed with distilled water. The vulva was held open by an assistant and the probe inserted 15 cm into the vagina. The probe was calibrated to ensure consistent placement. Once positioned the probe was gently inclined so the electrodes and the ventral vaginal mucosa were in contact and two readings of the electrical resistance were made.

The cows were observed for oestrus three times a day as described previously (5.1.2)

5.2.3 RESULTS

The resistance readings recorded varied from a high of 72 to a low of 25 Ohms (x10). The data are shown for individual cows observed in oestrus (figure 9) and for cows not seen in oestrus (figure 10). There was considerable variation in individual recorded resistances and declines in resistance to similar values which were not related to oestrus. Signs of vaginal infection were observed in 4 cows (66.7%) and, in 2 cows (33.3%), readings were not taken for days when inflamation was pronounced.

5.2.4 DISCUSSION

The vaginal probe was evaluated as a possible non-traumatic method of detecting oestrus in suckling Welsh Black cows. In the trial the electrical resistance of the mucus fell at oestrus in all three cows for which there was visual confirmation. However, those animals which did not show overt oestrus also showed similar declines which were clearly not consistent with their known oestrous cycles. This is in agreement with other reported findings (Gartland et al., 1975 ; Cavestany and Foote, 1985b).

Despite efforts to maintain asepsis during the test there were signs of infection in some of the probed animals and the trial was curtailed. This problem has also been reported by Peters and Ball (1987).

Published accounts of this method claim 70% detection rates (Peters, 1989) which could subsequently be confirmed by progesterone assay and that the probe was as efficient as visual detection (Foote et al., 1979). The current study showed that the instrument could identify oestrus in Welsh Black cows and may have application in beef breeding herds where visual detection of oestrus is not practicable. However, the stress induced by the daily readings and the possibility of introducing infection, combined with the variation in readings between cows which can be as great as the oestrus related changes in individual cows do not support the adoption of this method for routine oestrus detection and observation of standing oestrus remains the method of choice. Thus for large numbers of extensively managed cattle, as would be the situation in a MOET programme, a reliable and simple aid for oestrus detection has yet to be developed.

Figure 9: Vaginal resistance readings for cows synchronised in oestrus

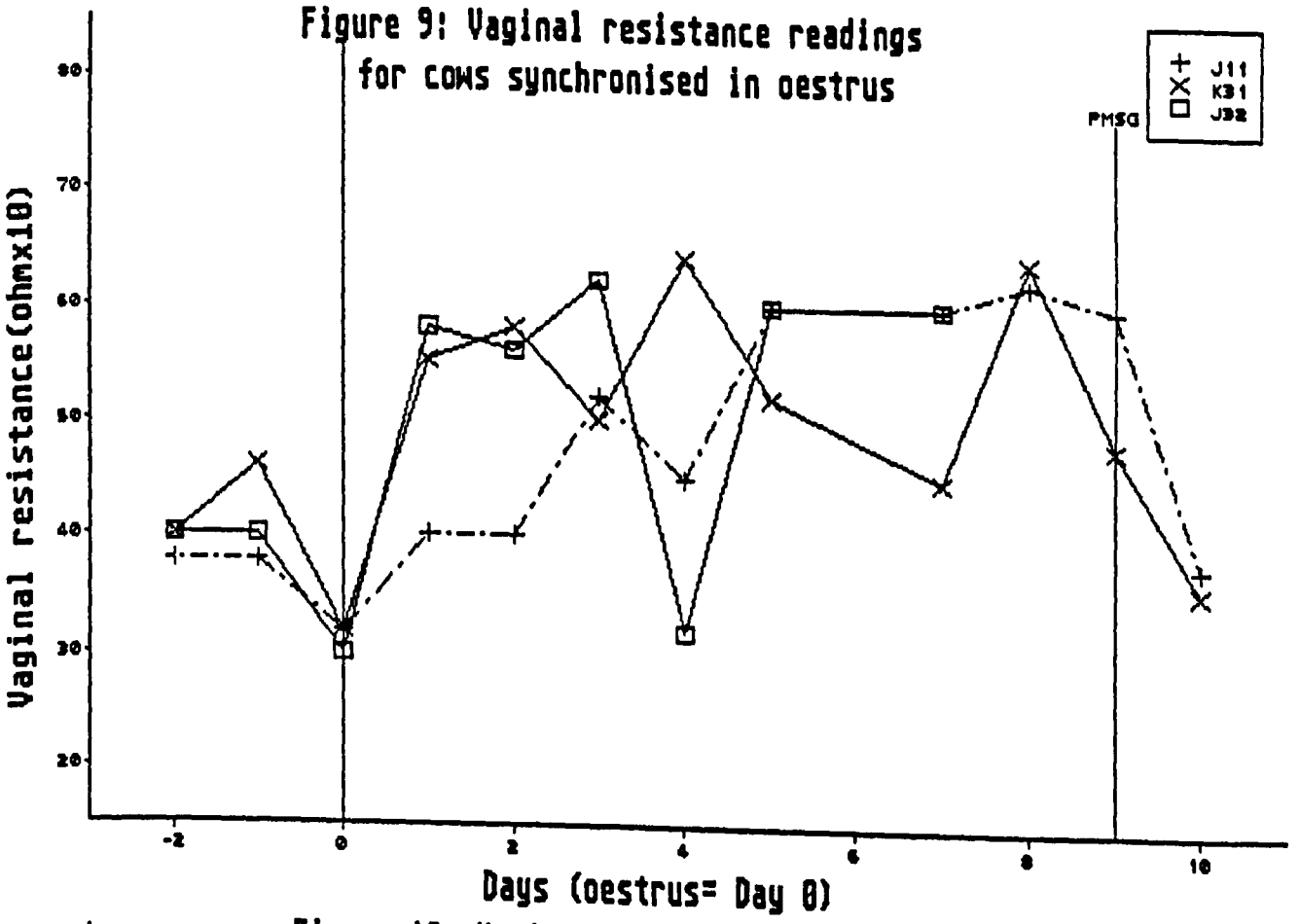
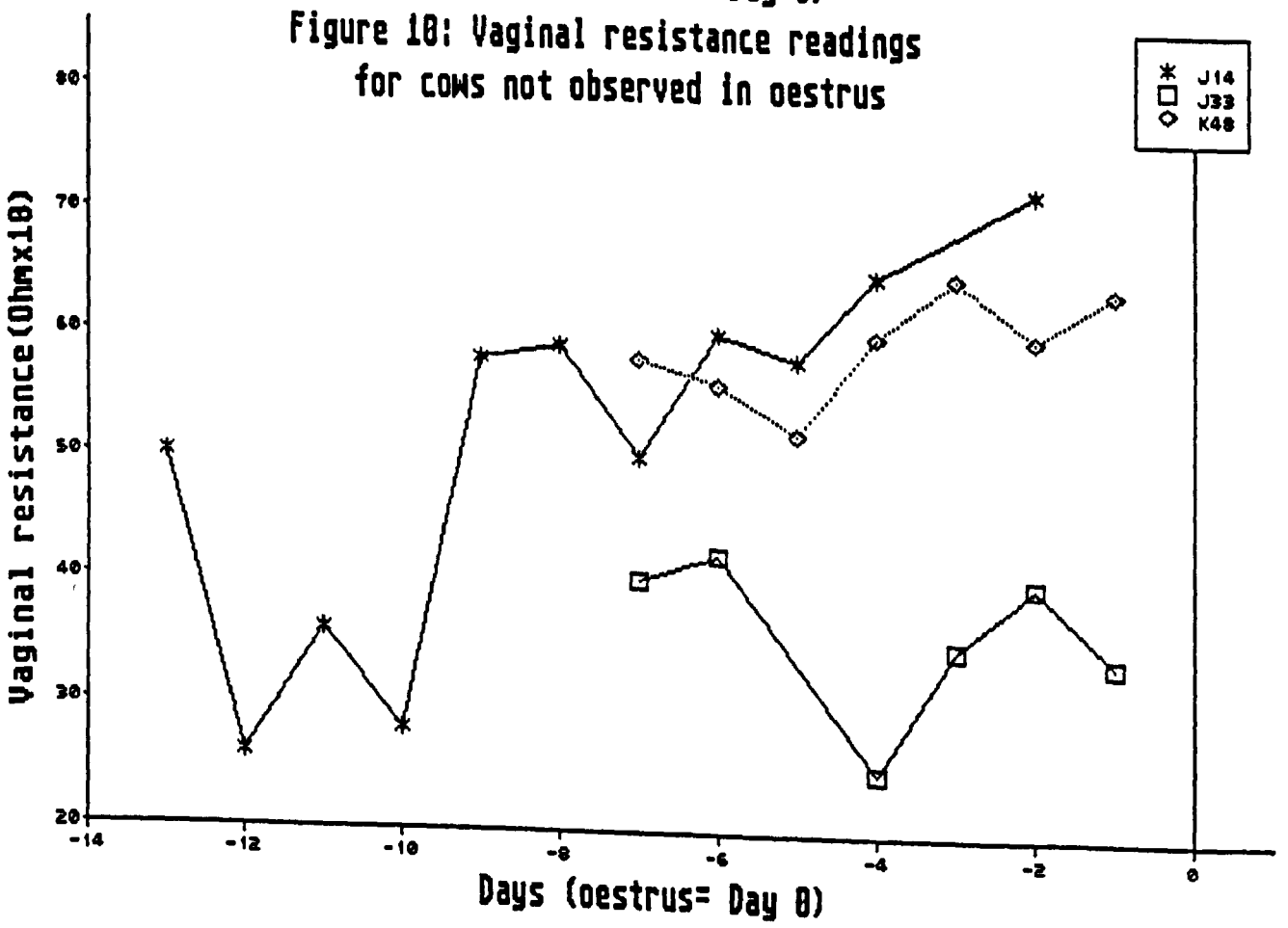


Figure 10: Vaginal resistance readings for cows not observed in oestrus



5.3 THE EFFECTS OF CONCENTRATE SUPPLEMENTATION AND hCG ADMINISTRATION ON SUPEROVULATION AND EMBRYO RECOVERY.

5.3.1 INTRODUCTION

Underfeeding beef cows affects their systemic hormonal secretion. Dunne et al. (1974) found that the CLs from cows fed restricted diets were lighter and had lower progesterone contents and concentrations than normally fed animals. Beal et al. (1978) observed that cattle fed restricted energy diets had reduced blood progesterone concentrations. Downie and Gelman (1976) found an association of falling bodyweight and low blood glucose with infertility in beef cows.

To investigate the effect of general nutritional supplementation of the normal winter diet on embryo quality a commercial concentrate supplement providing additional fat soluble vitamins, protein and energy was fed to a group of Welsh Black cows.

No published information was available on the endocrine responses of Welsh Black cows to superovulation. Examination of the patterns of blood hormone concentrations during superovulation has been suggested as a possible method of identifying poorly responding animals (Callesden et al., 1988b). The systemic blood concentrations of reproductive hormones of Welsh Black cows were monitored whilst they underwent superovulation to detect any aberrations in their secretion which could be associated with the poor responses to superovulation which had been observed previously.

Human chorionic gonadotrophin (hCG) has been proposed as an

aid to ovulation in superovulation protocols. The data obtained by others from trials using hCG have been equivocal. Moore (1975) found improved recovery of embryos from treated cows whilst Newcomb (1980) did not observe any benefit, either in terms of ovarian responses or embryo production, from hCG administration.

The effect of administering hCG to superovulated Welsh Black cows on the secretion of luteinising hormone, 17 beta-oestradiol, progesterone and on ovarian responses and embryo production was investigated.

5.3.2 MATERIALS AND METHODS

Ten Welsh Black cows, which had been used in the experiments detailed in sections 6.1 and 6.2, were used in a 2 x 2 factorial experiment. The factors to be tested were nutritional supplementation and hCG administration.

The cows had been calved for 10 months, superovulated with embryo recovery 1-2 times previously, and had their calves weaned at least 2 months earlier. They were randomly allocated to two groups. The supplemented group were housed and fed 1 kg per day of a protein, vitamin and mineral concentrate (Supercharge, Colborn Dawes; Table: 5.3) and 0.5 kg of rolled barley in addition to silage. The supplement provided an additional 19 MJ of ME , 434 g of CP per day. Silage was provided ad-libitum. Five non-supplemented cows were housed in their normal loose-housing winter quarters and fed a diet of silage only. The silages given to each group differed marginally in composition and nutritive value (Table 5.4).

The five Welsh Black cows fed a supplemented diet were housed in a neck-tie byre after being synchronised using PRIDs to provide their reference oestri. After 7 days of acclimatization, the cows were fitted with indwelling jugular catheters (Dobson, pers. comm.) as follows. The cows were sedated (ACP, C-vet) and an area over the vein was clipped free of hair, washed and sterilized with 70 % alcohol. A 12 gauge needle was inserted into the vein in the direction of the blood flow and a guide wire (William Cox plc) fed through it into the lumen for 10 cm. The needle was withdrawn over the guidewire and a catheter (60 cm polythene tubing, i.d. 1mm, Pertex Plastics) fed over the wire until approximately 8 cm of it lay in the vein lumen. The guidewire was then removed and the catheter sutured in place forming an external loop to allow for the head movements of the cow. Once correctly positioned blood was withdrawn to confirm catheter patency. The catheter was then flushed with sterile heparinized saline (5000 i.u. heparin/100 ml, 0.9% saline) and capped (male, Luer-lock cap).

Blood was collected, at predetermined times (figure 11), from the indwelling catheter. Ten ml blood samples were withdrawn into syringes and transferred immediately to heparinized vacutainers (Becton and Dickinson). Between samplings, the catheter patency was maintained with heparinized saline. The blood samples were centrifuged at 1000g for 30 min at 4°C. The resulting plasma was withdrawn and stored at -15°C until required for assay.

Human chorionic gonadotrophin was administered to three of

the housed supplemented cows and two of the standard (non-supplemented) diet cows according to the programme shown in figure 17; 1500 i.u. were given i/v immediately after the second insemination and 3000 i.u. were injected intramuscularly on day 3 after the second insemination. All the cows were superovulated as described previously (Chapter 3) with 2500 i.u. PMSG, and mated using AI. Embryos were recovered non-surgically seven days after AI and 3 weeks after the start of supplementary feeding.

The collected plasma samples were analysed using established methods of radioimmunoassay (RIA) at Liverpool University Veterinary Field Station, Leahurst (Dobson, 1983), through the courtesy of Dr H. Dobson, who provided the facilities there.

The samples were analysed for progesterone, 17 beta-oestradiol and LH using the methods of Dobson (1983) and subsequently some of these were reanalysed using solid phase no-extraction radioimmunoassay kits (Coata-count, Diagnostic Products Inc.) developed for the determination of the above hormones in human blood and also for FSH concentration.

5.3.2.1 ASSAYS OF HORMONES IN PLASMA

MATERIALS

All the glassware used in the analyses were cleaned by an overnight soak in a 2% (v/v) solution of Decon 90 (Decon Labs.) in tap water. After brushing and rinsing, the final 2 rinses being with distilled water, the glassware was dried in an hot air oven. All solvents and chemicals used were BDH Analar grade unless otherwise stated. Water for solutions was deionised. Diethyl ether was freed of peroxides by shaking with saturated

ferrous sulphate solution and after phase separation with distilled water. The ether was then distillated and was always used immediately. Hexane was distilled immediately before use.

Solutions:

1) Phosphate buffered saline (PBS) 0.1M phosphate buffer pH 7.0; 195 ml 0.2M $NaH_2 PO_4.2H_2O$ and 305 ml 0.2M $Na_2HPO_4.12H_2O$ and 9g NaCl. This solution was made up to 1 litre with deionized water and 0.1g sodium methiolate added as a preservative. To this solution was added 0.1% gelatine (PBS-gel) or 1% egg albumen (PBS-ea).

2) Charcoal suspension was prepared fresh by suspending 0.25g of activated Norvit A charcoal (Sigma) in 100 ml PBS-gel. This suspension was constantly stirred in an ice bath for one hour prior to use.

3) Toluene scintillator was prepared by mixing 1800 ml toluene and 5.4g 2,5 diphenyloxazole (PPO)

Standards of H^{3-} labelled progesterone and oestradiol markers were obtained from Amersham International UK plc. The radioisotopes were prepared by evaporating off the solvent in which they were supplied, then redissolving the marker in 25 ml of double distilled ethanol; stock solutions were stored at -15°C. Before use the appropriate marker was diluted with PBS-gel to give 8,000-10,000 cpm/100 µl. Unlabelled luteinising hormone was obtained from the National Institute of Health (N.I.H.), U.S.A.

ASSAY PROCEDURES

ASSAYS PERFORMED WITH EXTRACTION OF THE HORMONE

1) Progesterone. Samples of plasma (0.1 ml) were added in duplicate to glass tubes. Hexane (1 ml)was added and the tubes were vortexed for 1 minute. A solid CO_2/ethanol bath was then used to freeze the aqueous layer and the supernatant was decanted into a second series of glass tubes. These tubes were evaporated to dryness in a vortex-evaporator. Appropriate samples of progesterone standards (0, 20, 50, 100, 200, 300, 500 pg) and control samples were similarly treated.

Antibody (0.1 ml) and ^{3}H-progesterone (0.1 ml) were added to the assay tubes. These were then vortexed, covered with clingfilm and incubated overnight at 4°C. The following morning the tubes were placed in an ice bath and 0.5 ml of charcoal suspension was added. The solutions were vortexed and after 10 min were centrifuged at 3000g for 10 min at 4°C. The supernatant was decanted into plastic counting vials and 2 ml of scintillator fluid added. Beta radiation was counted in a Packard Tri-Carb scintillation counter (model 2450) with a counting efficiency of approximately 50%. All samples were counted until a preset count of 20,000 counts had been achieved, the elapsed time being recorded automatically.

2) 17 beta-Oestradiol. The assay method used for the estimation of 17 beta-oestradiol concentration was similar to that used for progesterone assay except that 1 ml samples of plasma were used and diethyl ether was used as the extraction

solvent. The aqueous layer was removed with a pipette as at 1 ml it was too large to freeze off. The standards were 0, 20, 30, 50, 75, 100 and 150 pg.

3) Luteinizing Hormone. Samples (0.1 ml), were set up in duplicate in LP3 plastic tubes (Luckham Ltd.). Triplicate standards were used (0, 0.05, 0.5, 1, 2, 3 and 5 ng). LH-antibody (0.1 ml) was added as necessary. The tubes were vortexed, covered with clingfilm and incubated for 24 hrs at 4°C. The following day 0.1 ml of ^{125}I-LH in PBS-ea was added to the tubes which were then vortexed, re-covered and incubated for a further 24 hrs at 4°C. Goat anti-rabbit gamma globulin (0.1 ml) was then added and incubation continued for a further 24 h. Chilled (4°C) water (1 ml) was then added and the tubes were centrifuged at 3000g for 30 min at 4°C. The supernatant was discarded and the tubes dried by draining. The Iodine-125 activity of the tubes was counted in a gamma counter (LKB Wallac Rackgamma II) for 1 min. The results were computed automatically and expressed as ng/ml.

ASSAYS PERFORMED WITHOUT EXTRACTION OF HORMONE

A) Progesterone and 17 beta-Oestradiol

Plasma samples were thawed and shaken and 100 µl aliquots were added to antibody coated tubes. 10 ml of I^{125} labelled hormone solution was added and the tubes were vortexed and incubated for 3 hours at room temperature. The supernatant was decanted and the inverted tubes tapped on absorbent paper to dry them. The activity of the bound labelled hormone was counted in a

gamma counter (LKB Wallac) for 1 minute. The standards were 0, 0.1, 0.5, 2, 10, 20 and 40 ng/ml for progesterone and 0, 20, 50, 150, 500, 1800 and 3600 pg/ml for oestradiol.

B) FSH AND LH

Plasma samples of 200 µl were added to polypropylene tubes and 100 ul of hormone antiserum added, the tubes were vortexed and incubated for 120 minutes in a water bath at 37°C. Then 100 µl of I^{125} labelled hormone solution was added, the tubes vortexed and incubated as before. 1 ml of cooled precipitating solution (goat anti-rabbit gamma globulin and polyethylene glycol in saline) was added. The tubes were centrifuged for 15 min at 3000g and the supernatant removed. The tubes were gently tapped dry onto absorbant paper and the precipitate counted in a gamma counter (LKB Wallac). The LH standards were 0, 3, 10, 20, 40, 100 and 200 mi.u./ml.

Figure11: Sampling timetable for the collection of blood samples to be used for hormone assay

Treatment	Day	Sampling
PRID out	-2	9.00/16.00
	-1	"
oestrus	0	"
	1	"
	2	"
	3	"
	4	"
	5	"
	6	9.00/16.00
	7	"
	8	"
	9	"
	10	"
	11	"
PMSG	12	9.00/16.00/22.00
	13	"
PG	14	"
	15	"
AI/hCG	16	every 4hrs
	17	"
	18	9.00/16.00/22.00
hCG	19	"
	20	"
	21	"
	22	9.00
Embryo collection	23	"

5.3.3 **RESULTS**

The ovarian responses of the 4 groups of cows is presented in table 5.1. Significant effects of nutritional suuplementation were detected on ovulation rate, number of embryos recovered, numbers of viable embryos recovered and on percentage embryo recovery. No effect of hCG administration was detected nor were any interactions observed among the variables studied. The data were reanalysed removing the effect of hCG (Table 5.2). The mean ovulation rate (4.0 cf 11.6CLs) mean numbers of embryos recovered (0.4 cf 6.4) and the mean number of viable embryos

recovered (0.4 cf 4.2) were significantly lower in the animals fed the supplemented diet than those on the standard diet.

One donor uterus (hCG treated and supplemented diet cow) was found to contain a sterile proteinacious solid which prevented fluid recovery. This observation has not been repeated and remains unexplained.

The crude protein (CP) content of the silage fed to the supplemented animals was lower than that offered to the control animals, the other dietary components studied were similar.

The refractory nature of the cows who clearly objected to the presence of humans engaged in sampling activity, reduced the life span of the indwelling catheters preventing collection of samples for as long as intended. Full collections were made from only one each of the treated and control cows.However, samples were collected from the other four cows up to oestrus.

The oestradiol and LH profiles obtained from the RIA assays from cows E21 and J31, for which full collections were possible, are shown in figures 12 and 13. The oestradiol assay performed by the method of extract (Liverpool) failed to detect any of the hormone, so only the results for the no-extract technique are shown (see below).

Mean progesterone concentration was higher after oestrus in the hCG treated cow (E21) than in the control (figure 14) but this was not significant as judged by a t-test.

The LH assays produced anomalous results. In one cow (E21) an LH surge was detected by the no-extract assay that coincided with one of the peaks identified using the extract assay

(figure 12). Another larger peak was detected only by the extract assay. For the other cow the LH peak from the no-extract assay had no such relationship with that found with the extract assay.

The oestradiol no-extract assay found no hormone when performed according to the protocol provided. However, when the assay was repeated after a 3 times concentration using an ether extraction and subsequent reconstitution in borate buffer of the pooled samples of plasma (Dieleman and Bevers, 1987), a positive concentration of oestradiol was detected.

No reactivity was found with the FSH no-extract assay. A reasonable correlation was found between the concentrations of progesterone determined using the no-extract kit and that obtained by the established extract method (corr=0.83).

The quality control data from the assays are summarised in table 5.5. The interassay coefficient of variation for the extract progesterone assay was 10.2%.

5.3.4 DISCUSSION

The addition of a high energy, vitamin and protein supplement did not improve the response of the cows to superovulation. This agrees with findings of other workers. Lammond (1970) found no benefits from improved nutrition with superovulated cattle. However, Lammond (1970) did observe a decline in ovulation rate when he fasted animals between PMSG administration and embryo collection. The effects of nutrition on well fed donors have been reviewed by Dunn (1980) and considered to be mimimal. The animals fed a standard diet in

this study received the normal winter ration for housed suckler cows at the University Farm. Such a diet is likely only to maintain an animal. Whilst the silage offered to the control animals was of a slightly higher CP concentration the supplementation given to the treated cows would have certainly more than compensated for the shortage in protein supply from silage. The supplementary protein was mainly in rumen undegradable form thus being a more efficient supply.

Published reports on the effects of undernutrition on the reproductive performance of beef cows are equivocal. Workers have found (Dunne et al., 1974) or not found (Spitzer et al., 1978) that energy restriction increased progesterone secretion. Gombe and Hansel (1973) found energy restriction increased LH secretion whilst Spitzer and others (1978) and Dunne and others (1974) did not. These differences have been attributed to different experimental environments and differing breeds and ages of cows (Spitzer et al., 1978).

The influence of crude protein intake on beef cow reproduction has been investigated by Garth Sasser and others (1988). Reduced feeding of protein increased the post-partum interval to first oestrus, to first service and to conception. These effects may be mediated by reduced gonadotrophin release from the anterior pituitary. In a study by Nolan and others (1988) pituitaries from animals fed a low protein diet had lower FSH and LH contents than animals fed higher protein diets.

This evidence suggests that there may be nutritional influences on the responses of productive cattle to superovulation treatments. Topps (1977) observed that when cows

fed low quality roughage were given a protein supplement their fertility increased from 36 to 75%. The nutrition of a superovulated cow may be analogous to that of a ewe where the effects of nutritional 'flushing' are well recognised (Robinson, 1986).

Callesden and others (1988b) suggested that blood progesterone concentration should be determined at four sampling times in order to identify poorly responding animals. These times were at gonadotrophin administration; at PG administration and at 24 and 48 hours post PG administration. The threshold concentrations suggested by Callesden and others (1988b) were above 1 ng/ml at gonadotrophin and PG administration and below 1 ng/ml for both times after PG administration. The mean blood progesterone concentrations of the cows used in this study were 2.3 (SEM=0.45), 2.73 (SEM=0.28), 0.53 (SEM=0.11) and 0.63 (SEM=0.15) ng/ml at these times indicating normality according to the values reported by Callesden and others (1988b).

The blood concentration of LH, determined using the extract RIA method, of the cows in this study was 3.66 (SEM=0.19)ng/ml for Days -6 to -1 before oestrus and a peak concentration of 19.05 (SEM=2.35)ng/ml, which occurred 112 (SEM=4)h after gonadotrophin administration and 56 (SEM=4)h after PG administration. The data reported by Callesden and others (1988b) were 3.9 (SEM=0.2)ng/ml, 38.8 (SEM=1.8)ng/ml, 98 (SEM=1)h and 40 (SEM=1)h respectively, which are in reasonable agreement.

The blood hormone concentrations determined in this study are thus in good agreement with those reported by Callesden and

others (1988b) for normal secretory patterns. This suggests that aberrant endocrine profiles did not limit the responses to superovulation on this occasion.

The refractiveness of the catheterized animals made them unsuitable for close handling procedures. As this was their first experience of intensive handling some difficulty was anticipated. However, the catheters were not robust enough to survive for long periods and tail venepuncture, which was the only alternative method of blood sampling, can only be used a limited number of times. There were complete data for only one hCG treated and one control cow. The treated cow had two LH peaks, one large and one small, and a single oestradiol peak whilst the control animal exhibited biphasic secretion of both LH and oestradiol (figure 13).

Attempts have been made to reduce embryonic mortality by selectively adminstering hCG; Sreenan and Diskin (1983) injected 2500 i.u. 10-11 days post mating and observed a slight improvement in conception rates. The first injection, given after the second insemination, was intended to promote a more complete ovulation. It was considered that the superovulation may have been stimulating an excess of follicles which the endogenous LH could not cope with, or, more likely, the long biological half life of PMSG was causing waves of follicular growth and, consequently oestradiol secretion (Abdul Saeed et al., 1989).

Reports have suggested that early progesterone supplementation could prove beneficial in improving embryo survival (Kimura et al., 1987). Two other investigations have

subsequently been published (Helmer and Britt, 1987; Stewart et al., 1987) but in neither was there any improvement in conception rate resulting from the administration of hCG. Maiden heifers were used in their studies and normal breeding methods were used whilst the investigation reported here involved superovulated multiparous cows which had been exhibiting low levels of fertility. Maurer and Echternkamp (1982) observed that cattle which produced normal embryos possessed higher peripheral progesterone concentrations than those with abnormal embryos at Days 3-4 post oestrus. No such relationship could be found with the data collected here. The second injection of hCG was designed to increase progesterone secretion. The hCG treated cow, for which there was complete data, did show higher concentrations of plasma progesterone compared to the control animal but the difference was not significant.

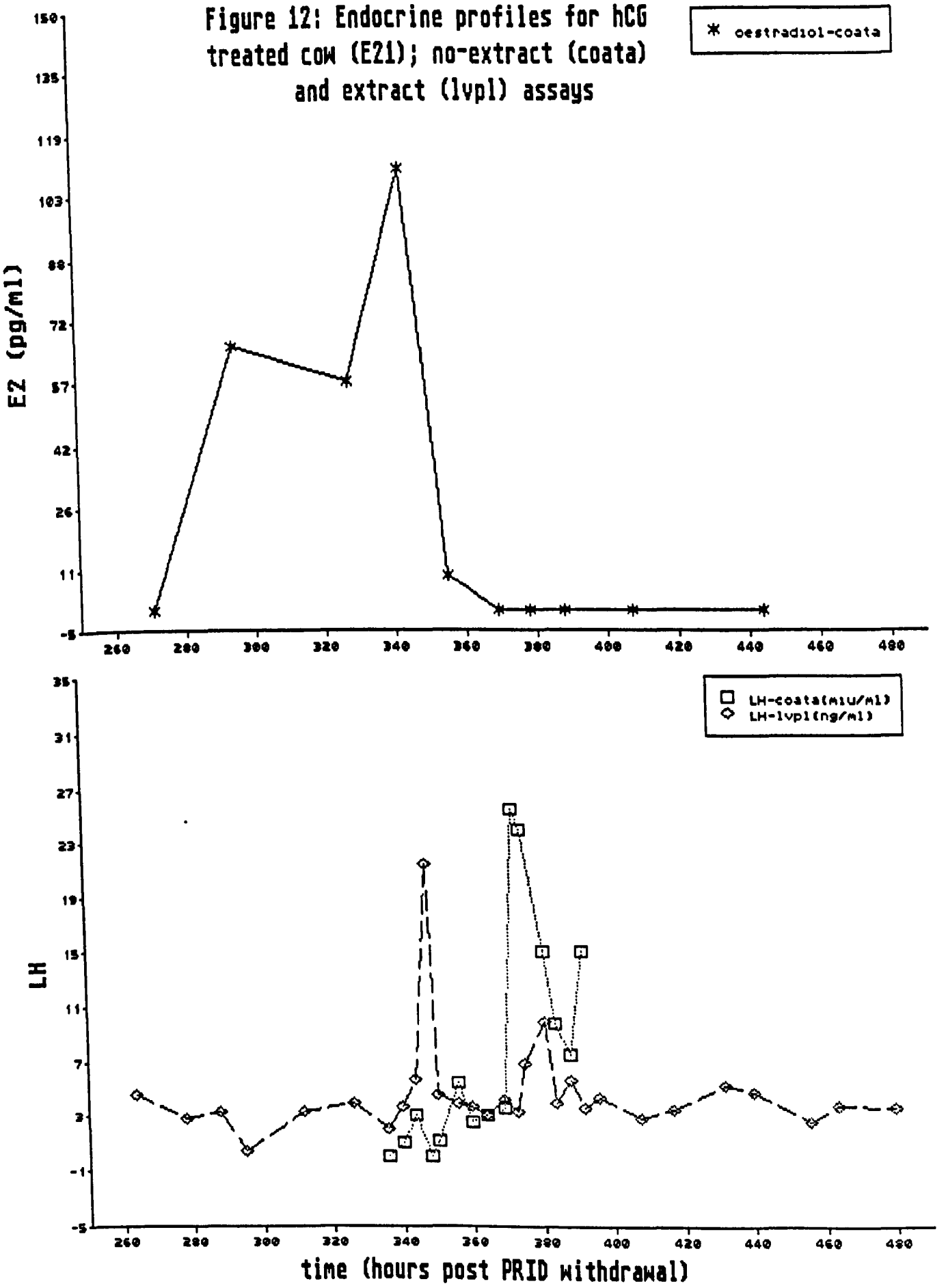
The results obtained by the progesterone coatacount assay kits indicate that, although developed for use on human plasma, they can be applied to the blood of cattle. The failure of the FSH assay was not unexpected as even specific bovine assays are unreliable (Dobson, pers. comm.). There have been earlier reports of the no-extract progesterone and oestradiol assays being used in bovine endocrinology (Dieleman and Bevers, 1987).

In conclusion, there was an unexpected and significant decline in the responses to superovulation in those cows which were housed and fed a concentrate. The animals had previously (2 winters) been housed in a neck-tie byre for the winter and appeared accustomed to this management. One cow resisted all attempts to be housed and was returned to group housing. However,

the blood sampling procedures may have been stressful to these animals. Alam and Dobson (1986) found increased physiological responses to these procedures. Stress is known to increase the adrenal cortical activity and hence inhibit the secretion of LH (Li and Wagner, 1981). The stress of restraint and blood sampling has been found to alter endogenous LH secretion in cows (Alam and Dobson, 1986) and ewes (De Silva et al., 1981). Doney and others (1976a,b) found that stress reduced ovulation rates and increased embryonic loss in ewes. Rhind and others (1984) reported conflicting data, where environmental and climatic stresses had no effect on ovulation rate or embryonic survival in ewes. Edwards and others (1987) found that stresses caused by transportation did not affect superovulatory responses.

When the responses of the cows were compared with their previous superovulatory responses (Table 5.6), interactions were detected between flush and feeding group on ovulation rate, numbers of recovered embryos and numbers of viable embryos recovered. This shows that rather than a decline in the responses of housed cows, there was an increase in the responses of the control, unsupplemented, cows.

Figure 12: Endocrine profiles for hCG treated cow (E21); no-extract (coata) and extract (lvpl) assays



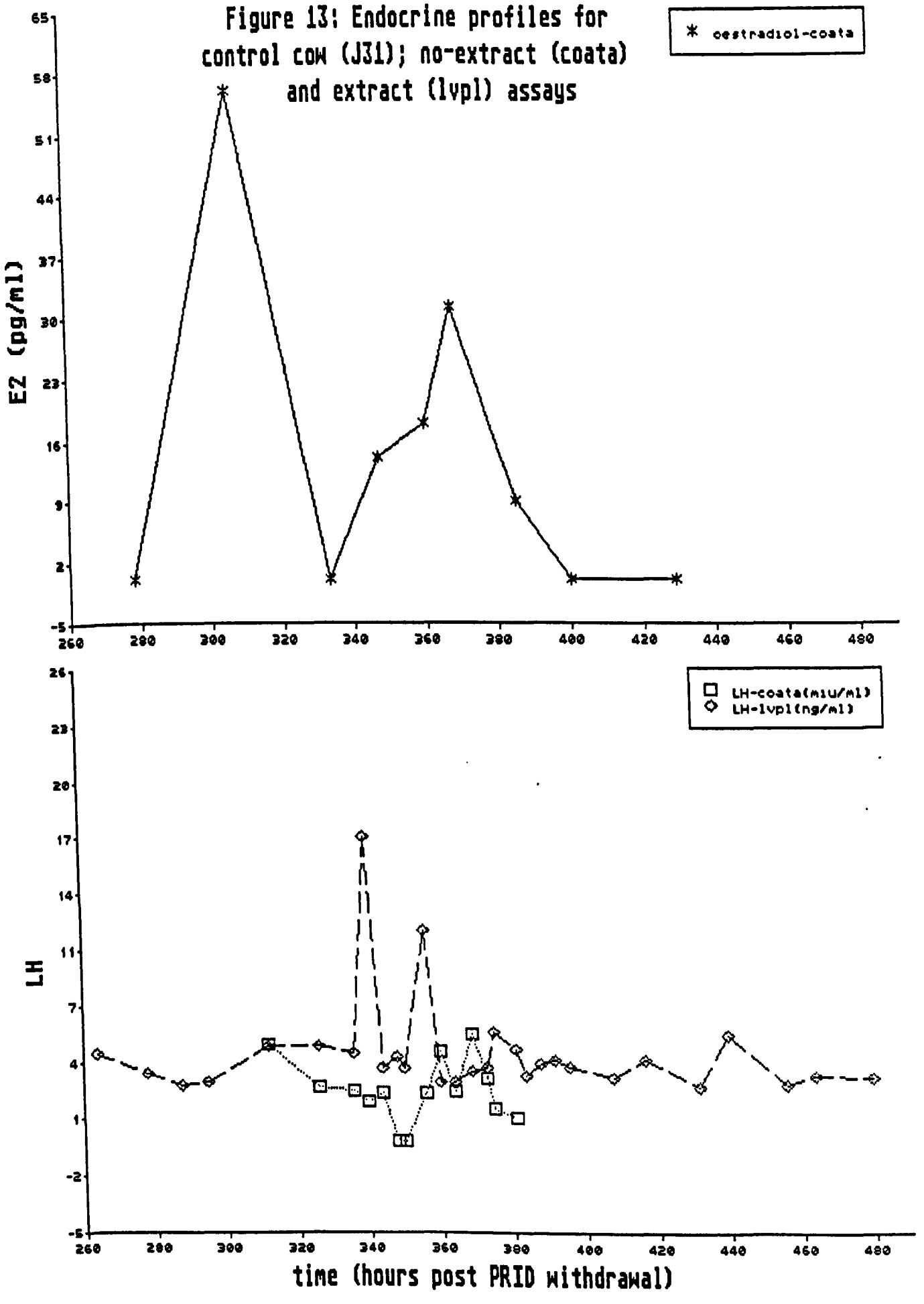


Figure 13: Endocrine profiles for control cow (J31); no-extract (coata) and extract (lvpl) assays
* oestradiol-coata
E2 (pg/ml)
65
58
51
44
37
30
23
16
9
2
-5
260
280
300
320
340
360
380
400
420
440
460
480
LH-coata(miu/ml)
LH-lvpl(ng/ml)
LH
26
23
20
17
14
11
7
4
1
-2
-5
time (hours post PRID withdrawal)

Figure 14: Plasma progesterone profiles for hCG treated and control cows

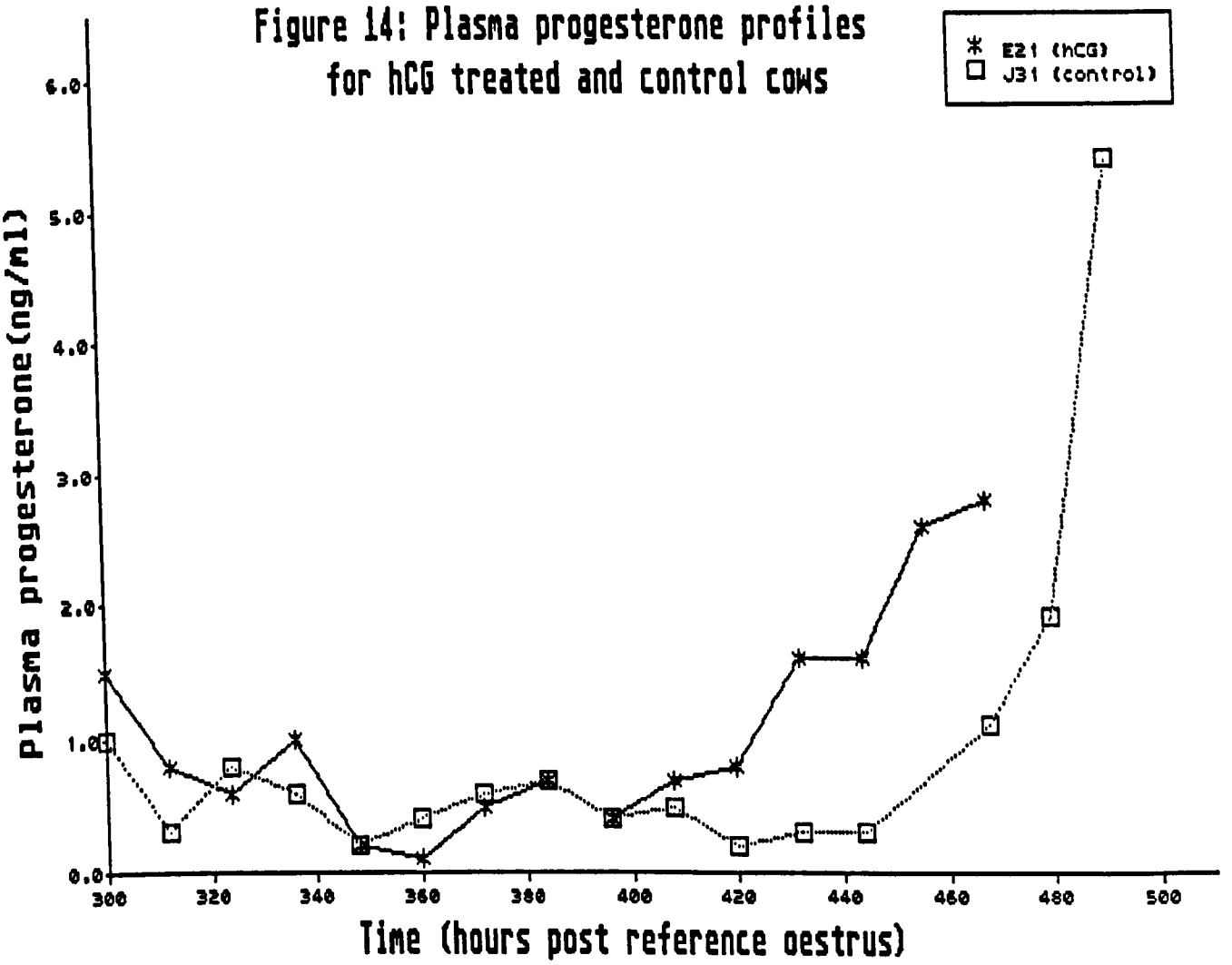


Table 5.1: Mean ovarian responses and embryo production from donors fed supplemented and control diets and administered hCG.

		Mean (SEM)				
	n	CL	Embryos	Viable embryos	% Viable* embryos	Embryo* recovery(%)
Supplemented diet						
hCG	3	5.0(2.6)	0.3(0.3)	0.3(0.3)	100.0(-)	8.3(8.3)
control	2	2.5(2.5)	0.5(0.5)	0.5(0.5)	100.0(-)	20.0(-)
Standard diet						
hCG	2	13.5(6.5)	7.0(2.0)	3.0(1.0)	51.1(28.9)	58.2(13.2)
control	3	10.3(0.3)	6.0(2.1)	5.0(2.3)	74.4(20.8)	59.1(21.5)
Overall		7.8	3.4	2.3	75.1	37.6
SEM		3.1	1.1	1.1	31.8	19.8
P hCG	=	ns	ns	ns	ns	ns
supplement	=	0.039	0.008	0.062	ns	0.095
hCGxsuppl.	=	ns	ns	ns	ns	ns

* calculated for each cow and then the mean taken (section 3.4)

Table 5.2: Mean ovarian responses and embryo production from groups of cows fed supplemented and standard diets

Diet	n	Mean (SEM) CL	Embryos	Viable embryos	% Viable* embryos	Embryo* recovery(%)
Suppl'd	5	4.0 (1.8)	0.4 (0.2)	0.4 (0.2)	100.0 (-)	11.3 (6.6)
Standard	5	11.6 (2.2)	6.4 (1.3)	4.2 (1.4)	65.1 (15.7)	58.7 (12.5)
Overall		7.8	3.4	2.3	75.1	37.6
SEM		2.2	1.0	1.0	20.2	10.9
P =		0.03	0.002	0.03	ns	0.02

* calculated for each cow and then the mean taken (section 3.4)

Table 5.3 Composition of the concentrate supplement (/kg DM) (Supercharge, Colburn Dawes)

Protein	370 g
UDP	240 g
ME	13 MJ
Ca	65 g
P	30 g
Mg	10 g
Co	20 mg
I	65 mg
Mn	1360 mg
Zn	850 mg
Fe	170 mg
Se	5 mg
Cu	160 mg
Vitamin A	76000 i.u.
D3	15000 i.u.
E	100 i.u.

Table 5.4: The components and feeding value of the silages offered to the supplemented and the standard diet animals

	Mean (SEM)				
	D.M.(%)	MADF(%DM)	CP(%DM)	Ash(%DM)	ME(MJ/kgDM)
Suppl'ed	24.73	34.00	11.29	6.6	10.72
SEM	(6.32)	(1.99)	(0.80)	(0.6)	(0.52)
Standard	26.67	32.77	12.81	6.67	10.85
SEM	(2.03)	(1.18)	(0.38)	(1.41)	(0.45)
P =	ns	ns	0.09	ns	ns

Table 5.5: Hormone assay results

		Mean (std. dev.)			
	no of assays	Samples /run	Recovery (%)	% binding at 0	NSB (%)
			Extract assay (Liverpool)		
Prog.	5	38(16)	63.9(4.5)	44.1(7.2)	at 500 pg 24.6(4.2) 3.6(0.6)
E2	1	20	81.7	24.3	at 150 pg 13.3 6.9
LH	1	98	-	61.9	at 5 ng 21.6 2.1
			No-extract assay		
E2	1	20	-	at 0 100	at 3600pg/ml 8.1
FSH	1	20	-	100	at 100 mi.u./ml 14.9
Prog.	1	20	-	100	at 100 ng/ml 12.3
LH	1	20	-	100	at 200 mi.u./ml 15.0

Table 5.6: Mean ovarian responses and embryo production from cows fed supplemented and control diets; comparison with their previous flushing.

	n	Mean (SEM) CL	Embryos	Viable embryos	% Viable* embryos	Embryo* recovery(%)
Group 1 flush 1	5	6.4 (2.9)	3.4 (2.0)	0.4 (0.4)	16.7 (16.7)	48.3 (12.6)
supplemented flush 2	5	4.0 (1.8)	0.4 (0.2)	0.4 (0.2)	100.0 (-)	11.3 (6.6)
Group 2 flush 1	5	5.4 (1.8)	3.0 (0.8)	0.8 (0.7)	25.0 (14.4)	64.8 (13.7)
Standard flush 2	5	11.6 (2.2)	6.4 (1.3)	4.2 (1.4)	65.1 (15.7)	58.7 (12.5)
Overall		7.8	3.4	2.3	75.1	37.6
SEM		2.2	1.3	0.8	17.1	12.3
P group	=	ns	ns	0.015	ns	0.022
flush	=	ns	ns	0.043	0.004	0.104
group x flush	=	0.07	0.02	0.043	ns	ns

* calculated for each cow and then the mean taken (section 3.4)

5.4 THE RELATIONSHIP BETWEEN PLASMA PROGESTERONE CONCENTRATION ON THE DAY OF PMSG ADMINISTRATION AND THE SUBSEQUENT OVARIAN RESPONSES AND EMBRYO RECOVERY

5.4.1 INTRODUCTION

The development of reliable methods for the determination of peripheral progesterone has led to proposals that animals could be screened to identify those bearing subnormal corpora lutea at gonadotrophin administration. This could prevent the unnecessary superovulation and embryo recovery of suboptimal animals in superovulation programmes (Britt and Holt, 1988). In this investigation the concentration of progesterone in the plasma of Welsh Black cattle was determined at the time of initation of superovulation treatment. This was compared with the resulting ovarian responses and embryo production, to investigate the feasibility of identifying poorly responding Welsh Black cows prior to undergoing superovulation.

5.4.2 MATERIALS AND METHODS

Welsh Black cows (n=24), were selected at random from the animals undergoing superovulation in this study. They were synchronised in oestrus as described previously. Samples of blood were collected by tail venepuncture into evacuated heparinised tubes (Vacutainer, Breton and Dickinson) on the day of PMSG administration. The samples were centrifuged at 1000g for 30 min at 4°C. The plasma was withdrawn and stored frozen until

required for assay.

Progesterone concentration was determined using enzyme immuno-assay (EIA) kits (Ovucheck, Camb.) and extract radioimmuno-assay (RIA) procedure (Dobson, 1983).

The cows were superovulated with 2500 i.u. PMSG and bred by AI. The method of embryo recovery was as described previously (Chapter 3). The estimated ovarian responses and the embryo recovery data were compared with the determined plasma progesterone concentrations.

5.4.3 RESULTS

The cows were divided into groups according to the plasma progesterone concentration thresholds suggested by Callesden et al. (1988b) who selected 1.0 ng/ml as the threshold. Also data were categorised on the basis proposed by Goto et al. (1988) who used a value of 3.0 ng/ml. The responses to superovulation and the subsequent embryo production are summarised in table 5.4. The cows had a mean plasma progesterone concentration of 4.0 (SEM=0.65) ng/ml. They responded to superovulation producing a mean of 9.0 (SEM=1.29) CLs. A mean of 4.2 (SEM=0.89) embryos were recovered per cow. Of these 1.3 (SEM=0.45) were classified as transferrable. The overall mean recovery of embryos was 45.2% (SEM=7.1). The mean percentage of transferrable embryos from the total number of recovered embryos was 36.6% (SEM=9.6). No significant correlations were found between plasma progesterone concentration at PMSG administration and either ovulation rate, numbers of embryos recovered, numbers of viable embryos recovered, % viable embryos or embryo recovery rate (%). Figure

15 is presented as an example of a typical scatter diagram for these characteristics.

5.4.4 DISCUSSION

The concentrations of plasma progesterone found in the cows were consistent with published values. Callesden and others (1988b) found a mean concentration of 4.3 ng/ml at time of gonadotrophin administration. Two reports have suggested different threshold values for plasma progesterone concentration at gonadotrophin administration below which embryo viability falls. Callesden and others (1988b) considered 1.0 ng/ml to be necessary whilst Goto and others (1988) proposed 3.0 ng/ml as the critical value. Calculations were performed using both threshold figures (Table 5.7). Mean embryo viability was lower in cows which had a plasma progesterone concentration below 1 ng/ml but the difference was not significant to those above this concentration at the 10 % level of statistical signficance.

The absence of correlation between plasma progesterone at PMSG adminstration and ovarian responses and embryo production found here agrees with previous reports (Sreenan and Gosling, 1977; Bevers and Dieleman, 1987). The availability of kits for determining progesterone concentration in both milk and plasma has led to reports outlining their application in screening donors and recipients (Britt and Holt, 1988). The data presented in this study indicates that Welsh Black cows have progesterone concentrations similar to other cows but do no support the use of plasma progesterone kits to screen cows for embryo donation at

time of gonadotrophin administration.

Table 5.7: Mean ovulation rate and recovery of embryos from superovulated Welsh Black cows; influence of plasma progesterone concentration at PMSG administration.

		Mean (SEM)					
Conc. of prog.	n	Prog. (ng/ml)	CLs	Embryos	Viable embryos	Recovery rate (%)	% Viable embryos
≤ 1.0 ng/ml	7	0.4	9.8	6.0	0.6	50.9	15.4
(SEM)		(0.15)	(2.3)	(3.1)	(0.4)	(16.0)	(11.8)
> 1.0 ng/ml	17	5.0	8.8	3.7	1.5	41.0	42.2
(SEM)		(0.6)	(1.5)	(0.8)	(0.6)	(8.0)	(11.4)
P =		0.003	ns	ns	ns	ns	ns
≤ 3.0 ng/ml	11	1.2	8.0	3.9	0.8	41.9	40.6
(SEM)		(0.3)	(2.0)	(1.6)	(0.3)	(11.2)	(16.6)
> 3.0 ng/ml	13	6.35	9.9	4.5	1.7	47.9	34.2
(SEM)		(0.7)	(1.8)	(0.96)	(0.79)	(9.8)	(12.2)
P =		0.000	ns	ns	ns	ns	ns

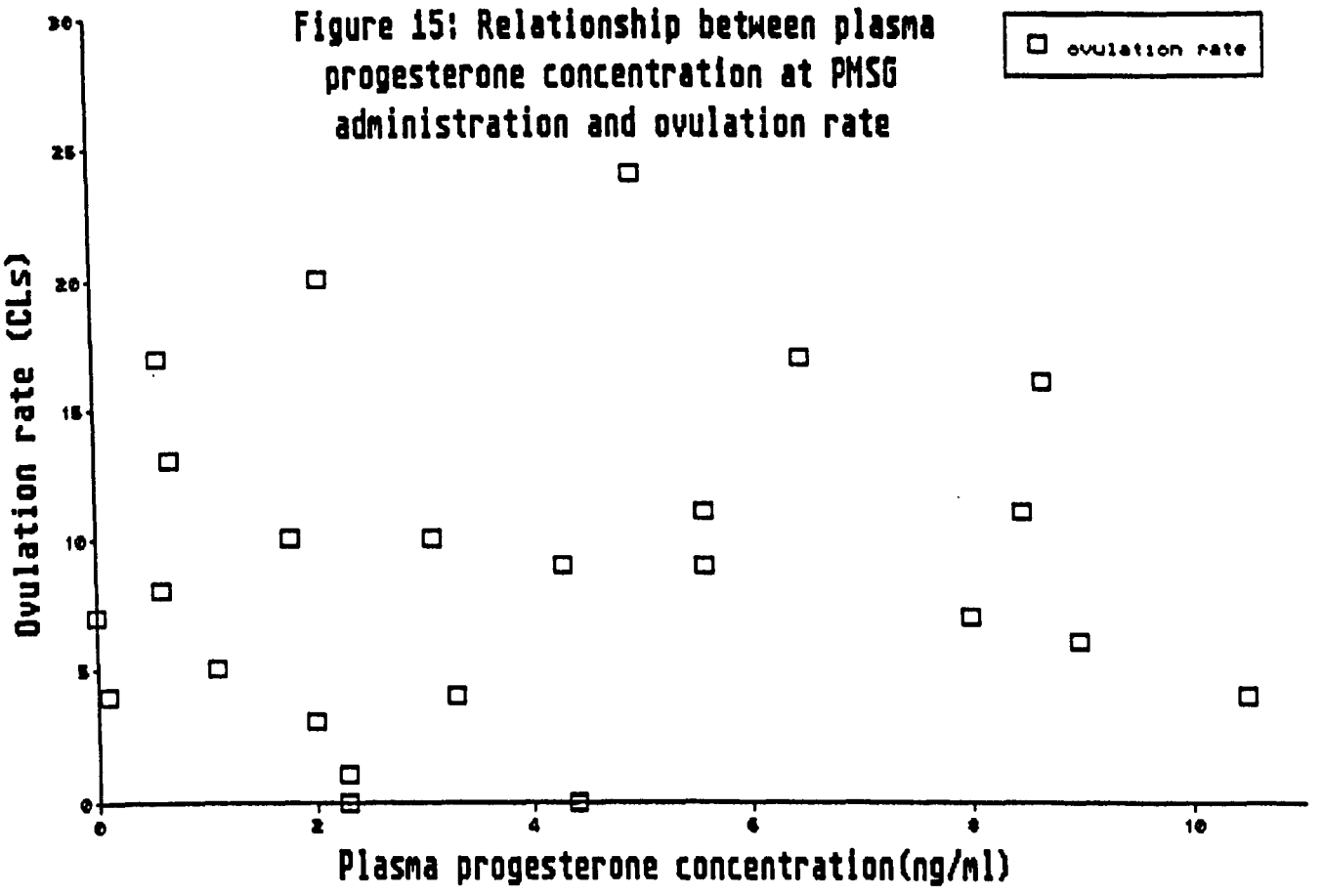


Figure 15: Relationship between plasma progesterone concentration at PMSG administration and ovulation rate
ovulation rate
Ovulation rate (CLs)
30
25
20
15
10
5
0
0
2
4
6
8
10
Plasma progesterone concentration(ng/ml)

CHAPTER 6

THE EFFECT OF SUCKLING AND SEASON ON EMBRYO PRODUCTION FROM SUPEROVULATED WELSH BLACK COWS

6.1 THE INFLUENCE OF INTERVAL FROM CALVING TO FLUSHING ON SUPEROVULATION AND EMBRYO PRODUCTION.

6.1.1 INTRODUCTION

There is no published information on the effect of dates of calving and the interval between calving and first superovulation and embryo collection on Welsh Black cows. The season of calving has been reported as influencing the postpartum interval to first oestrus. Animals calving in November to April took longer to return to cyclicity than May to October calving beef cows (70.8 cf 35.9 d; Peters and Riley, 1982a). Such data is crucial for the practical application of MOET programmes in cattle breed improvement where the maintenance of tight calving patterns is normally required for economic reasons and to provide accurate contemporary comparisons for selection decisions. This study was designed to investigate the role of date of calving and interval to first superovulation on the responses of Welsh Black cows and thus examine the benefits and drawbacks of removing donors from the herd and distrubing their normal calving pattern, in the interests of ensuring that full and repeated cyclicity had been achieved.

6.1.2 MATERIALS AND METHODS

A total of 12 first or second calving cows were selected. Seven of these came from the early (April-May) calving group and five from the late calving (June-July) group. The cows were synchronized using PRIDs. Superovulation was induced using 2500 i.u. PMSG. Embryo recovery was performed seven days after

insemination. Ovarian responses were assessed by palpation per rectum. The mean time interval between calving and embryo recovery was 128.3 d in the early calving group and 85.4 d in the late calving group. Embryo assessment was performed according to previously stated criteria (section 3.4).

6.1.3 RESULTS

The responses of the cattle from the calving groups were analysed with further division of these groups into early and late treatment groups (Table 6.1).

The animals responded to superovulation producing a mean ovulation rate of 6.1 CLs. The ovulation rate of the animals in the late treatment groups were higher than the early treated animals ($p=0.1$). More embryos were recovered from the late calving animals (7.6 cf 2.3; $P=0.1$) however, neither the yield of viable embryos nor the mean embryo viability were significantly different.

6.1.4 DISCUSSION

This study found only small differences in the responses of early and late calving animals to superovulation and embryo collection when treated from days to days postpartum. This suggested that the time interval was of limited influence and agrees with the findings of Dorn and others (1989) who observed no differences in the superovulatory responses of beef cows when treatment commenced either 23-33 or 60-90 days post-partum.

The trend towards superior embryo production from the early calving cows could be related to the availability of high quality

grass which would allow earlier forage ingestion by the calves reducing the nutritional demand on the dam. The late calving animals did not have access to such grazing increasing the nutritional burden on the cows. Seasons are known to have significant effects on beef cow reproduction (Peters and Riley, 1982a; Hansen and Hauser, 1983). The literature which has been presented on superovulated beef cows is equivocal with workers detecting (Lerner et al., 1986) and not finding (Hasler et al., 1987) seasonal effects in the responses of superovulated cows. Further and larger scale investigations will be required to establish and evaluate this effect.

The data presented here suggests that there is no benefit from extending the calving-first flush interval for spring calving Welsh Black cows and consequently animals selected as donors could be superovulated, flushed and then mated in line with the target annual calving so as to be consistent with the calving pattern of such herds.

Table 6.1: The interval from calving to embryo recovery; mean ovarian responses and embryo production from early and late calving Welsh Black cows

Group	n	interval calving -flush	CLs	Embryos	Viable embryos	% Viable embryos*	Embryo* recovery (%)
Early calving							
early treat	4	139.5	4.0	2.3	1.0	38.9	61.2
late treat	3	113.3	6.3	2.3	1.0	50.0	36.9
Late calving							
early treat	3	72.0	4.7	3.7	0.7	16.7	75.0
late treat	2	105.5	12.0	7.0	0	0	53.8
Overall		110.4	6.1	3.4	0.75	26.7	59.0
SEM		15.2	2.5	1.7	0.75	25.1	19.7
P early/late calving	=	0.04	ns	0.1	ns	ns	ns
early/late treatment	=	ns	0.1	ns	ns	ns	ns
calvingxtreat	=	0.08	ns	ns	ns	ns	ns

* calculated for each cow and then the mean taken

6.2 THE EFFECT OF WEANING ON SUPEROVULATION AND EMBRYO PRODUCTION.

6.2.1 INTRODUCTION

The proportion of embryos, which were viable, recovered from superovulated Welsh Black cows was persistently lower than expected. Discussions with other workers (Sreenan, pers. comm.) suggested that the nursing status of the donor may be a factor. The effect of abrupt weaning on the responses to superovulation of Welsh Black cows was examined to identify any benefits from drying off cattle prior to the implementation of a superovulatory protocol.

6.2.2 MATERIALS AND METHODS

Six cows were selected from suckling animals which had previously been superovulated but produced few viable embryos. The calves were removed from their dams, on 13.10.87, approximately 2 weeks before gonadotrophin administration and 3 weeks before embryo collections were to be carried out. The cows were synchronized using PRIDs, and superovulation was induced with a single injection of 2500 i.u. PMSG. Embryo recovery was performed seven days after insemination. Ovarian responses to superovulation were assessed by rectal examination. Collected embryos were assessed by the criteria described previously (section 3.4).

The cows' responses to superovulation and the subsequent embryo production were compared with data from their previous superovulation and embryo collection which took place in either

August or September when they were suckling calves.

6.2.3 RESULTS

The cows did not respond significantly differently to superovulation or to embryo collection after calf removal from the previous occasion whilst still suckling. The mean ovulation rate post-weaning was 7.8 CLs (SEM=2.5) compared to 9.33 CLs (SEM=2.5) whilst suckling, with a mean of 4.7 embryos (SEM=2.8) recovered compared to 6.0 (SEM=2.3). There was no improvement in the number of viable embryos recovered (0.5 cf 0.8) nor in the percentage of viable embryos recovered (21.7 cf 15.4). The results are detailed in table 6.2.

6.2.4 DISCUSSION

The viability of embryos recovered from the recently dried off cows remained extremely low. Any treatment effect would be confounded with variation caused by season and number of flush. This latter variable however, is currently reported to have little effect on superovulation or embryo recovery (Christie *et al.*, 1979; Ali Dinar *et al.*, 1987).

The limited number of animals available for this study did not permit the use of controls to remove any influence of the post-partum interval to flushing which was significantly different between the two periods. However, the low percentage of viable embryos (21.7%) recovered in the previous flush made it reasonable to expect any benefits from the treatment to be identifiable.

Suckling is known to inhibit ovulation (McNeilly *et al.*,

1982) but its effect on the responses of superovulated cattle has not been elucidated. This investigation revealed no benefit from drying off Welsh Black cows on subsequent superovulatory responses or embryo production. The work of Lusby and others (1981), where increased fertility and reduced post-partum interval to conception was observed in heifers dried off 6-8 weeks post-partum compared to the normally suckling controls, suggests an effect not identified in this experiment. A more detailed investigation would provide information on the influence of nursing status on the superovulatory responses of Welsh Black cows, which are primarily selected on their 'mothering ability'.

Table 6.2: The mean (SEM) ovarian responses and embryo production from Welsh Black cows superovulated before and after calf weaning.

	Suckling	Weaned	P
no of animals	6	6	
Date of embryo collection	6.8-18.9.87	6.11-20.11.87	
days post calving	129.9 (9.8)	188.6 (14.3)	0.1
No of CLs	9.3 (2.5)	7.8 (2.5)	ns
No of Embryos	6.0 (2.3)	4.7 (2.8)	ns
No of viable Embryos	0.8 (0.4)	0.5 (0.3)	ns
% viable Embryos	21.7 (12.0)	15.4 (11.8)	ns

6.3 THE EFFECT OF SEASON AND SUCKLING ON EMBRYO QUALITY FROM SUPEROVULATED WELSH BLACK COWS

6.3.1 INTRODUCTION

The proportion of viable embryos may be reduced when donor cows are suckling (Sreenan, pers. comm., 1988). Suckling is known to stimulate the release of prolactin (McNeilly, 1987) which causes a reduction in the pulsatile secretion of LH and consequently a lowering of ovarian activity (Dorrington and Gore-Langton, 1981). This response to suckling could interact unfavourably with the process of superovulation and affect embryo viability in nursing cows.

Abrupt weaning of calves in mid-lactation did not improve the response to superovulation of the dams (section 6.2). This study was designed to investigate the long term effect of early calf removal, over a 5 month period, on the responses of superovulated Welsh Black cows.

6.3.2 MATERIALS AND METHODS

Seventeen multiparous spring calving Welsh Black cows were randomly allocated as they calved to three treatment groups. The calves of Group 1 cows (n=6) were weaned two days after birth. Groups 2 (n=6) and 3 (n=5) were allowed to suckle their calves freely. Groups 1 and 2 were superovulated and flushed in the summer (July or August), after completing at least one oestrous cycle and at a mean of 85 days post calving, and again in the autumn (November) at 180 days post calving. Group 3 cows were

flushed once only in the autumn 140 days into their lactation. The cows were superovulated with 2500 i.u. PMSG. The ovulation rate of the cows was assessed by rectal palpation of the ovaries and the presence or absence of large follicles (larger than 10 mm) noted (Christie et al., 1979).

Recovered embryos were assessed morphologically and categorised as viable if they were of grade 2 or better.

Data were analysed by a General Linear Model in which the main effects of treatment groups and season of flush, and their interactions were examined.

6.3.3 RESULTS

Results are presented in table 6.3. One cow in Group 2 was withdrawn after one flush. The season of embryo recovery had a significant effect on the numbers of embryos recovered with more embryos being recovered in summer than in autumn. The results are presented for the dry and pooled suckling groups of cows in table 6.4. Donors which were dry produced higher proportions of viable embryos compared to suckling donors (51.5 cf 27.4%; P=0.02). The season of flushing affected the number of viable embryos recovered, with fewer being observed in autumn (1.1 cf 2.9; P=0.02). The incidence of follicles larger than 10 mm was influenced by the nursing status of the donors (P=0.1), with those donors which were suckling possessing more follicles in both the seasons studied.

6.3.4 DISCUSSION

The effect of season on embryo quality was pronounced but difficult to explain, as many seasonal factors are operating (Janson, 1980; Faust et al., 1988). Availability of grass was greater for the summer collection so a better plane of nutrition could have been a factor. The cows were in better condition in the summer, July and August. Milk yield was not measured but would be expected to be declining by the second flush. However, this could not have been a primary factor as the fall in embryo quality for the dry cows was as least as pronounced as for those suckling calves.

The detrimental effect of suckling on embryo quality in superovulated cows which was found in this study has not been reported previously.

The role of suckling on reproduction in cattle has been investigated, especially with respect to the longer postpartum anoestrus in suckling cows compared to milked animals (Smith et al., 1981). An endocrinological basis for that observation has been elucidated; suckling and lactation inhibit ovulation at the hypothalamic, pituitary and ovarian levels (Lamming et al., 1981; McNeilly et al., 1982). Interaction of superovulation and suckling at the ovarian level may be responsible for the poor quality of embryos recovered in the present investigation. Primarily, suckling releases prolactin which lowers ovarian activity while superovulatory treatment aims to increase it. As the responses to superovulation were uniform between groups, follicular development was not repressed by suckling. Studies in

humans show that prolactin inhibits oestrogen production, reducing granulosa cell numbers and producing a hypofunctional CL (Franchimont and others, 1988). This may be analogous with the first CL formed postpartum in cattle which is characterized by being short lived and having impaired function (Lamming and others, 1981) possibily due in part to altered follicular development. Superovulation overrides the normal process of dominant follicle selection and it is possible that abnormal ovarian steroid concentrations received by the hypothalamus-pituitary axis interfere with the normal feedback controls. Braden and others (1989) found that the granulosa and thecal cells from the first preovulatory follicle after calf removal possessed fewer gonadotrophin receptors. These may later give rise to hypofunctional CLs.

The role of such CLs in cattle infertility has been considered and attempts have been made to demonstrate a relationship between them (Kimura et al. ,1987), and to improve fertility with progesterone supplementation (Sreenan and Diskin, 1983). It has been reported that cows which can be shown to have normal embryos had higher progesterone levels at days 3-4 post oestrus than those with abnormal embryos (Maurer and Echternkamp, 1982). Most embryonic mortality in first service dairy cows occurred before day 5 and was associated with a significantly changed uterine environment (Wiebold, 1988).

Suckling reduced embryo viability compared to weaned cows. This finding has important implications for MOET beef breed improvement schemes (Land and Hill, 1975), where the requirements for assessing mothering ability for the selection of

donors and the need to reduce generation time are incompatible.

Table 6.3: Mean ovarian responses amd embryo production from suckling and dry groups of cows in two seasons.

Group	season	n	Mean (SEM) CL	Foll.	Embryos	Viable embryos	% Viable embryos*	Embryo recovery* (%)
1 (DRY)	summer	6	8.0 (2.2)	0.5 (0.5)	5.0 (1.2)	3.8 (0.9)	83.6 (8.3)	71.6 (9.3)
	autumn	6	6.8 (2.7)	0.3 (0.2)	4.3 (1.9)	0.7 (0.2)	36.5 (18.0)	54.2 (14.0)
2 (SUCKLING)	summer	6	10.7 (3.1)	1.0 (0.6)	5.5 (2.6)	1.8 (1.0)	39.8 (19.9)	40.9 (12.9)
	autumn	5	7.1 (3.1)	3.4 (2.1)	3.2 (1.5)	0.8 (0.6)	12.5 (12.5)	32.0 (18.4)
3 (SUCKLING)	autumn	5	6.8 (2.7)	1.6 (0.7)	5.8 (2.6)	0.8 (0.8)	8.3 (8.3)	62.0 (17.4)
Overall			7.9	1.3	4.8	1.6	60.3	55.8
SEM			2.8	1.0	3.2	0.8	17.3	16.8
P group		=	ns	ns	ns	ns	0.05	ns
season		=	ns	ns	ns	0.007	0.03	ns
group x season		=	ns	ns	ns	ns	ns	ns

* calculated for each cow and then the mean taken

Table 6.4: Mean ovarian responses and embryo production from suckling and dry cows.

Group	season	n	Mean (SEM) CL	Foll.	Embryos	Viable embryos	% Viable embryos*	Embryo recovery* (%)
DRY	summer	6	8.0 (2.2)	0.5 (0.5)	5.0 (1.2)	3.8 (0.9)	83.6 (8.3)	71.6 (9.3)
	autumn	6	6.8 (2.7)	0.3 (0.2)	4.3 (1.9)	0.7 (0.2)	36.5 (18.0)	54.2 (14.0)
SUCKLING	summer	6	10.7 (3.1)	1.0 (0.6)	5.5 (2.6)	1.8 (1.0)	39.8 (19.9)	40.9 (12.9)
	autumn	10	7.1 (2.0)	2.5 (1.3)	4.5 (1.4)	0.7 (0.5)	10.1 (6.5)	47.0 (13.0)
Overall			7.9	1.3	4.8	1.6	60.3	55.8
SEM			2.5	0.9	1.9	0.7	13.2	15.2
P group		=	ns	0.1	ns	ns	0.015	ns
season		=	ns	ns	ns	0.004	0.016	ns
group x season		=	ns	ns	ns	ns	ns	ns

* calculated for each cow and then the mean taken

CHAPTER 7

FACTORS AFFECTING EMBRYO RECOVERY AND EVALUATION OF EMBRYO TRANSFER TECHNIQUES

7.1 THE RELATIONSHIP BETWEEN WEIGHT, CONDITION SCORE AND CERVIX DIAMETER AND THE PASSAGE OF AN INTRODUCER THROUGH A CERVIX

7.1.1 INTRODUCTION

A major constraint which has been observed in this study on the use of non-surgical MOET techniques is the requirement that both donor and recipient animals possess cervices large enough to permit the passage of the introducer and catheter. Occasionally animals were found to have cervices which were impassable to instrument passage after they had been superovulated and prepared for embryo collection or prepared as recipients for embryos. An attempt was made to identify suitable animals, using easily measured parameters which correlated with the penetrability of the cervix to standard equipment.

7.1.2 MATERIALS AND METHODS

A total of 44 beef heifers (Friesian and Friesian crosses) were used in the experiment. The cattle had been purchased and it was estimated that they were approximately 16 months old. The animals were drawn from loose housing. Any that were visually detected in oestrus were discarded. The weights and condition scores of selected cattle were recorded. The perineum of each was cleansed and swabbed with 70% alcohol. A sterile (boiled) blunt ended AI gun (o.d. 5mm, I.M.V.), was introduced up to the os cervix and, aided by the hand in the rectum, passed if possible into the body of the cervix. Complete passage was avoided, as was forced passage. If the introducer could be progressed 2/3 to

3/4 of the length of the cervix complete passage was considered possible and a positive result recorded. Complete passage was not attempted to minimise the risks of infection.

After the introducer had been withdrawn, the diameter of the cervix was measured at its narrowest point using a pair of hand held, flat, blunt-ended calipers. These were placed over the cervix from the rectum, adjusted and withdrawn. The opening of the calipers was then measured.

The data were analysed, by logistic regression (Whitaker, pers. com.), to examine the relationship between cervix penetrability and heifer weight, condition score and cervix diameter. The relationship between penetrability and the individual parameters were examined by chi-square analysis.

7.1.3 RESULTS

No significant relationship was found between cow weight, condition score or cervix size and the ability to pass the cervix (figure 16 and 17). Cow weight was positively correlated with cervix diameter (c=0.7).

It can be seen from figure 16 that only 4 animals with a cervix diameter greater than 9 mm could not be penetrated, while none of those of smaller size would permit passage of the introducer.

Applying a threshold diameter of 11 mm would reduce the number of false positives to 3 but would also identify 4 false negatives; ie. assessed as impassable when actually passable.

Chi-square analysis (Table 7.1) revealed the cervix diameter

to be consistently more significantly related to cervix penetrability than either liveweight or condition score.

7.1.4 DISCUSSION

No published information was available on this subject and the problem of small cervix size has been ignored. The assessment of both donors and recipients for use in non-surgical MOET has depended upon subjective rectal examination of the reproductive tract. The use of objective simple equipment to screen animals for non-surgical embryo recovery and transfer would be advantageous as the cost of preparing donors and recipients is high. Whilst cervical dilators can be used to aid the penetration of otherwise impassable cervices this would be contra-indicated in potential recipients which need minimal handling. The measurement of cervix diameter, using cheap, accurate calipers, offers a preliminary screening method which should prove useful for assessing potential donors and recipients. This study was performed on heifers and the method requires further evaluation for use in cows which are less likely to be difficult to penetrate.

Table 7.1: Chi-square analysis of cervix size, heifer weight and condition score on cervix penetrability.

Threshold value	Chi-sq statistic	False positive	False negative	Probability (1 d of Freedom)
cervix				
8 mm	11.5	9	0	0.005
10	26.6	5	2	0.005
11	31.8	3	4	0.005
13	19.9	3	6	0.005
16	8.2	1	13	0.05
weight				
235 kg	2.6	11	2	ns
250	21.0	5	3	0.005
270	15.8	3	7	0.005
350	3.3	3	13	0.1
condition score				
2.0	5.2	11	0	0.025
2.25	10.6	5	6	0.005
2.5	3.9	3	13	0.025
2.75	2.1	3	15	ns
3.5	0.01	2	23	ns

Figure 16: the relationship between cervix size, heifer weight and the ability to pass the cervix

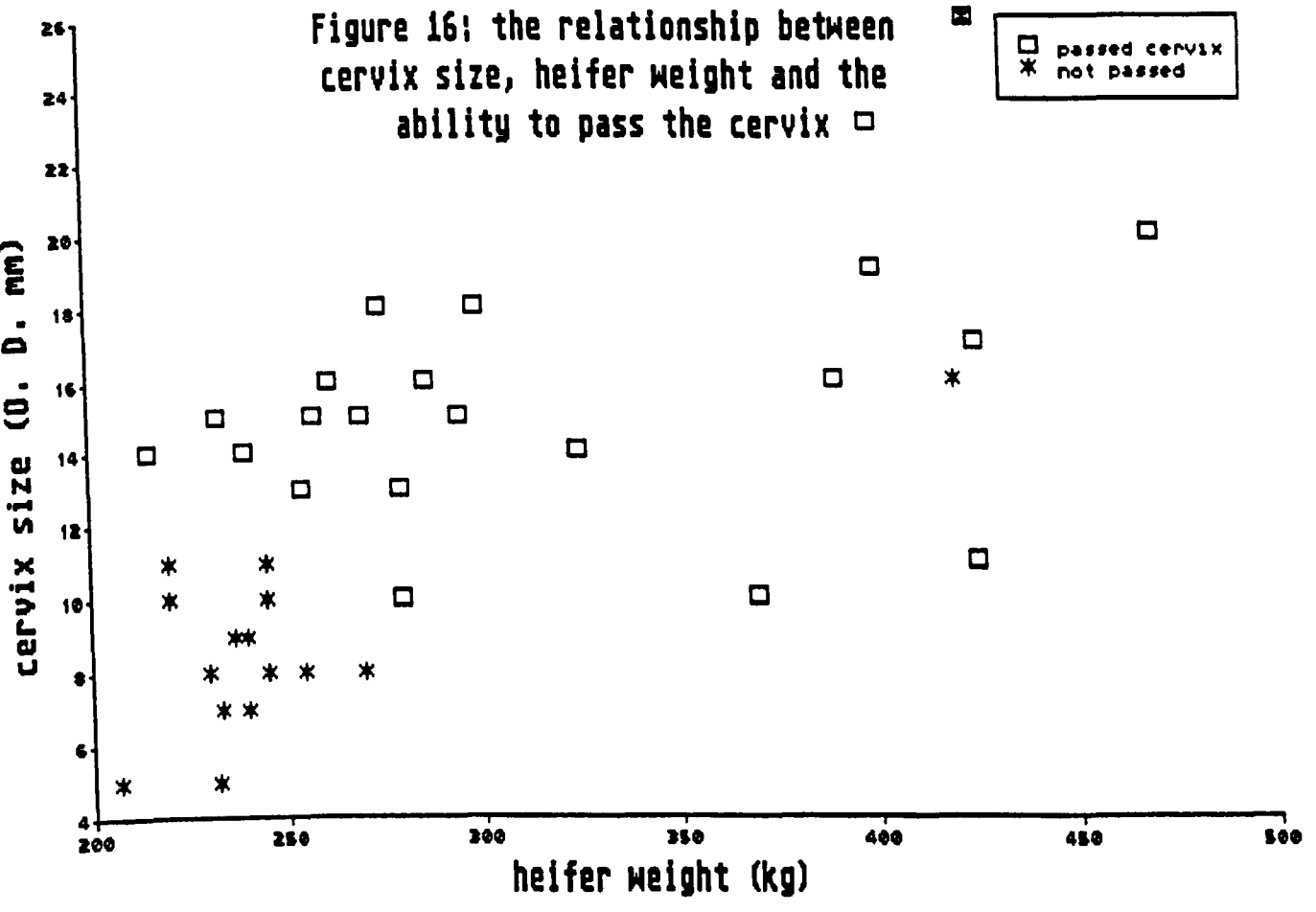
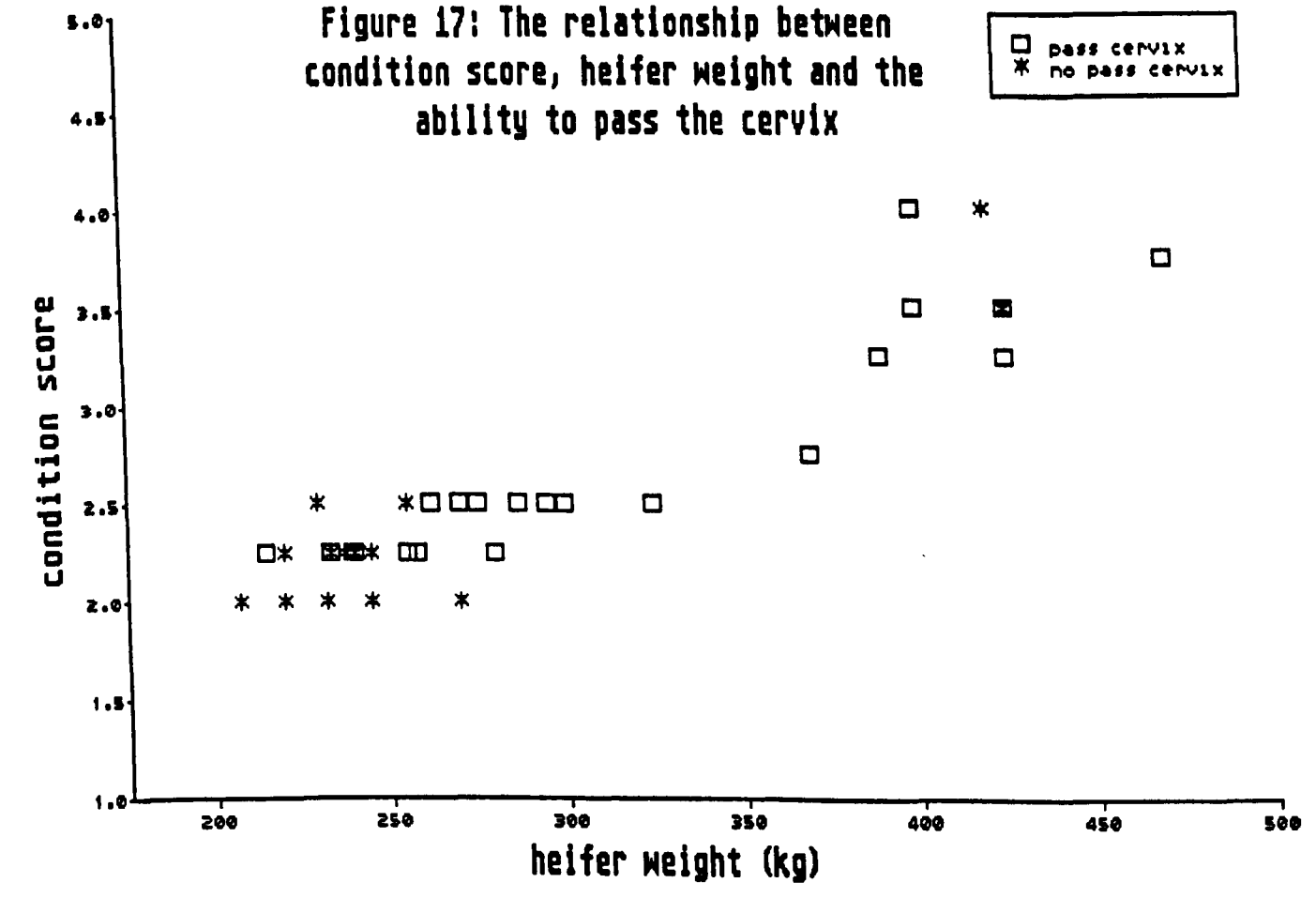


Figure 17: The relationship between condition score, heifer weight and the ability to pass the cervix



7.2 THE EFFECT OF CATHETER TYPE AND UTERINE HORN ON EMBRYO RECOVERY

7.2.1 INTRODUCTION

Multiple ovulation and embryo collection were first applied to cows in the Welsh Black Group Breeding scheme in 1985. Four catheter types were used to collect embryos. Data collected during three years of study are presented below and analysed retrospectively to identify the factors influencing the recovery of superovulated embryos.

7.2.2 MATERIALS AND METHODS

A total of 27 Welsh Black cows were used in this study. The animals had been treated to provide a reference oestrus and superovulated in the manner described previously (section 3). Embryos were collected non-surgically seven days after mating. The ovulation rate of the cows was estimated by the average of 2 independent estimates of ovarian stimulation per rectum. All catheters were used in a non-systematic way throughout the study period. The embryos recovered were assessed using previously defined criteria. The data collected were analysed with catheter types and uterine horn as fixed effects.

9.7.1 RESULTS

Data are presented for the embryo recovery percentage (embryos recovered/CLs) with respect to the catheter types used and the uterine horn flushed in table 7.2. The catheter type used did not significantly affect embryo recovery, which averaged

51.3%. Recovery from the two sides of uterus did not differ.

7.2.4 DISCUSSION

Many systems have been devised for non-surgical embryo recovery (Elsden, 1976; Newcomb and others, 1978; Sreenan, 1978; Newcomb, 1979; Rasbach, 1979; Seidel, 1981) but comparisons of the different catheters types are rare. Newcomb (1980) found the 3-lumen, circulation system to be superior to the 2-lumen, to and fro, system when collecting on Day 7 after oestrus. On day 8 the 2-way system appeared more successful. In this study embryos were collected on day 7 only and 4 types of catheter were used; 3-lumen, 2-lumen (Gibbon), long 2-lumen (Foley) and short 2-lumen (Foley). No difference was detected between the different catheters in their efficiency of embryo recovery. Recovery was equally successful from the right and left uterine horns.

These results suggest that when correctly positioned, there are no differences between the efficacy of the catheter types used, for embryo recovery. In this study the catheter of choice would be the 2-lumen Foley which was the easiest to place and therefore least likely to be traumatic.

Table 7.2: The effect of type of catheter on embryo recovery from superovulated Welsh Black cows.

Recovery catheter	n	Embryo recovery (%) Mean (SEM) uterine horn left	right
3-way (3-WAY)	17	27.3(15.7)	37.5(16.5)
GIBBON (2-WAY)	35	57.0(10.4)	39.1(10.9)
FOLEY (2-WAY,RUSCH)	31	56.9(21.3)	54.9(15.7)
FOLEY (2-WAY,SHORT)	4	65.7(23.3)	72.2(30.1)
P catheter	=	ns	ns
horn	=	ns	ns
catheter x horn	=	ns	ns

7.3 EVALUATION OF EMBRYO TRANSFER TO RECIPIENTS

7.3.1 INTRODUCTION

During the programme of superovulation and embryo recovery on Welsh Black cows a series of embryo transfers were performed. Data is presented here on the results of these transfers over three years of embryo recoveries.

7.3.2 MATERIALS AND METHODS

The methods of both surgical and non-surgical embryo transfer have been described previously (section 3.3.5). All surgical transfers were performed on Friesian heifers from the College dairy herd replacements. All non-surgical transfers were performed on multiparous cows from the College dairy herd.

7.3.3 RESULTS

The results from a total of 14, 7 nonsurgical and 7 surgical, transfers performed over 3 years are summarised in table 7.3. The mean success in transferring embryos was 38.5%. No successful non-surgical transfers were performed. The pregnancy rate with surgical transfers was 71.4%. Embryos stored frozen and transferred surgically had a pregnancy rate of 50%. Embryos surgically transfered fresh had a mean pregnancy rate of 80%.

7.3.4 DISCUSSION

The failure of embryos transferred using non-surgical techniques to continue development is difficult to explain. The operator had wide experience of both AI and embryo transfer methods and considered all the embryos had been correctly placed

in the uterus. The quality of the embryos could have been a factor but the most likely explanation is that the recipient dairy cows were of low fertility and unable to sustain embryo development.

The heifers used in surgical transfer would be expected to have higher fertility than the dairy cows and the higher uterine placement achieved with surgical procedures may have aided weaker embryos unable to continue development lower in the uterus. The success of the surgical method, and to a less proven extent the freezing method, was encouraging and shows the possibilities of transferring embryos into heifers using these methods. The calves produced from embryo transfer techniques have been shown to be of the same quality as those produced through conventional breeding methods (King et al., 1985a,b).

Table 7.3: Transfers of frozen and fresh Welsh Black embryos by surgical and non-surgical methods.

Recipient	date (m/yr)	Donor	Embryo grade (develop. stage)	Method of transfer	Calf birth
181	9.86	C1	3 (blasto.)	non-surgical	no
841	11.86	C11	4 (morula)	non-surgical	no
270	11.86	C11	3 & 2(morulae)	non-surgical	no
148	11.86	C11	2 (2 morulae)	non-surgical	no
32H	8.87	K31	4 (morula)	surgical	no
- H	8.87	J39	4 (morula)	surgical	yes
55H	8.87	J39	4 (morula)	surgical	yes
40H	8.87	J11	4 (blasto.)	surgical	yes
58H	8.87	J11 K31	2 (morula) 4 (blasto.)	surgical surgical	no yes
33H	9.87	J33	3 froz.(blasto)	surgical	no
69H	9.87	J33	3 froz.(blasto)	surgical	yes
169	11.87	J31	2 (2 morulae)	non-surgical	no
94	11.87	J31	4 (blasto.)	non-surgical	no
273	11.87	E21	4 (morula)	non-surgical	no

* Note 'H' after recipient number denotes dairy heifer.

CHAPTER 8

THE DETERMINATION OF FEED INTAKE IN WELSH BLACK CATTLE USING n-ALKANE MARKERS.

8.1 INTRODUCTION

The use of multiple ovulation and embryo transfer methods in beef breeding demands accurate identification of superior animals due to the increased influence the selected sires and female donors will have on the breeding herd. The selection criteria used vary between breeds according to their production characteristics. The Welsh Black breed has been developed as a hill suckler animal and the maintenance costs of a herd, especially winter feed costs, are important factors in herd profitability. Thus the feed conversion efficiency (FCE) of an animal has a direct effect on hill farm economics as well as being germane to beef production in general. The estimation of FCE, and its subsequent application in selection, requires a knowledge of feed intake and this presents difficulties at the farm level.

Many methods have been proposed for the estimation of feed intake by ruminants. The most successful have been those which use a marker either dosed or incorporated in the animals' diet (Kotb and Luckey, 1972). The most widely used marker is chromic oxide (Cr_2O_3) which is used to estimate faecal output. There is known to be diurnal variation in the faecal excretion of this compound, unless it is continually administered, and this renders it unsuitable for the estimation of individual intakes (Meijs, 1981). The n-alkane marker has been reported as having considerable advantages over this and other markers as it has been reported as showing no cyclical variation in excretion and behaving identically to natural plant alkanes in the gut (Mayes *et al.*, 1986). The method, proposed by Mayes and others (1986),

is based on the faecal recovery of even chain length alkanes, usually dotriacontane (C_{32}) which are administered daily, and of the naturally occurring odd chain length alkanes, usually C_{31} and C_{33} derived from feed. This method assumes that the faecal recovery of the dosed alkane is the same as the natural ones. The low digestibility of the alkanes is assumed to be the same and can therefore, be disregarded (Table 8.1). The naturally occurring alkanes are constituents of cuticular wax (Tulloch, 1981).

Table 8.1: Reported faecal recoveries of n-alkanes.

Authors	Mean faecal recovery C_{31}	C_{32}	C_{33}	C_{35}	C_{36}
Mayes et al. (1986, sheep)	0.854	0.889	0.891	0.931	-
Wilkinson and Mackie (1988, cattle)	-	0.791	0.827	-	0.914
Gosden and Moseley (1984, sheep)	0.931	1.024	1.015	1.086	-
mean	0.893	0.901	0.911	1.009	

The object of the experiments reported here was to adopt and evaluate the n-alkane method to determine the herbage intake of Welsh Black cattle in order to identify superior animals. Such animals would be those that used forage most efficiently and would therefore be selected for use in multiple ovulation and embryo transfer schemes.

The alkane method of feed intake estimation was applied to:

a) 6 sheep in metabolism cages

b) 4 bulls and heifers housed and fed a winter diet of silage and concentrate

c) 4 cows grazing pasture in summer

Samples collected in these experiments were stored frozen before analysis. Unfortunately the deep freeze containing most of the samples suffered a failed compressor and the samples contained in it thawed and were spoiled.

8.2 MATERIALS AND METHODS

8.2.1 PREPARATION OF THE PELLETS CONTAINING C_{32} AND C_{36} ALKANES

The C_{32} (dotriacontane) and C_{36} (hexatriacontane) alkanes to be administered to the cows were incorporated into paper pellets by the following procedure. Whatman no. 45 filter paper (100g) was spread onto an aluminium tray (80 x 40 cm) which was loaded into a 40°C drying oven. 12g of C_{32}, and for the cows grazing pasture (see section 8.6) 10g of C_{36}, alkanes were dissolved in 400 ml of warm (30°C) heptane (B.D.H. plc.). The solution was added to the tray containing the papers. Uniform absorption was achieved by gentle shuffling of the papers and solvent. The solvent was evaporated from the impregnated paper by standing the tray in a freely ventilated room. The impregnated papers were removed and dried individually in an oven at 60°C. The dry paper was shredded in an office shredder (Krug and Priester, Gmbh) and

6g portions of the matrix were compacted in a tube (i.d. 2.5 cm) to form the pellets which were wrapped in tissue paper and secured using starch/sucrose paste as adhesive.

8.2.2 ANALYSIS OF n-ALKANES

Samples of dry, milled faeces (1-2g) and forage (3-4g) were weighed into clean 50 ml beakers. 200µl of heptane containing 0.6 mg C_{34} (tetratriacontane) alkane , were added to each sample. Alkanes were extracted using the method of Tulloch and Weenink (1969). Twenty ml of petroleum spirit (B.P. 40-60°C) were added and the mixture was gently swirled for 30 mins at room temperature. Aliquots (3ml) of the solvent were aspirated into a 10ml glass syringe and elutriated through Florasil (Macey and Barber, 1970) using prepacked filters (Waters, Millipore plc) into 5ml test tubes. The elutriate was evaporated under a nitrogen stream and redissolved in 200 ul heptane. 1-2 µl of this was injected onto a 2.2 m x 4mm glass column containing 3% Dexsil 300 on 80-100 mesh Chromosorb WHP (Phase Sep plc) in a model 104 gas chromatograph fitted with a flame ionization detector (Pye Unicam, Camb.). The chromatograph oven was maintained at 295°C and the carrier gas, nitrogen, had a flow rate of 35 ml/min. The carbon chain lengths of the alkane peaks were deduced from their retention times relative to standards. The concentration of these alkanes were calculated relative to the internal standard (C_{34}).

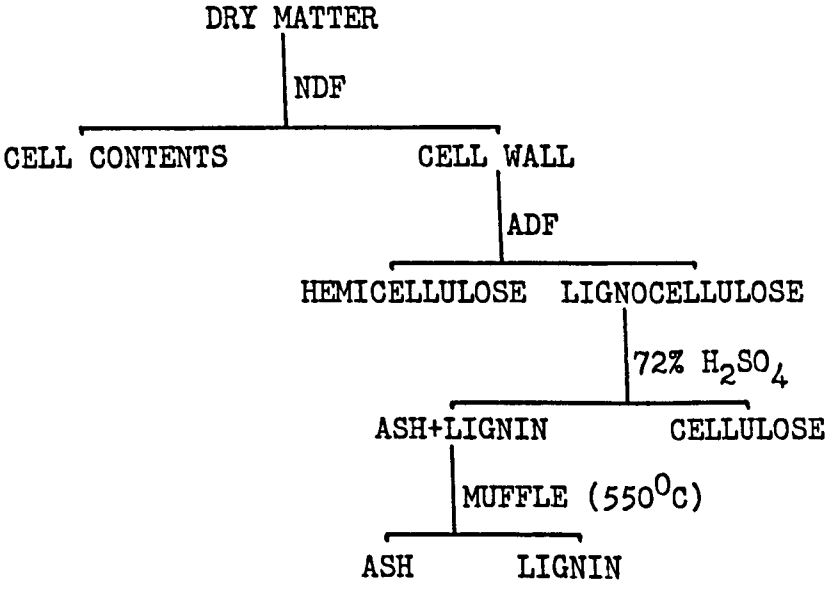
The alkane contents of the dosed pellets were determined using the same methods, described above, after Soxhlet extraction of whole, perforated, pellets with petroleum spirit

(B.P. 40-60°C).

8.2.3 **FIBRE ANALYSIS**

Fibre fractions of forage were determined using the method of Halliday (1985) (figure 18) and the modified acid detergent method (MAFF, 1979).

Figure 18: Fibre fractionation scheme



8.2.3.1 **MATERIALS**

Neutral detergent : 37.2g of disodium EDTA, 13.6g disodium tetraborate and 60g of sodium dodecyl sulphate were dissolved in approximately 1 litre of warm water. 9.12g of disodium hydrogen phosphate (anhydrous) were added to 20 ml of 2-ethoxy ethanol in 500 ml of warm water. The two solutions were mixed and made up to 2 l. The pH was adjusted to 6.9-7.1.

Acid detergent : 20g of cetyltrimethylammonium bromide (CTAB) were dissolved in 2l of 0.5M H_2SO_4 (0.53ml conc. H_2SO_4 in 1.5l water made up to 2l)

72% sulphuric acid: 403 ml of conc. H_2SO_4 added to 274 ml water.

8.2.3.2 METHODS

Approximately 0.5 g of dry samples of forage and faeces were added to glass filter crucibles (porosity 1, Corning) and their weight recorded. To determine the NDF, 0.25g of sodium sulphite was added to the sample and the loaded crucible was placed in a 100ml beaker containing 50ml of ND reagent. The beaker was covered, using polythene film secured with a silicone rubber band, and incubated overnight at 90°C.

After incubation the residue was washed four times with near boiling water and twice with acetone (B.D.H.). The outside of the crucible was also rinsed. The crucible was dried for two hours at 90°C, allowed to cool in a dessicator, and weighed. The crucible and the residue were then placed in a 100ml beaker containing 50ml of AD reagent and the protocol repeated as for ND digestion. After cooling and weighing, the AD residue in its crucible was placed in a 100ml beaker containing 50 ml of 72% H_2SO_4. The beaker was covered, using polythene film secured with a silicon rubber band, and incubated for one hour at 25°C. The residue was washed thoroughly with cold water and rinsed with acetone before drying and weighing. Ash was determined after heating the crucibles in a muffle furnace at 550°C overnight. The crucibles were allowed to cool and then weighed.

Modified acid detergent fibre (MADF) was determined by the method described by MAFF (1979). The lignin content of the residue was determined using the method described previously.

8.2.4 ESTIMATION OF INTAKE AND DIGESTIBILITY

Herbage and silage intakes were calculated from the ratios of C_{32}:C_{31} and C_{32}:C_{33} using the formula of Mayes and others (1986) (figure 19). Herbage intakes were also estimated, for the grazing cows only, using C_{36} as an external marker for faecal output estimation (LeDu et al.,1982). The excretion pattern of the dosed alkanes indicated that equilibrium was reached by 7 days from the start of dosing. Digestibility of the forage was estimated from the forage and faecal concentrations of C_{31} and C_{33} alkanes corrected for the digestibility of the alkane (Table 8.1).

Figure 19: formula for intake determination from the alkane concentrations of forage, faeces and pellets

$$\text{Herbage intake} = \frac{\dfrac{(F_i)}{(F_j)}}{\dfrac{(D_j)}{H_i}}$$

H_i- natural odd chain alkane conc. (mg/kg DM) -Herbage

F_i- -Faeces

F_j- even chain alkane concentration (mg/kg DM) -Faeces

D_j- amount of alkane, j, dosed by pellet (mg/day)

8.3 THE EFFECT OF METHOD OF DOSING n-ALKANE TO SHEEP ON THE ESTIMATED FEED INTAKE AND THE DIURNAL EXCRETION AND DIGESTIBILITY OF ALKANES

8.3.1 INTRODUCTION

The use of the n-alkane marker in feed intake determination for sheep (Mayes et al., 1986) has made the estimation of individual intakes and digestibilities a practical possibility. Established methods for the estimation of feed intake are not sufficiently accurate for such individual determinations (Cordova et al., 1978). The efficacy of the n-alkane technique was tested with respect to the accuracy of the individual feed determinations, the diurnal excretion of the alkane marker and the method of marker delivery.

8.3.2 MATERIALS AND METHODS

Six Welsh Mountain ram lambs were placed in metabolism crates. After a seven day acclimatization period the daily silage allowance was reduced to 75% of the individual ad lib. intake to ensure refusals were minimised. The animals were dosed in a latin square design experiment with the dosing periods being separated by two weeks and two replicates per dosing method. The C_{32} was administered by three routes; dosed as pellets containing impregnated filter paper; incorporated into the silage on impregnated filter paper or incorporated into the silage as flakes of the pure compound.

Samples of faeces were collected at 9.00 am and 4.00 pm with daily total faecal collections. Samples of the offered silage and

the collected faeces were stored frozen.

8.3.3 RESULTS

The stored samples of faeces and food were irrecoverably damaged when the freezer in which they were stored failed in operation. No results were therefore, obtained from this experiment.

8.4 THE APPLICATION OF THE n-ALKANE MARKER FOR INTAKE ESTIMATION TO WELSH BLACK CATTLE IN A GROUP FED AND HOUSED PERFORMANCE TEST

8.4.1 INTRODUCTION

The work of Mayes and others (1986) has validated the use of n-alkane markers for intake determination in sheep. In this study the marker was used to determine forage intake in cattle in a situation analogous to performance testing, where intake measurements have more usually been achieved using individual feeding stations.

8.4.2 METHOD

Four cattle, 2 bulls and 2 heifers, were selected from a group of 10 housed Welsh Black yearling cattle. The selected animals were each given 1 paper pellet containing C_{32} alkane daily; for 2 periods of 10 days during January to Febuary.

Alkane dosing and faecal collections were performed in the morning between 9 and 10 am. The animals were restrained in a crush and the pellets administered with a standard cattle bolus gun. Grab samples of faeces were taken and the animals weight recorded at the same time.

A diet of ad lib. silage supplemented with a rolled barley/soya concentrate was available to all the animals in the housed group, including animals not in the trial, through a barrier fence. Samples of the late cut arable silage and the concentrate offered were collected. All feed and faeces samples were stored frozen until required for analysis. The alkane content of the pellets,

diet and faeces were determined using the methods described previously. The interval between dosing periods was 4 weeks.

8.4.3 RESULTS

The collected samples of faeces and silage were stored in a freezer which failed. Consequently analyses were not able to be performed on all samples.

There were no differences in the fibre contents and feeding value of the offered silages (Table 8.6). The alkane contents of the silages were significantly different (Table 8.7) and the estimation of silage intakes were calculated using the appropiate alkane concentration. Feed conversion efficiency was calculated for each animal for the two dosing periods using combined mean growth rate for both the periods (Table 8.4).

There were no differences between the C_{32} alkane contents of the pellets used in the two dosing periods (305 cf 314 mg C_{32}; Table 8.8). No measurable concentration of alkane was found in the lipid fraction extracted from the barley/soya concentrate. The mean estimates of silage intake for each animal in each of the two dosing periods ranged from 7.83 to 10.18 kg DM (Table 8.2) and, in relation to their metabolic liveweight (MLW), 91.8 to 158.3 g/kg MLW (Table 8.3). The digestibility of the silages was calculated from the concentrations of C_{31} and C_{33} in the silage and faeces and corrected for the mean digestibility of the alkane (Table 8.1). The resulting digestibility coefficients are shown in table 8.5 for each animal.

8.4.4 DISCUSSION

It was possible to estimate the herbage intake of individual animals using the n-alkane technique and consequently estimate the FCE of the animals. This estimate does not allow for the small concentrate intake of the animals but is sufficient for comparative purposes. The calculated FCE are consistent with those calculated for Welsh Black cattle by Southgate and others (1982), who calculated the mean FCE to be 8.8% with winter housed animals gaining 0.72 kg/day. The decline in intake between the two dosing periods, separated by 37 days and approximately 30 kg of growth, cannot be explained. There was no difference between the quality of the silage samples, although the alkane content was significantly lower in the first period (Table 8.7). It is possible that increased amounts of concentrate were consumed with a concurrent reduction in silage intake. The estimated values of herbage intake per kg of MLW agreed with those observed by Bruckental and others (1987) where only slightly larger animals to those in the present study were used (mean 364.2 cf 283.4 kg). Bruckental and others (1987) dosed their animals with magnesium ferrite ($MgOFe_2O_3$) and estimated that 81-131 g/kg MLW of herbage were consumed when grazing pasture. The present study observed a range in intake of 91.8-158 g/kg MLW.

The estimated mean digestibility coefficients of the silage and the estimated intakes were used to verify the result (MAFF, 1977), using mean values: body weight=283.4 kg; digestibility of silage=50.2%; daily weight gain=0.45 kg/day. The daily metabolisable energy requirements were 43.45 MJ. The ME supplied

by an intake of 8.86 Kg DM, was 56.42 MJ. These figures do not take account of the concentrate intake of the animals which could not be measured. Despite this, the calculated estimates of silage intake and digestibility as determined from the n-alkane analyses were reasonable.

Table 8.2: Mean daily silage intake of bulls and heifers calculated from the ratios of C_{32}:C_{31} and C_{32}:C_{33} in silage and faeces.

Animal	Body wt(kg)	Intake (kg DM/Day) C_{32}:C_{31}	C_{32}:C_{33}	Mean(SEM)
		First dosing period.		
176	299.6	9.82	10.54	10.18 (0.39)
183	264.6	8.76	9.65	9.20 (0.69)
186	263	10.84	9.84	10.34 (0.76)
181	276.1	8.64	7.01	7.83 (1.0)
		Second dosing period.		
176	308.3	6.75	6.76	6.76 (0.01)
183	278.5	7.0	7.66	7.33 (0.33)
186	282	11.69	11.54	11.44 (2.3)
181	297	7.61	7.77	7.69 (1.2)
Between animals P=		0.013	ns	0.0427
Between periods P=		ns	ns	0.0495

Table 8.3: The relationship between metabolic liveweight and silage intake

	Period 1			Period 2		
Animal	Body wt	$wt^{.75}$	gDM/MLW	wt	$wt^{.75}$	gDM/MLW
176	299.6	72.0	141.3	308.3	73.6	91.8
183	264.6	65.6	140.2	278.5	68.2	107.5
186	263.0	65.3	158.3	282.0	68.8	167.7
181	276.1	67.7	115.7	297.0	71.5	107.6
		c = -0.604			c = -0.240	

Table 8.4: Feed conversion efficiency (%) of the dosed animals

	Period 1	Period 2
176	3.93	5.92
183	6.74	5.42
186	5.32	7.50
181	9.58	9.75

P between periods = ns
P between animals = ns

Table 8.5: Digestibility of silage, for the two dosing periods, calculated from the concentration of C_{31} and C_{33} in the silage and faeces for each animal

Mean digestibility of silage (%)

	Period 1		Period 2	
Animal	C_{31}	C_{33}	C_{31}	C_{33}
176	51.9	48.9	49.4	42.7
181	40.7	45.9	53.1	47.1
186	56.3	40.7	48.5	43.6
183	52.3	53.2	51.6	49.2
Overall	50.0	47.4	50.5	45.21
SEM	3.0	3.3	3.0	2.7
P animals =	ns	ns	ns	ns
P periods =	ns	ns	ns	ns
P animals x period =	ns	ns	ns	ns

Table 8.6: Fibre content and feeding value of silages from the two dosing periods.

	n	Cell contents (MADF)	Cellulose	Lignin	Ash	DM	ME
Period 1	3	35.95	28.95	6.55	0.75	30.4	10.3
Period 2	4	37.82	30.36	6.86	1.26	19.0	10.2
Overall		37.29	29.96	6.77	1.11	20.0	10.2
SEM		1.4	1.2	1.46	0.31	1.5	0.5
P =		ns	ns	ns	ns	ns	ns

Table 8.7: Alkane content of silage

	n	Mean content (mg/kg DM) C_{31}	C_{33}
Period 1	10	30.49	9.14
SEM		2.88	0.96
Period 2	4	45.69	11.5
SEM		2.43	0.6
P=		0.006	0.105

Table 8.8: Alkane content of the dosed pellets (C_{32} mg)

Period	n	Mean (SEM)
Month 1	4	305 (28)
Month 2	5	314 (40)
P=		ns

Figure 20: Formula for the calculation of ME from oven-dried silage

$$ME = 0.16 \left(99.6 - 1.04 \left(\frac{MADF(\%) \times DM(\%)}{(DM(\%) + 1.9)}\right)\right)$$

(Chamberlain, pers comm)

8.5 ESTIMATION OF THE HERBAGE INTAKE AND DIGESTIBILITY OF HERBAGE BY SUCKLING WELSH BLACK COWS AT PASTURE.

8.5.1 INTRODUCTION

Foraging ability and digestive efficiency are important components of beef cattle performance. However, food intake and its digestibility are difficult to estimate in individual grazing cattle and consequently are not included in the assessment of cattle performance. The methods used for intake estimation of grazing cattle are compromised by several factors eg. diurnal variation in marker excretion (LeDu et al., 1982). n-Alkane markers (Mayes et al., 1986) have been suggested as providing greater accuracy in intake determination and enable the digestibility of the diet to be estimated without recourse to invasive techniques or in-vitro studies. n-Alkanes are being evaluated for intake estimation in a variety of species (Lamb, S. pers. comm.) including camelids (Moseley, G. pers. comm.).

An additional investigation was performed into the use of neck mounted pedometers to assess the grazing behaviour of Welsh Black cows. The high capital and labour costs associated with grazing behaviour research has limited its use in animal evaluation. However, the pedometer, when fitted to the neck of an animal provides an inexpensive and easily interpreted monitor of grazing activity. This system has successfully been applied to the measurement of dairy cow grazing behaviour. Phillips and Denne (1988) found a positive correlation between pedometer reading and grazing time. This experiment will evaluate the application of this method to beef cattle.

In this study the n-alkane marker system was used to estimate the intake and digestibility of herbage by grazing cows which were also equipped with neck mounted pedometers to monitor their grazing behaviour.

8.5.2 METHOD

Four suckling cows were selected for an evaluation of the n-alkane marker for intake estimation in grazing cows. The cows were dosed with C_{32} and C_{36} alkanes daily in pellets as described previously.

The cows were fitted with pedometers by the method described by Phillips and Denne (1988). The daily readings were recorded each morning at 9-10 am for 16 days.

All dosing and collections were performed in the morning between 9 and 10 am. The animals were restrained in a crush and the pellets administered with a standard cattle bolus gun. Grab samples of faeces were taken at the same time. Hand plucked samples of pasture were collected in a 'W' pattern from each of the 3 fields available to the cows, for 3 days mid dosing cycle. At the same time measurements of herbage height were taken using a falling plate reader (Kibon and Holmes, 1987). Faecal and forage samples were stored frozen before being dried at 60°C in a circulating air oven, and milled through a 1 mm screen.

The alkane contents of the samples were determined as described before (section 8.2.3).

The intakes of the cows were calculated by 2 separate procedures. In the first the method and formula of Mayes and

others (1986) was used (section 8.2.5). In the second C_{36} was assumed to be completely recovered in the faeces (Table 8.1) and could therefore, be used as a faecal marker to estimate total faecal output. Intake was determined from this and the estimate of digestibility calculated from the herbage concentrations and faecal concentrations of C_{31} and C_{33}, corrected for the digestibility of the alkanes (Table 8.1).

8.5.3 RESULTS

The collected samples of faeces and herbage were stored in a freezer which failed. Consequently analyses were performed on the limited number of samples which were recovered.

The estimates of herbage intake calculated for individual cows ranged from 23.7 to 33.2 kg DM/day (Table 8.9). The herbage digestibility coefficients calculated from the corrected concentrations of C_{31} and C_{33} in the herbage and faecal samples, and the herbage intake of individual cows determined using these estimates and the faecal concentration of C_{36}, are shown in table 8.10. The two estimates of intake were correlated (c=0.998). The herbage intake of individual cows is expressed in relation to the animals' metabolic liveweight in table 8.11 and ranged from 194.7 to 326.8 g/kg MLW . The fibre content, sward height and alkane contents of the three fields were not significantly different (Table 8.13). The n-alkane content of the pellets was 355 mg (SEM=22) of C_{32} and 316 (20)mg of C_{36}. The differences in alkane content between the pellets were not significant.

The mean pedometer reading ranged from 729 to 1005 (Table 8.12). Significant differences were observed between cows. The

mean pedometer readings were correlated to the estimated mean daily dry matter intakes (c=0.786 for mean $C_{31,33}$:C_{32} and 0.952 from the faecal recovery of C_{36}). There was no relationship between pedometer reading and cow bodyweight.

8.5.4 DISCUSSION

Significant differences in herbage intake between the cows could be discerned in the herbage intakes calculated from the recoveries of C_{32}. The results obtained from the n-alkane dosing in this study would have allowed discrimination between the dosed animals.

The alkane concentration and the fibre fractions of the herbage did not vary between the three fields to which the cows had access. One animal, K48, consistently appeared to digest the herbage to a lesser extent than her fellows (Table 8.10). All the cows digested the herbage to a greater extent than would be anticipated from the fibre content of the available pasture (MAFF, 1986). This may have been due to selection within the sward. However, the sward heights of the pasture (Table 8.13) were below the minimum of 7 cm suggested to maintain suckling beef cows (Wright and Whyte, 1989) and so selective grazing was probably not a factor.

The herbage intakes of the cows revealed one animal (J32) which was consuming more than the other cows both in gross intake and in intake per unit of metabolic liveweight.

Animals with the largest food intakes possessed the highest pedometer readings, suggesting that they grazed more actively

than animals with lower food intakes. This method of grazing and intake evaluation offers new possibilties for assessing grazing behaviour and foraging ability in extensively managed cattle.

The estimated herbage intakes expressed in relation to the metabolic weight of the cows (Table 8.11) were higher than expected from most published accounts. Cordova and others (1977) reported a range of 40-60 g DM/$kg^{0.75}$. Bruckental and others (1987) measured a dry matter intake of 6.8 to 13.6 kg and a intake of 81.4 to 131.8 g/kg MLW with cows grazing pasture. Hyder and others (1968) published results similar to those found in the present study (135-204 cf 194-326 g DM/$kg^{0.75}$). The intake of dry matter expressed for a mean animal in this study, 643.3 kg body weight and an intake of 28.3 kg DM, provides approximately 249 MJ ME/Day, the digestibility of the pasture being 74.12 (MAFF, 1977). The maintenance requirement would be 66 MJ per day. Thus there would be an energy surplus, available for production, of approximately 183 MJ. Assuming a milk yield of 10 litres a day the cows would be gaining 5.8 kg each day. Whilst these are average figures, there has been an overestimation in the intake of the cows. This could be attributed to diurnal variation in the excretion of C_{32} and/or C_{36} producing low marker concentrations in the faeces at sampling. Recent reports have suggested that alkanes exhibit cyclic variation in excretion in cattle (Dillon and Stakelum, 1989).

The methods investigated in these studies cannot be considered ideal for selection purposes. The number of days of recording in order to detect a true 5% difference from the mean

were calculated for each method using the least significant difference equation for unequal numbers of replicates (Whitaker, pers. comm.). The numbers of days were 41.2 and 52 for the housed and grazed animals respectively. The number of days of pedometer recording were 230. To detect a difference of 10% from the mean with 95% probability the numbers of days of dosing or recording was estimated to be 7, 12 and 46 respectively. These figures reflect the within animal variation which was found with these methods of intake assessment.

Table 8.9: Herbage intake of cows estimated from herbage and faecal concentrations of alkanes.

Cow	Body wt(kg)	Herbage intake(kg DM/ Day) $C_{32}:C_{31}$	$C_{32}:C_{33}$	Mean (SEM)
K31	587.5	28.1	33.8	31.2 (1.2)
K48	660	23.7	30.9	27.3 (3.6)
J11	682.5	35.7	45.4	42.9 (4.6)
J32	573.4	32.9	45.7	42.5 (0.2)
	Overall	31.4	41.0	38.2
	SEM	6.1	4.0	4.2
	P=	ns	0.06	0.07

Table 8.10: Intake calculated from the faecal recovery of C_{36} and the digestibility of herbage estimated from C_{31} and C_{33} alkanes.

Cow	Mean digestibility of herbage (%) C_{31}	C_{33}	Intake (kg DM/Day)
K31	79.01[a]	82.33	25.37
K48	68.21[b]	77.13	20.8
J11	78.32[a]	83.43	37.1
J32	74.92[a,b]	77.13	38.3
P=	0.005	0.095	

values in the same column, with differing superscripts are significantly different at the 5% level

Table 8.11: Relationship between metabolic liveweight and intake (using C_{32} concentration).

Cow	wt	$wt^{0.75}$	intake (g DM/MLW/Day)
K31	587.5	119.3	231.3
K48	660.0	130.2	194.7
J11	682.5	133.5	240.2
J32	573.4	117.2	326.8

c = -0.642

Table 8.12 Mean pedometer readings

Cow	No. of days	Mean (SEM) Pedometer counts
K31	16	729 (58)[a]
K48	16	756 (88)[a]
J11	16	1005 (39.4)[b]
J32	15	989 (46)[a,b]
	P=	0.001

values with differing superscripts within the same column are significantly different at the 5% level.

Table 8.13: Fibre and alkane content and sward height of the available pasture.

Field	n	Mean (SEM) (g/kg DM) Cell contents	Hemi+Cellul.	Lignin
1	4	311.8 (13.3)	428.6 (6.7)	192.2 (12.2)
2	5	349.5 (45.5)	396.5 (30.5)	220.7 (14.2)
3	2	362.3 (76.0)	389.6 (62.6)	206.9 (10.4)
	P=	ns	ns	ns

		(mg/kg DM) C_{31}	C_{33}
1	9	38.88 (3.56)	24.12 (2.6)
2	4	44.82 (7.11)	32.92 (6.0)
3	2	41.0 (23.0)	21.78 (8.1)
P=		ns	ns

		Mean sward height (cm)
1	23	4.41 (0.27)
2	39	3.88 (0.12)
3	24	4.04 (0.21)

CHAPTER 9

GENERAL DISCUSSION

9.1 INTRODUCTION

The objectives of this study were i) to develop and apply methods of multiple ovulation, embryo recovery and embryo transfer with Welsh Black cattle, and ii) to evaluate a novel method of intake measurement for use in the selection of superior, efficient Welsh Black animals.

9.2 THE WELSH BLACK GROUP BREEDING SCHEME

The Welsh Black nucleus herd, Haulfryn, was started in 1978 in parallel with the establishment of similar schemes for Lleyn and Welsh Mountain sheep. The sheep Group Breeding schemes have been better received by farmers because of the lower individual value of sheep compared to cattle, the higher profitability of sheep enterprises compared with cattle in the last decade and the logistical difficulties of transporting cattle.

Open nucleus schemes achieve the greatest gains when 10% of the base population is in the nucleus and when half the replacement females arise from the base (James, 1977). The movement of mature cattle inferred by these parameters, especially with the wide geographical distribution of base herds in the Welsh Black scheme, would incur large transport costs and would be impractical. Additionally, persuading the base herds to release their best individuals to the nucleus can be difficult.

Whilst several reports have been published on the benefits of MOET schemes in reducing generation intervals and increasing the selection intensity on females (eg. Nicholas and Smith, 1983) the modus operandi of the proposed scheme in the Welsh Black

nucleus was to surmount practical problems encountered in operating an open nucleus. Thus MOET techniques were be used to allow the base herds direct access to the genetic material, in the form of embryos, from the nucleus, rather than semen which is of more limited application. Such a scheme would also enable the nucleus herd to acquire progeny from superior animals in the base herds without withdrawing them from the farms. Additionally the nucleus would act as a genetic repository for the entire breed by the introduction of embryos from herds outside the scheme.

As there was no expertise in this area in U.C.N.W., the initial studies were directed to the practical adoption of the techniques from published reports and personal communications. Accordingly the first attempts at superovulation and embryo recovery were applied to dairy and beef heifers. These animals proved unsuitable, possessing very small cervices which could not be passed by the introducer. The animals were not reliably superovulated. It was decided that resources would be more usefully employed into investigations on Welsh Black cows.

The first collections of Welsh Black embryos were from four cows C1, C3, C11 and A79, selected on their index scores (MLC). Adequate embryo recovery rates were achieved, 44.8%, indicating that the positioning of the catheters and the recovery of instilled fluid were satisfactory. The low embryo quality permitted only three transfers, none of which continued development. The selection indices are based on the 200 day weight of the calves. To demonstrate obvious genetic merit the cows have had to produce several superior calves and these high index cows were multiparous and among the oldest in the nucleus.

The succeeding animals in the programme were mainly drawn from the first and second calvers in the herd. This expanded programme allowed more meaningful investigations to be performed on younger animals.

9.3 DETECTION OF OESTRUS IN SUCKLING BEEF COWS

The long anoestrous associated with suckling beef cows (Tervit et al., 1977) reduced the time available to perform embryo collections while still maintaining the treated animals in the spring calving period of the nucleus. The intention was to flush the early calving animals once and then to return them to the herd. The later calving animals would be repeatedly flushed and returned to the herd the following year.

A major determinant of herd fertility is the detection of oestrus (Schofield, 1988). This is particularly true of beef herds where, in comparison to dairy herds, the labour input, and hence the time available for oestrus detection, is reduced. The desire to retain the calving pattern of the nucleus herd necessitated the detection of the first oestrus post partum. This occurs approximately 60 days after calving in suckling beef cows (Tervit et al., 1977) and has been reported to be influenced by season of calving (Peters and Riley, 1982b).

The selected animals were monitored for milk progesterone concentration, and pedometers were to have been used to monitor physical activity. The pedometers had been validated previously for oestrus detection in dairy herds when attached to the hind leg (Schofield, 1988). However attempts to fit pedometers

similarly failed, and it was considered that the physical risks involved were not justified.

The interpretation of the results obtained from the milk progesterone assays were equivocal. It has been suggested that the variation in fat content of milk samples may affect the assay (Claus et al., 1978). The kits are designed for use in dairy herds where representative homogenous milk samples are easily obtained. The samples obtained by hand milking in this study had variable milk fat concentrations. Intersample variation could have been reduced by performing the assay on defatted milk but the manufacturers of the assay, when approached, were unable to provide suitable information. It has been reported (Holdsworth et al., 1980) that milk fat variation is not a major source of error in milk progesterone assays for dairy cows however similar results have not been reported for beef animals. The progesterone profiles obtained exhibited the characteristic surges in concentration which precede the first oestrus and were related to observed oestri.

The vaginal probe was considered to reduce the physical risks to the operator and produce results which were simple to interpret. The cows used for this trial were undergoing superovulation which took precedence over the probe evaluation. Consequently when indications of vaginitis occurred, the measurements ceased. Despite this, successful detection of oestrus was achieved by the probe. The advantage of the probe over the progesterone assay was that it produced rapid results. LH surges have been monitored using a vaginal resistance probe to identify optimal insemination time (Canfield and Butler, 1989)

and this may be useful in breeding superovulated cattle. However, the oestrus detection method requires validation, especially with respect to the detection of first oestrus post-partum which is essential for MOET methods to be employed, whilst retaining the calving patterns, to beef herds.

The milk progesterone assay kits and the vaginal probe were able to identify oestrus cattle but the labour required and stress incurred in their execution did not support their adoption over repeated observations. The progesterone kits may be useful in identifying animals which are approaching first oestrus post partum which cannot be predicted from observations.

9.4 ENDOCRINE PATTERNS IN SUPEROVULATED COWS

It was hoped elucidation of the hormonal patterns would help explain the poor embryo quality phenomenom. Thus repeated samples of blood were taken, from five cows, through indwelling jugular catheters for hormone assay. This procedure had not been used at U.C.N.W. previously and several problems were encountered with maintaining the catheters. The byre was unsuitable as there was no front access to the animals. All feeding and sampling had to be carried out in the area between adjacent animals. One atavistic individual was returned to the herd. The remaining cows were unfamiliar with close human contact and the process of blood sampling caused them obvious distress. One cow, in oestrus, escaped her chain and removed her catheter and those of two others. Consequently complete records could not be obtained for all the cows.

The entire hormone profiles which were obtained suggested that the luteinizing effect of the hCG prevented further follicular development as no secondary surge in oestradiol concentration was observed. A small secondary increase in LH concentration could be discerned but there was no associated increase in oestradiol concentration. Biphasic secretion of both LH and oestradiol was observed in the control hormone profile.

The administration of hCG had no effect on the ovarian responses nor on the production of embryos but it did increase progesterone secretion. This latter observation has been repeated, with a corresponding improvement in conception rate, by Breul and others (1989). Very few embryos were recovered in this series of collections, especially from the supplemented diet animals, and any advantage in administering hCG may have been missed.

9.5 FEED SUPPLEMENTATION

The supplementation of the donors winter diet with a high mineral, protein and vitamin concentrate did not improve their superovulatory responses. The ovulation rates of treated animals were decreased as were the numbers of embryos and the numbers of viable embryos when compared to donors fed the standard diet. The silage fed to the standard and supplemented diet animals was a low quality, third cut grass and arable silage. Such forage represents the usual winter diet of suckler beef cows. Further analysis of data has revealed that there were seasonal variations in the responses to superovulation in the Welsh Black

cattle used in the present study (section 9.7.1). The pattern of response observed indicates that an improvement in embryo viability would be expected in cows superovulated in early spring. Thus, the effect of blood sampling and, to a lesser extent, housing may have stressed the cows fed supplemented diet and prevented the normal seasonal pattern being expressed. Whilst these stresses were caused by experimental procedures and would not be encountered in farm situations, the superovulation protocol may be less successful in cattle unused to handling and human contact.

9.6 RESPONSES TO SUCKLING AND WEANING

The low numbers of viable embryos recovered in the first embryo collections could not be explained on the basis of existing knowledge. Discussions with other workers suggested that the nursing status of the donor may influence the viability of the recovered embryos. However, when the calves were removed from the donors, 2 weeks before gonadotrophin administration, no improvement in superovulatory response was detected over the previous superovulation. The interval between superovulations was approximately 2 months. Either the dry period was too short or the superovulatory responses of a donor were not affected by its nursing status.

An expanded investigation (section 6.3) into the effect of suckling on the viability of recovered embryos found a significant improvement in embryo quality when cows were dried off. This experiment also confirmed the seasonal variation in

embryo quality observed previously. The group of cows (Group 3) flushed once only in the autumn, to assess the effect of postpartum interval to superovulation, showed no differences in their responses to the groups treated twice.The variation in embryo quality which was influenced by season and by the nursing status of a donor has important implications for the application of MOET methods by farmers. The seasonal decline in embryo quality indicates that cows selected as donors should be flushed in early spring. As suckling beef herds in the U.K. are either spring or autumn calving, a change in calving pattern will be required which may be impractical. Similarly, early weaning of calves to improve embryo quality may meet with farmer resistance. The desire to assess mothering ability has prevented the acceptance of early weaning for animals to be performance tested (Simm et al., 1985) and similar reservations could apply in this circumstance.

9.7 EMBRYO PRODUCTION

In this study the four catheter types used were equally effective for non-surgical embryo collection. This observation must be viewed in relation to practical considerations. The catheter type used was usually decided after rectal examination of the uterus. Many of the donor animals possessed flaccid, extended tracts in which some catheter types could not be positioned correctly.

Cervix diameter measurement could prove useful in eliminating animals with cervices too small for non-surgical embryo recovery and transfer. Normally such cattle would be

examined, per rectum, for abnormalities of the reproductive tract before treatment. The caliper measurement of cervix diameter, as part of this examination, would allow the penetrability of the cervix to be estimated without the risk of introducing infection by a test with a probe.

9.7.1 THE EFFECTS OF MONTH, METHOD OF BREEDING, METHOD OF OESTRUS SYNCHRONISATION AND PARITY ON OVARIAN RESPONSES AND EMBRYO PRODUCTION FROM WELSH BLACK COWS

Multiple ovulation and embryo collection were first applied to cows in the Welsh Black Group Breeding scheme in 1985. Retrospective analysis of the data collected during three years of study is presented below with the objective of evaluating those factors which affect the production of embryos from superovulated Welsh Black cows.

A total of 27 Welsh Black cows had been superovulated and as a group a total of 60 embryo collections had been carried out on them. Animals which had had their calves weaned early (section 6.3) were not included in the analysis. For each superovulation the animals had been treated to provide a reference oestrus using two methods of oestrus synchronisation, either PRID or PG. Those animals treated with a PRID and PG were grouped with those receiving a PRID alone. Superovulation was induced in the manner described previously. The donors had been bred by three methods, by AI timed from the injection of PG (fixed time), by AI timed from observed oestrus (detected time) or by natural service

(bull). Embryos had in all cases been collected seven days after mating. The ovulation rate of the cows had been estimated from the average of 2 independent estimates of ovarian stimulation per rectum and the embryos recovered had been assessed using previously defined criteria. The data collected were analysed with month, parity, method of breeding, method of oestrus synchronisation and flush as fixed effects.

Table 9.1 summarises the data from the total of 60 flushes. These are presented in relation to the month in which they were performed. Eighteen flushes were carried out which produced no embryos and therefore no embryo quality data. In figure 21, the embryo quality data for the 42 embryo-yielding flushes is presented separately for each of the three years. There was a significant variation in embryo quality throughout the year, with particularly poor quality being obtained in the Autumn. This was a consistant observation for each of the 3 years studied. The mean ovulation rate was estimated to be 7.3 and the mean number of embryos recovered was 3.7. This represents an overall recovery rate of 50.7%. The mean number of embryos recovered which were classified as good was 1.2, giving a mean percentage of good embryos of 32.5%. There was an annual increase in the percentage of embryos recovered, 37.1; 48.6; 55.8%, estimated from the assessed ovulation rate, but the difference between years was non-significant.

The method of breeding had no influence on embryo production (Table 9.2) neither did the method of oestrus synchronization influence the superovulatory response (Table 9.3).

Ovulation rate and the number of embryos recovered were significantly affected by the number of donor parities. The percentage of embryos recovered was not influenced by the donors number of parities (Table 9.4). The effect of donor parity was not consistant with published work, where better responses were achieved with younger cattle. The present study observed better responses with cattle with 4 and 8 parities. However, the numbers of cattle used in the study were small, biased towards first and second calvers and the underlying pattern of variation may have been obscured.

Significant monthly variations were detected, for the 32 flushes for which there was suitable data, in the numbers of unfertilized embryos (P=0.086) (figure 22). There were indications of monthly effects on the numbers of good embryos (P=0.109) and on the percentage of unfertilized embryos (P=0.103) in this dataset. The numbers and percentage of embryos which were fertilized and failed to develop were unaffected by month.

Histograms are presented showing the ovulation rate observed for each cow at each superovulation and the numbers of embryos recovered at each flush (figures 23 and 24). Gordon (1983) considered that successful superovulation had to induce 3 or more CLs. On this basis, 26.4% of the Welsh Black cattle treated in the present study did not respond to superovulation. The incidence of none or one embryo being recovered was 36.1%, and the incidence of none, one or two embryos being recovered was 43.1%.

The success of superovulation and embryo recovery from

Welsh Black cattle was less than expected for other breeds (Seidel, 1981; Iritani, 1988) or theoretically required for genetic improvement (eg. Nicholas and Smith, 1983; Land and Hill, 1975). The overall mean number of good quality embryos recovered per flush was only 1.2. The pregnancy rate following embryo transfer is currently reported as 83% (Wagner, 1987). The majority of the viable embryos in this study were not transferred to recipients but were frozen, so no reliable figure for success rate is available. However, assuming the quoted success rate could be obtained, 6 flushes a year per donor would produce on average 6.0 progeny per year. This compares with the 8 progeny per donor per 3 months suggested by Nicholas and Smith (1983) as being necessary. Repeated superovulation and flushing did not have any detrimental effects in this study, in broad agreement with previous workers, in that there was no indication of a decline in response. Ali Dinar and others (1987) superovulated cows up to 10 times and found the ovulation rate increased slightly. Christie, Newcomb and Rowson (1979) observed an initial decrease in ovulation rate but no further decline in subsequent treatments. In contrast, Lubbadeh and others (1980) and Halser and others (1983) found repeated superovulations had deleterious effects on fertilization and good embryo production. However, the interval between treatments may be significant in this aspect as Lubbadeh used 16 days intervals whilst Ali Dinar and others used 50 days. In the present study the mean interval was 101 d (std. dev.= 54).

Bindon and others (1986) suggested that more prolific breeds of cattle would respond better to superovulation than less

profilic ones. Dairy breeds of cattle have higher frequencies of twinning (Morris and Day, 1986) and higher responses to superovulation (Betteridge, 1977; Gordon, 1982) than beef breeds. The Welsh Black Group Breeding Scheme herd had a twinning frequency of 0.004 (Owen J.M., pers. comm.), which is comparable with that reported for other beef breeds (Morris and Day, 1986). This suggests that the ovulation rates achieved with Welsh Black cattle in the present study may also be applicable to other beef breeds when superovulated. If so, the value of MOET methods in beef breed improvement will require further appraisal. However, as the data on breed responses to superovulation increases, it may be possible to select for induced ovulation rate. This factor has been shown to be controlled by major genes in mice (Spearow, 1988)

Workers have differed in the methods used to mate superovulated cattle. Scanlon and others (1968) used natural service whilst Christie and others (1979) employed AI. In the present study initial trials used AI after a fixed time from PG injection. The adoption of a timed insemination scheme based on the observed time of standing oestrus (Donaldson, 1985) did not lead to a decline in the viability of recovered embryos and natural service was as successful as AI in the present study. These findings are necessary prerequsites for the practical implementation of multiple ovulation and embryo transfer methods for beef breeding herds where the extensive production environment precludes frequent observation for the detection of oestrus.

Analysis of the data shows that the superovulatory responses achieved were not affected by the synchronisation method used and consequently that successful superovulation requires only that a reference oestrus is determined.

Significant effects of age have been reported on ovulation rate in superovulated cattle (Moore, 1975; Newcomb et al., 1978) and on embryo recovery (Newcomb, 1980). No similar relationships were observed in this study although significant effects were detected for parity on ovulation rate and the numbers of embryos recovered.

The significant monthly variation of embryo quality found in this work has important implications for the application of a MOET scheme. Seasons are known to have significant effects on beef cow reproduction (Hansen and Hauser, 1983) but information on superovulated cattle is inconclusive. The dataset in the present study was not sufficently large to permit the removal of the effects of individual cows or the number of flushes on embryo quality. However the consistant effect of month (Figure 21), obtained from different samples of cows, each containing animals which were flushed either for the first or second time in the autumn supports the contention that the seasonal effect is a real one. Hasler and others (1987) examined the results from an ET unit in the eastern USA. No monthly differences were found in superovulatory response but there was a difference in pregnancy rate of the recipients, being highest in July and lowest in November and February. Other workers have described a seasonal effect on superovulation with the best superovulatory responses being recorded in summer and spring (Crister and others, 1979;

Lerner and others, 1986). However examples of conflicting data where there was no seasonal or monthly variation have also been published (Massey and Oden, 1984; Shea and others, 1984). There is strong evidence of a seasonal effect in hot environments which contrasts with the equivocal data for temperate climates. Superovulating European Dairy Cattle in the hot months of the year in Saudi Arabia was less successful than in the colder months (Almeida, 1987; Gordon and others, 1987).

The present study differs to previous work in that it relates to suckling beef cows of a single breed. The poorest embryo quality was found to occur in the early autumn/winter period when the cows were still suckling (weaning occurred at the start of January), feed quality was declining as winter rations were introduced, and daylight hours were reducing. Each of these factors may contribute to the observed effect.

Table 9.1: Mean superovulatory response and embryo production from superovulated Welsh Black cows classified by month.

Month	No. of flushes	Mean (SEM) CLs	Embryos recovered	Viable embryos recovered	% Viable embryos*	Embryo* recovery (%)
JAN	3	6.0 (2.7)	3.0 (1.7)	2.33 (1.9)	66.7 (33.3)	34.3 (17.8)
FEB	5	4.0 (1.8)	0.4 (0.2)	0.4 (0.2)	100.0 (0)	11.3 (6.6)
MAR	5	11.4 (2.4)	6.4 (1.3)	4.2 (1.4)	65.1 (15.7)	61.0 (14.4)
MAY	6	8.8 (3.9)	3.0 (1.2)	1.5 (0.8)	46.9 (21.9)	41.4 (17.4)
JUL	4	6.0 (1.1)	2.0 (0.4)	1.0 (0.7)	50.0 (28.9)	33.2 (1.8)
AUG	8	9.9 (2.5)	5.5 (1.9)	2.0 (0.7)	38.0 (11.0)	58.7 (12.3)
SEP	9	5.8 (2.2)	2.9 (1.3)	0 (-)	0 (-)	44.0 (14.2)
OCT	5	7.0 (2.8)	5.8 (2.6)	0.8 (0.8)	8.3 (8.3)	62.0 (17.4)
NOV	15	7.0 (1.4)	3.7 (1.2)	0.9 (0.5)	23.1 (11.0)	52.4 (11.3)
P=		ns	ns	0.02	0.006	ns
Overall		7.3	3.7	1.2	36.4	45.7
SEM		2.6	1.8	0.8	17.5	15.8

* calculated for each cow and then the mean taken

Table 9.2: Effect of breeding method on superovulatory responses

Breeding method	n	Mean (SEM) CLs	Embryos	Viable embryos	% Viable embryos*
Fixed time	13	4.3 (1.2)	2.1 (0.6)	0.4 (0.2)	21.4 (10.7)
Detected time	33	7.9 (1.1)	4.1 (0.8)	1.5 (0.4)	42.6 (8.4)
Natural service	14	9.3 (1.5)	5.1 (0.9)	1.8 (0.6)	34.7 (11.9)
P =		ns	ns	ns	ns

Table 9.3: Effect of reference oestrus synchronisation method on superovulatory responses

Synchronisation method	n	Mean (SEM) CLs	Embryos	Viable embryos	% viable embryos*
PRID	50	7.7 (0.9)	4.0 (0.8)	1.3 (0.3)	43.3 (20.8)
PG	10	5.6 (1.0)	2.5 (0.6)	1.2 (0.7)	43.3 (6.5)
P =		ns	ns	ns	ns

* calculated for each cow and then the mean taken

Table 9.4: Mean superovulatory response and embryo production from superovulated Welsh Black cows classified by their number of parities°.

Parities	No of cows	No of treat.	CLs	Embryos	Embryo recovery(%)*
1	2	8	2.3	0.75	33.9
2	16	33	8.3	4.6	56.0
4	5	11	11.4	5.3	47.5
6	2	5	2.8	1.2	60.0
7	3	8	5.1	2.1	31.9
8	3	4	9.3	7.5	42.5
9	2	3	6.7	4.0	61.8
P=			0.009	0.028	ns
Overall			7.4	3.9	50.1
SEM			2.24	1.7	15.3

° including cows from section 6.3

* calculated for each cow and then the mean taken

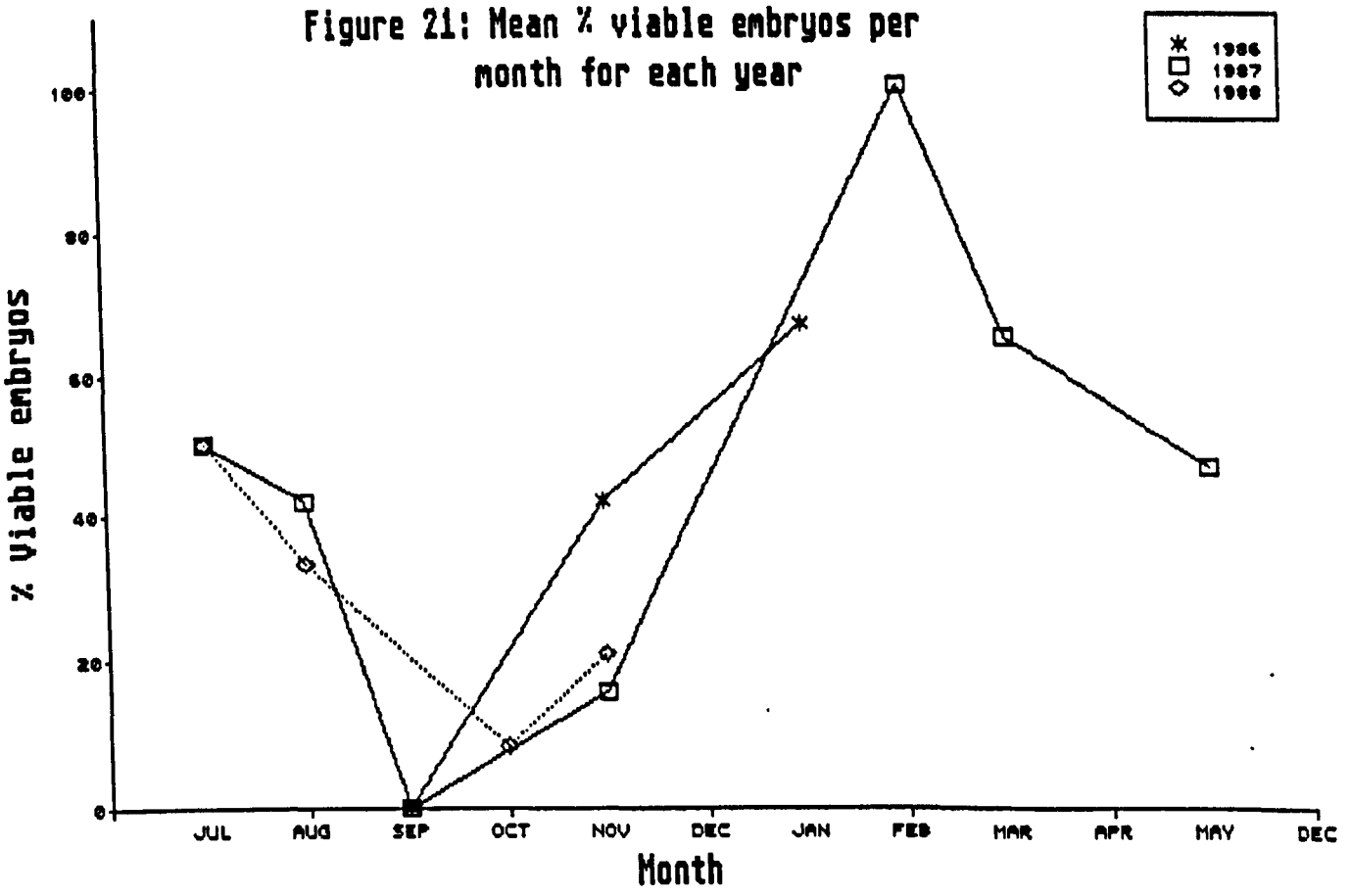


Figure 21: Mean % viable embryos per month for each year
1986
1987
1988
% Viable embryos
100
80
60
40
20
0
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
DEC
Month

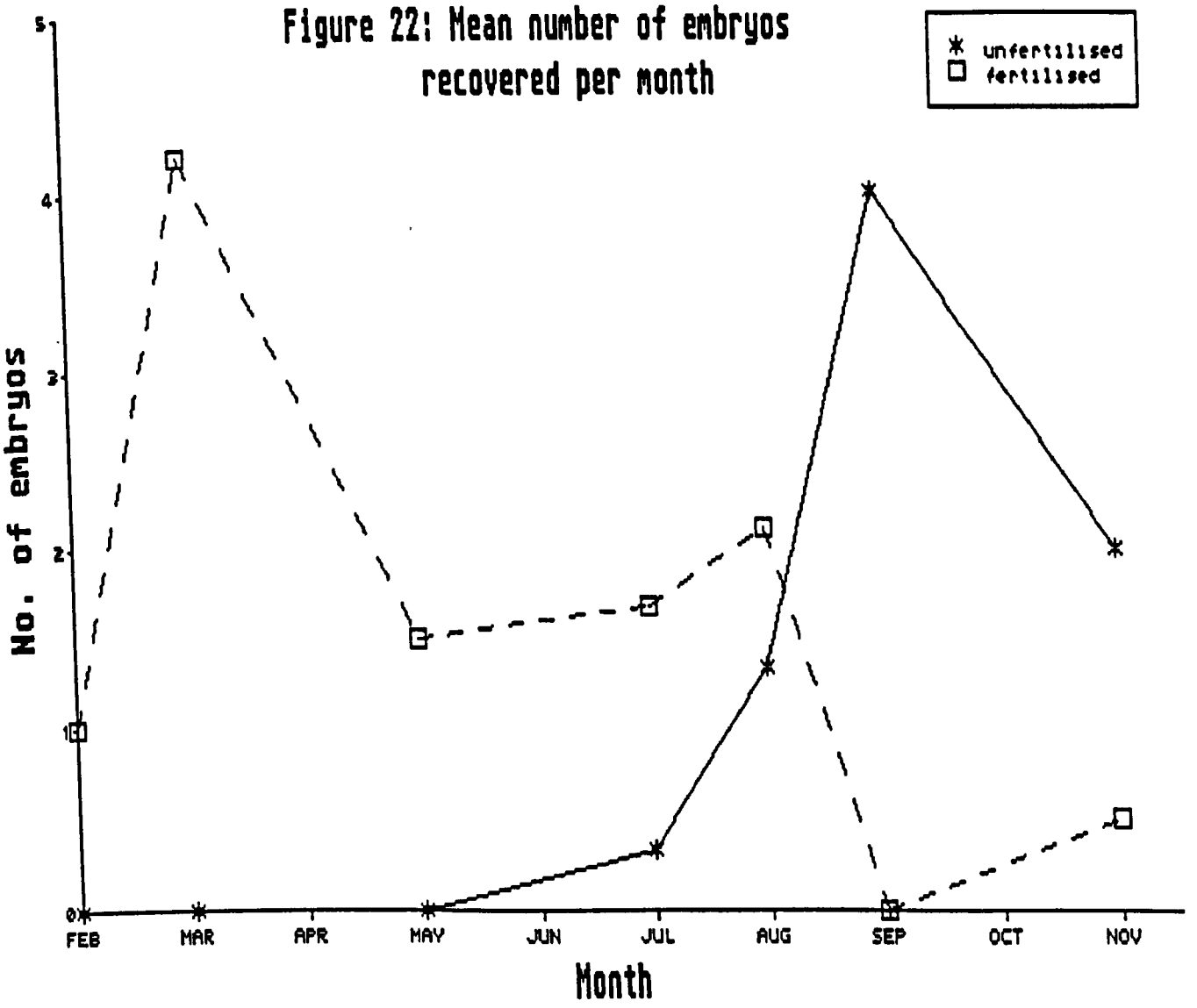


Figure 22: Mean number of embryos recovered per month
unfertilised
fertilised
No. of embryos
0
1
2
3
4
5
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
Month

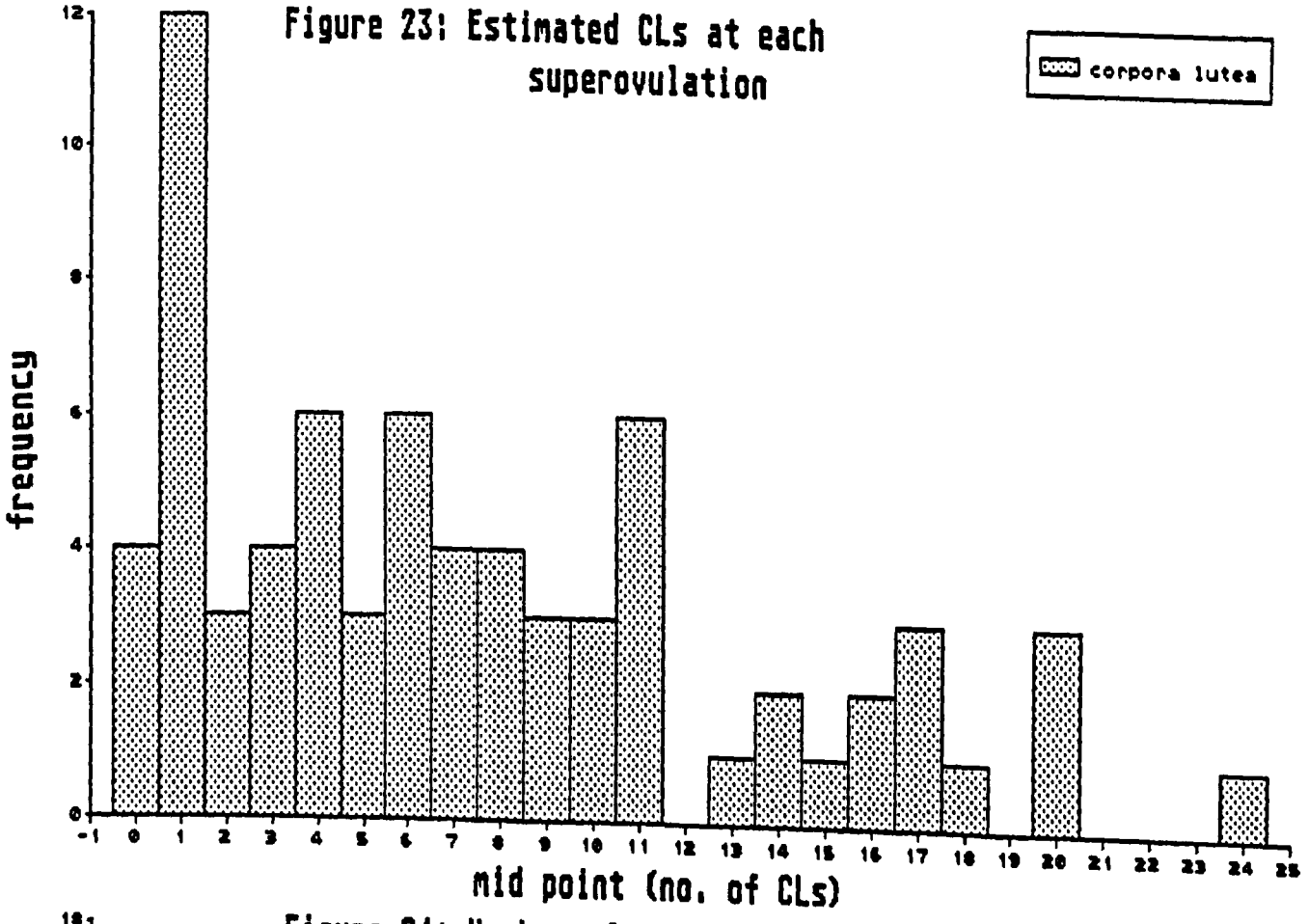


Figure 23: Estimated CLs at each superovulation
corpora lutea
frequency
mid point (no. of CLs)

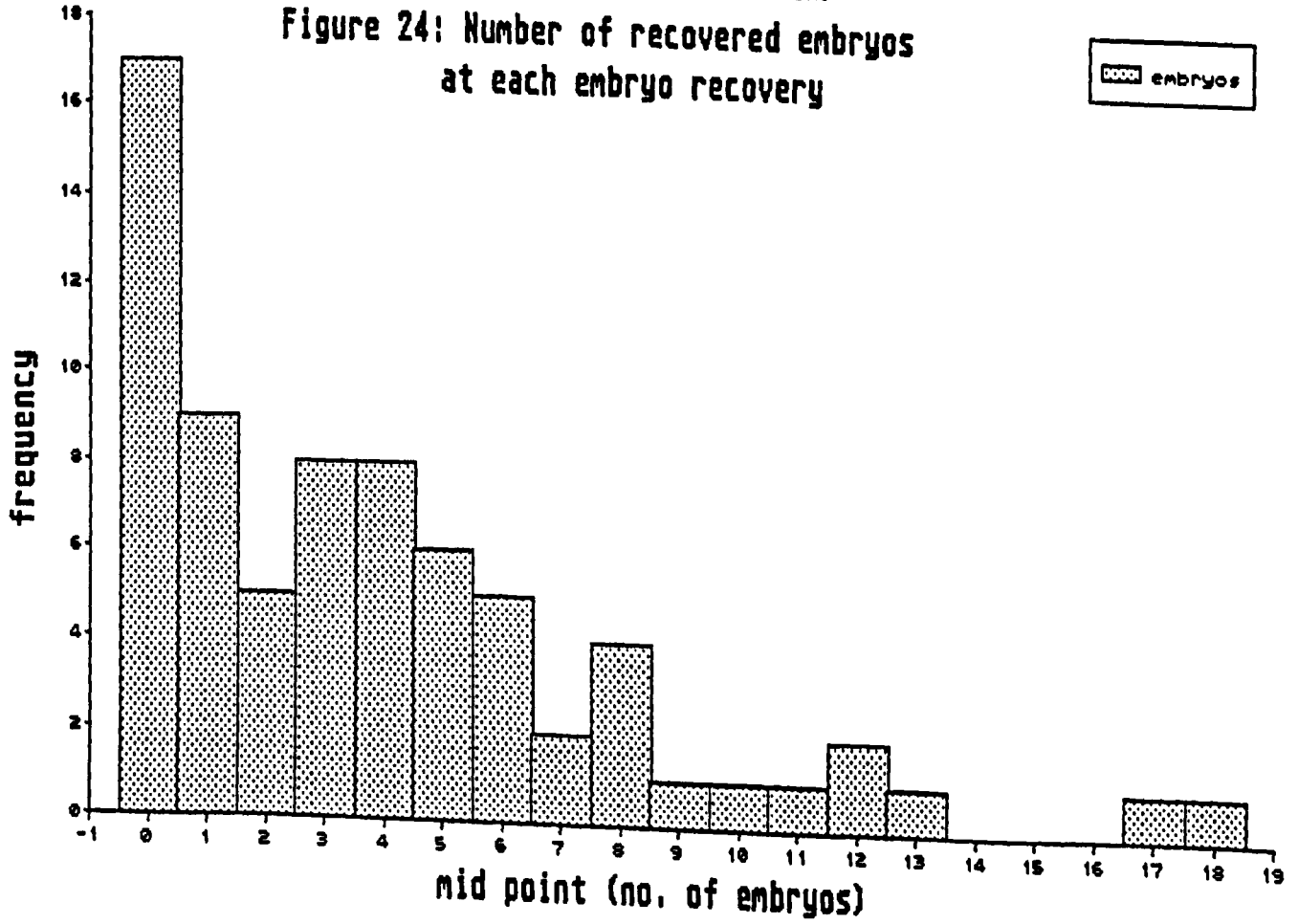


Figure 24: Number of recovered embryos at each embryo recovery
embryos
frequency
mid point (no. of embryos)

9.8 FEED INTAKE IN BEEF CATTLE

In situations of adequate quantities of high quality feed, as in Western Europe and North America, selection to improve economic efficiency favours animals with genetic potential for the primary production traits. Such animals also have high maintenance requirements and are less economically efficient when nutrients are restricted. This is especially pertinent in the range environment where the amount of forage that can be harvested can be limited. Thus selection goals must be assessed in terms of the feed resources available. The optimum weighting of performance traits for use in selection requires knowledge of the inter-relationships among dietary characteristics, feed intake and the rate and composition of gain in market animals (Notter, 1986). Housed animals directly utilise harvested feed at an efficiency which determines the profit. Grazing animals rely on a feed resource which can vary in quality and quantity within and between years and which is harvested by the animal along poorly understood pathways. Profit in this situation is defined by cost per unit of animal production.

Further, reports suggest that there are breed differences in appetite, related to fasting metabolic rate (Webster, 1985), in genetic potential for growth (Ferrel and Jenkins, 1985) and that there are breed x nutritional environment interactions (Holloway et al., 1985).

These findings have important considerations for the performance testing of animals. Traditionally performance testing of beef animals has meant the testing of housed bulls at

a central station (Krauslich, 1974). The efficacy of this system has not been demonstrated (Parnell et al., 1986). Langholz (1984) compared the performance of sires under two systems; short term station tests and long term testing at pasture. Significant sire reranking occurred between the environments. Improvement schemes in New Zealand based on testing at pasture, more closely related to the production environment, have demonstrated large differences between sires for production traits (Baker and Carter, 1976). Menissier and others (1984) examined performance testing on pasture for European beef breeds and emphasised the role of feed intake capacity in selection criteria in these conditions. There is a requirement to assess the consumption of an animal in relation to its performance in the producing environment (Brown et al., 1980). This has been partially achieved through on-farm performance tests although feed intake cannot currently be measured during such tests.

Accordingly, methods which can be used to estimate the forage intake of cattle would have a large impact on selection criteria and methods. The expanded programme of on-farm testing of cattle would benefit from a low cost intake recording method. On-farm tests in the U.K. utilise the feeding system of the host farm. n-Alkane markers could be used in such a situation for periods during the test to determine the forage intake of the cattle and hence derive their feed conversion efficiency (FCE). It is possible to envisage a central analytical facility where the samples of faeces and forage from the tests could be sent for intake determination. This would be considerably cheaper than the

cost of individual feeders and would permit the assessment of environment interactions. At a more fundamental level the marker could be used to discriminate the intake components of grazing cattle and from this develop more accurate selection criteria based on factors such as feed intake capacity, grazing ability and composition of gain. However the results obtained with alkane markers in this study indicate that the present methods of marker delivery produce large within animal variation in faecal recovery and further development will be necessary to elucidate the factors which affect alkane excretion. The pedometers produced readings which were correlated to the estimated intake of the cows. However, the large variation in readings would require too long a sampling period for any statistical differences to be revealed. Improved methods of mounting the pedometers might reduce this variation and increase their efficacy.

The development of accurate selection criteria for individual or groups of environments would assist the identification of superior animals. The grouping together of farmers applying such technologies to overcome the deficiencies inherent with small pedigree herd size synergised with MOET techniques, for germ plasm distribution and in breeding schemes (eg. Nicholas and Smith, 1985), presents one pathway for beef breed improvement.

9.9 CONCLUSIONS

This work has examined the value of two techniques to beef cattle improvement.

The results from three years multiple ovulation, embryo recovery and embryo transfer have shown that;

a) embryo quality from superovulated Welsh Black cows was poorest from early autumn to late winter.

b) suckling reduced embryo quality from superovulated Welsh Black cows.

c) the measurement of cervix diameter was an objective and accurate method of identifying animals with cervices too small to allow non-surgical procedures to be applied.

e) PMSG/PG induced superovulation was successful in producing a reliably increased ovulation rate irrespective of seasonal effects. The responses to superovulation were not affected by the breeding method nor the synchronisation method applied.

f) the catheter types used were equally efficient at recovering embryos.

g) nutritional supplementation did not improve the superovulatory responses of treated Welsh Black cows. No improvement was achieved by the administration of hCG. Stress may have a detrimental effect on superovulation.

h) good pregnancy rates were possible using surgical transfer of fresh and, based on limited data, frozen embryos.

The adoption of current MOET methods in genetic improvement schemes for Welsh Black cattle can not be recommended from the data acquired in this study.

From intake studies performed using n-alkane markers the following conclusions were drawn;

1) the analysis of the n-alkane content of forage and faeces can be carried without recourse to expensive and complicated field equipment.

2) the n-alkane marker can be used to discriminate between the estimated feed intakes of individual cattle in a group housed and fed system although more convenient marker delivery methods are required.

3) the n-alkane marker was unsuccessful at estimating the intake of herbage by grazing cows; withthe proviso that the samples analysed were not entirely satisfactory.

REFERENCES

ABDUL SAEED, S., HUTCHINSON, J.S.M., BROADBENT, P.J. and DOLMAN, D.F. (1989). Hormonal profiles in superovulated Hereford x British Friesian heifers. Theriogenology 31, 253 abstr.

ABOUL-ELA, M.B.E., TOPPS, J.H. and MACDONALD, D.C. (1983). Relationships between intravaginal electrical resistance, cervico-vaginal mucus characteristics and blood progesterone and LH. Anim. Reprod. Sci. 5, 259-273.

ABRAMS, R.M., THATCHER, M.W., CHENAULT, J.R. and WILCOX, C.J. (1975). Bovine vaginal circulation: Changes during estrous cycle. J. Dairy Sci. 58, 1528-1530.

AITICHISON,T.E. (1982). Genetic contribution of embryo transfer. Advanced Animal Breeder 30, 4-14.

ALAM, M.G.S and DOBSON, H. (1986). Effect of various veterinary procedures on plasma concentrations of cortisol, luteinising hormone and prostaglandin F2 alpha metabolite in the cow. Vet. Rec. 118, 7-10.

ALFURAIJI, M.M., BROADBENT, P.J., HUTCHINSON, J.S.M., DOLMAN, D.F. and ATKINSON, T. (1988). Effect of time of administration of monoclonal anti-PMSG on superovulatory response and embryo quality in cattle. Theriogenology 31, 165 abstr.

ALI DINAR, M., DISKIN, M.G., McDONAGH, T. and SREENAN, J.M. (1987). Oestrus and ovarian responses in repeatedly superovulated cows. Theriogenology 27, 201 abstr.

ALLDEN, W.G. and YOUNG, R.S. (1959). Summer nutrition of weaner sheep: Herbage intake following periods of differential nutrition. Aus. J. Agric. Res. 15, 989-1000.

ALLEN, D.M. (1985). Trends in beef populations in Great Britain. 36th Annual EAAP meeting, Thessaloniki, Greece.

ALLEN, S.E. and FOOTE, R.H. (1988). An enzyme linked immunoassay of milk progesterone as a diagnostic aid in embryo transfer programs. Theriogenology 29, 893-903.

ALMEIDIA, A.P. (1987). Seasonal variations in superovulatory response to PMSG in dairy cows. Theriogenology 27, 204 abstr.

ANDERSON,G.B.(1983). Embryo transfer in domestic animals. Ad. Vet. Sci. Comp. Med. 27, 129-162.

AVERY, T.L., FAHNING, M.L. and GRAHAM, E.F. (1962). Investigations associated with the transplant of bovine ova. II. Superovulation. J. Reprod. Fert. 3, 212-217.

BAK, A., GREVE, T. and SCHIMDT, M.
(1988a). Effect of superovulation on reproduction. Theriogenology 31, 169 abstr.

(1988b). Effect of superovulation on milk production. Theriogenology 31, 170 abstr.

BAKER, A.A. and JILELLA, D. (1980). The surgical fixation of catheter in the uterine horns of superovulated cows for the collection of ova. Animal Prod. in Aus. 13, 489.

BAKER, R.L. and CARTER, A.H. (1976). The value of on-farm performance selection of Angus and Hereford bulls. Proc. N.Z. Soc. Anim. Prod. 36,216-221.

BEAL, W.E., SHORT, R.E., STAIGMILLER, R.B., BELLOWS, R.A., KALTENBACH, C.C and DUNNE, T.G. (1978). Influence of dietary energy intake on bovine pituitary and luteal function. J. Amin. Sci. 46, 181-188.

BECH ANDERSEN, B., de BAERDEINACKER, A., BITTANTE, G., BONAITI, B., COLLEAU, J.J., FIMLAND, E., JANSEN, L., LEWIS, W.H.E., POLITICK, R.D., SEELAND, G., TEEHAN, T.J. and WERKMEISTER, F. (1981). Performance testing in AI: Report of a working group of the Commission on cattle production. Livest. Prod. Sci. 8, 101-119.

BECKERS, J.F. (1987). Isolation and use of a porcine FSH to improve the quality of superovulation in cattle. Theriogenology 27, 231 abstr.

BENJAMIN, R.W., CHEN, M., DEGEN, A.A., ABDUL AZIZ, N. and AL-HADADA, M.J. (1977). Estimation of the dry- and organic matter intake of young sheep grazing a dry Mediterraenean pasture and their maintenance requirements. J. Agric. Res. 88, 513-520.

BETTERIDGE, K.J. (1977). Embryo transfer in farm animals. Canada department of agriculture. Monograph 16.

BEVERS, M.M. and DEILEMAN, S.J. (1987). Superovulation of cows with PMSG: variation in plasma concentrations of progesterone, oestradiol, LH, cortisol, prolactin and PMSG and in number of perovulatory follicles. Anim. Reprod. Sci. 15, 37-52.

BICHARD, M. (1971). Dissemination of genetic improvement through a livestock industry. Anim. Prod. 13, 401-411.

BINDON, B.M., PIPER, L.R., CAHILL, L.P., DRIANCOURT, M.A. and O'SHEA, T. (1986). Genetic and hormonal factors affecting superovulation. Theriogenology 25, 53-70.

BOOTH, W.D., NEWCOMB, R., STRANGE, H., ROWSON, L.E.A. and SACHER, H.B. (1975). Plasma oestrogen and progesterone in relation to superovulation and egg recovery in the cow. Vet. Rec. 97, 366-396.

BOWEN, R.A., HOWARD, T.H., ELSDEN, R.P. and SEIDEL, G.E., Jr. (1983). Transfer of embryo from cattle infected with blue tongue virus. Theriogenology 19, 115 abstr.

BRADEN, T.D., KING, M.E., ODDE, K.G. and NISWENDER, G.D. (1989). Development of preovulatory follicles expected to form short lived corpora lutea in beef cows. J.Reprod. Fert. 85,97-104.

BRADFORD, G.W. and KENNEDY, B.W. (1980). Genetic aspects of embryo transfer. Theriogenology 13, 13-26.

BREUL, K.F., SPITZER, J.C. and HENRICKS, D.M. (1989). Systemic progesterone concentration following human chrionic gonadotrophin administration at various times during the estrous cycle in beef heifers. J. Anim. Sci. 67, 1564-1572.

BRITT, J.H. and HOLT, L.C. (1988). Endocrinological screening of embryo donors and embryo recipients: A review of research with cattle. Theriogenology 29, 189-202.

BROADBENT, P.J. and HUTCHINSON, J.S.M. (1989). Realising the potential of embryo transfer in cattle. Report of North of Scotland College of Agriculture 1989. pp 9-16.

BROWN, C.J., JOHNSON, Z. and BROWN, H. (1980). Estimates of partial efficiency of young beef bulls. J. Anim. Sci. 51, 830-836.

BRUCKENTAL, I., LEHRER, A.R., WEITZ, M., BENARD, J., KENNIT, H. and NEUMARK, H. (1987). Faecal output and estimated voluntary dry matter intake of grazing beef cows, relative to their liveweight and to the digestibility of the pasture. Anim. Prod. 45, 23-28.

BUTLER, J.E. and BIGGERS, J.D. (1988). Review of embryo viabilty assessment. Theriogenology 31, 115-126.

CALLESDEN H., GREVE, T. and HYTTEL, P. (1987). Premature ovulations in superovulated cattle. Theriogenology 28, 155-166.

CALLESDEN, H., BAK, H., GREVE, T., AVERY, B., GOTFREDSEN, P., HOLM, P. PEDERSEN, J.O., SCHIMDT, M., SMITH, S. and SVANBERG, N. (1988a). Use of PMSG antisera in superovulating dairy heifers. Theriogenology 31, 179 abstr.

CALLESDEN, H., GREVE, T. and HYTTEL,P. (1988b). Pre-ovulatory evaluation of the superovulatory response in donor cattle Theriogenology 30, 477-488.

CANFIELD, R.W. and BUTLER, W.R. (1989). Accuracy in predicting LH surge and optimal insemination time in Holstein heifers using a vaginal resistance probe. Theriogenology 31, 835-842.

CASSIDA, L.E. (1940). cited by GORDON, I. (1983). In: Controlled Breeding in Farm Animals, Pergamon Press, Oxford.

CAVESTANY, D. and FOOTE, R.H. (1985a). Reproductive performance of Holstein cows administered GnRH analog HOE 766 (Buserelin) 26 to 34 days postpartum. J. Anim. Sci. 61, 224-233.

CAVESTANY, D. and FOOTE, R.H. (1985b). The use of milk progesterone and electronic vaginal probes as aids in large dairy herd reproductive management. Cornell Vet. 75, 441-453.

CHAMBERLAIN, D.G. and THOMAS,P.C. (1983). A note on the use of chromium sesquoxide as a marker in nutritional studies with dairy cows. Anim. Prod. 36, 155-157.

CHENAULT, J.B., THATCHER, W.W., KALRA, P.S., ABRAMS, R.M. and WILCOX, C.J. (1975). Transitory changes in plasma progestins, estradiol and luteinising hormone approaching ovulation in the bovine. J. Dairy Sci. 58, 709-717.

CHRISTIE, W.B., NEWCOMB, R., and ROWSON, L.E.A. (1979). Ovulation rate and egg recovery in cattle treated repeatedly with pregnant mares serum gonadotrophin and prostaglandin. Vet. Rec. 104, 281-283.

CHURCH, R.B. and SHEA, B. (1976). Some aspects of bovine embryo transfer. In: Egg Transfer in Cattle (Ed. Rowson, L.E.A.) C.E.C., Brussels. pp 73-86.

CHURCH, R.B., and SHEA, B.F. (1977). The Role of Embryo Transfer in Cattle Improvement Programmes. Can. J. Anim. Sci. 57, 33-45.

CLARK, H. (1985). Tissue flux in perennial ryegrass grazed by sheep. PhD thesis, University of Wales.

CLAUS, R., KARG, H., ZWIANER, D., VON BUTLER, I., PIRCHNER, F. and RATTENBERGER,E.(1983). Analysis of factors influencing reproductine performance of the dairy cow by progesterone assay in milk fat. Br. Vet j. 139, 29

COLE, H.H. and HART, G.H. (1930). The potency of blood serum of mares in progressive stages of pregnancy in effecting sexual activity of immature rats. Amer. J. Physiol. 98, 57-61.

COOK, A.S., WEBSTER, B.W., TERRANOVA, P.F. and KEEL, B.A. (1988). Variation in the biologic and biochemical characteristics of hMG. Fertil. Steril. 49, 704-712.

COOPER, M.J. (1974). Control of oestrous cycles of heifers with a synthetic prostaglandin analogue. Vet. Rec. 95, 200-203.

CORDOVA, F.J., WALLACE, J.D. and PIEPER, R.D. (1978). Forage intake by grazing ruminants: A Review. J. Range Management 31, 430-438

COWEN, P. and SUSNIK, U. (1987). Effect of superovulation on lactating Holsteins. Theriogenology 28, 783-788.

CRISTER, J.K., GUNSETT, F.C., WINCH, R.P., ECLTS, C.L. and GINTHER, O.J. (1979). Factors affecting ova transfer in Limousin, Maine Anjou and Simmental cattle. Theriogenology 11, 95.

DeSILVA, A.W.M.V., ERAL, S.A., DUNN, T.G. and KALTENBACH, C.C. (1981). Comparison of serum prolactin and cortisol in sheep bled by 2 methods. J. Anim. Sci. 53 Suppl. 1, 306 abstr.

DEL CAMPO, M.R. and GINTHER, O.J. (1980). Mechanism involved in the survival of embryos in cattle. 9th Int. Con. Anim. Reprod. Artificial Insem. 16-20th June, 1980, III.

DICKERSON, G.E. and WILHEILM, R.L. (1983). Quantative genetic engineering more efficient animal production. J. Anim. Sci. 57 Suppl. 2, 248-257.

DIELEMAN, S.J. and BEVERS, M.M.(1987). Effects of monoclonal antibody against PMSG administered shortly after the preovulatory LH surge on time and number of ovulations in PMSG/PG-treated cows. J.Reprod. Fert. 81, 533-542.

DIELEMAN, S.J., BEVERS, M.M., WURTH, Y.A., GIDEN, J.Th. and WILLEMSE,A.H. (1989).Improved embryo yield and condition of donor ovaries in cows after PMSG superovulation with monoclonal anti-PMSG administered shortly after the preovulatory LH peak. Theriogenology 31 473-487.

DILLON, P. and STAKELUM, G. (1989). Herbage and dosed alkanes as a grass measurement technique for dairy cows. Ir. J. Agric. Sci. 28, 104 abstr.

DOBSON, H. (1983). A radioimmunoassay laboratory handbook. Liverpool University Press.

DOBSON, H., MIDMER, S.E. and FITZPATRICK, R.J. (1975). Relationship between progesterone concentration in the milk and plasma during the bovine oetrous cycle. Vet. Rec. 96, 222-223.

DONALDSON, L.E. (1968). The efficiency of several methods of oestrus detection in cattle. Aus. Vet. J. 44, 496-498.

DONALDSON, L.E. (1985a). LH and FSH profile at superovulation and embryo production in the cow. Theriogenology 23 (3), 441-447.

DONALDSON, L.E. (1985b). Estimation of superovulation response in donor cows. Vet. Rec. 117, 33-34.

DONALDSON, L.E. (1985c). Effect of insemination regumen on embryo production in superovulated cows. Vet. Rec. 17, 35-37.

DONALDSON, L.E. (1985d). Matching of embryo stages and grades with recipient oestrous synchrony in bovine embryo transfer. Vet. Rec. 117, 489-491.

DONALDSON, L.E. (1988). Porcine, equine and ovine FSH in the superovulation of cattle. Theriogenology 31, 183 abstr.

DONALDSON, L.E. (1990). FSH-P batch variation. Theriogenology 33, 215 abstr.

DONALDSON, L.E., BASSETT, L.M. and THORBURN, C.D. (1970). Peripheral plasma progesterone concentration of cows during puberty, oestrous cycles, pregnancy and lactation, and the effects of under-nutrition or exogenous oxytocin on progesterone concentration. J. Endocrin. 48, 599-614.

DONEY, J.M., GUNN, R.G., SMITH, W.F. and CARR, W.R. (1976a). Effects of premating environmental stress, ACTH, cortisone acetate or metyrapone on oestrus and ovulation in sheep. J. Agric. Sci. 87, 127-132.

DONEY, J.M., SMITH, W.F. and GUNN, R.C. (1976b). Effects of post mating environment stress or administration of ACTH on early embryonic loss in sheep. J. Agric. Sci. 87, 133-136.

DORN, C.G., BAKER, J.F., KNUTSON, R. and KRAEMER, D.C. (1989). Embryo recovery from early postpartum beef cows. Theriogenology 31, 184 abstr.

DORRINGTON,J. and GORE-LANGTON, R.E.(1981). Prolactin inhibits oestrogen synthesis in the ovary. Nature, London. 290:600-602.

DOWLING, D.F. (1949).Problems of the transplantation of fertilised ova. J. Agric. Sci. 39, 374-395.

DOWNIE, J.G. and GELMAN, A.L. (1976). The relationship between changes in bodyweight, plasma glucose and fertility in beef cows. Vet. Rec. 99, 210-212.

DROST, M., TAN, H.S., MacMILLAN, K.L. and THATCHER,W.W. (1988). Successful asychronous embryo transfer in cattle. Theriogenology 31, 186.

DUNNE, T.G. (1980). Relationship of nutrition to successful embryo transfer. Theriogenology 13, 27-39.

DUNNE, T.G. and KALTENBACH, C.C. (1980). Nutrition and the postpartum interval of the ewe, sow and cow. J. Anim. Sci. 51 Suppl. 2, 29.

DUNNE, T.G., RONE, J., KALTENBACH, C.C., van der WALT, L.A., RILELY, M.L. and AKBAR, A.M. (1974). Hormone changes during underfeeding of beef cows. J. Anim. Sci. 39, 206 abstr.

EDMUNDS, H. (1981). Welsh Black cattle. J. Royal. Agric. Soc. Eng. 142, 24-32.

EDWARDS, L.M., RAHE, C.H., GRIFFITH, J.L., WOLFE, D.F., MARPLE, D.N., CUMMINS, K.A. and PITCHETT, J.F. (1987). Effect of transportation stress on ovarian function in superovulated Hereford heifers. Theriogenology 28, 291-299.

ELSDEN, R.P., HASLER, J.F. and SEIDEL, G.E. Jr. (1976). Non-surgical recovery of bovine eggs. Theriogenology 6, 523-529.

ESSLEMONT, R.J. (1973). A study of the economic and husbandry aspects of the manifestation and detection of oestrus in large herds. PhD thesis, University of Reading.

ESSLEMONT, R.J. and BRYANT, M.J. (1974). Economic and husbandry aspects of the manifestation and detection of oestrus in cows II. ADAS Quartely Review 13, 19-26.

FAUST, M.A., McDANIEL, B.T., ROBISON, O.W. and BRITT, J.H. (1988). Enviromental and yield effects on reproduction in primiparous Holsteins. J. Dairy Sci. 71:3092-3099.

FERREL, C.L. and JENKINS, T.G. (1985). Cow type and the nutritional environment: nutritional aspects. J. Anim. Sci. 61, 725-741.

FRANCHIMONT, P., DEMOULIN, A. and VALCKE, J.C. 1989. Endocrine, Paracrine and Autocrine control of follicular development. Horm. Metabol. Res. 20, 193-203.

FOOTE, R.H., ALLEN, S.E. and HENDERSON, B. (1988). Buserelin in a superovulatory regimen for Holstein cows 2. yield and quality of embryos in commercial herds. Theriogenology 31, 385-392.

FOOTE, R.H., OLTENACU, E.A.B., MELLINGER, J., SCOTT, N.R. and MARSHALL, R.A. (1979). Pregnancy rate in dairy cows inseminated on the basis of electronic probe measurements. J. Dairy Sci. 62, 69-73.

FRANKLIN, I.R. (1982). Ppoulation size and the genetic improvement of animals. In: Future developments in the genetic improvement of animals (Eds. Barker, J.S.F., Hammond, K and McClintock, A.E.M.), Academic Press, Sydney, Australia.

GARCIA, G.J.K., ELSDEN, R.D. and SEIDEL, G.E., Jr. (1983). Efficacy of PGF2 alpha for reducing the return to oestrus interval in superovulated cattle. Theriogenology 19, 129 abstr.

GARCIA-WINDER, M., LEWIS, P.E., BRYNER, R.W., BAKER, R.D., INSKEEP, E.K. and BUTCHER, R.L. (1988). Effect of age and Norgestomet on endocrine parameters and production of embryos in superovulated beef cows. J. Anim. Sci. 66, 1974-1981.

GARTH SASSER, R., WILLIAMS, R.J., BULL, R.L., RUDER, C.A. and FALK, D.G. (1988). Post partum reproductive performance in crude protein restricted beef cows: return to estrus and conception. J. Anim. Sci. 66, 3033-3039.

GARTLAND, P., SCHIAVO, J., HALL, C.E., FOOTE, R.H. and SCOTT, N.R. (1975). Detection of oestrus in dairy cows by electrical measurement of vaginal mucus and by milk progesterone. J. Dairy Sci. 59, 982-985.

GARY, L.A., SHERRIT, G.W. and HALE, E.B. (1970). Behaviour of Charolais cattle on pasture. J. Anim. Sci. 34, 203-206.

GOMBE, S. and HANSEL, W. (1973). Plasma luteinising hormone and progesterone levels in heifers on restricted energy intakes. J. Anim. Sci. 37, 728-733.

GOODMAN, R.L. and KARSCH, F.J. (1981). A critique of the evidence on the importance of steriod feedback to seasonal changes in gonadotrophin secretion. J.Reprod. Fert. Suppl.30, 1-13.

GORDON, I. (1982). Synchronization of estrus and superovulation in cattle. In Mammalian Egg Transfer (Ed. Adams, C.E.). pp 81-118. CRC Press, Boca Raton, Florida.

GORDON, I.(1983). Controlled breeding in farm animals. Pergamon, Oxford.

GORDON, I., BOLAND, M.P., McGOVERN, H. and LYNN, G. 1987. Effect of season on superovulatory responses and embryo quality in Holstein cattle in Saudia Arabia. Theriogenology 27, 231 abstr.

GOSDEN, A.F. and MOSELEY, G. (1983). Naturally occurring forage hydrocarbons as intake markers. Welsh Plant Breeding Station, Annual Report, 1983, pp109-110.

GOTO, K., OHKUTSU, S., NAKANISHI, T., OGAWA, K., TASAKI, M., OHTA, H., INOHAE, S., TATEYANA, S., KAWABATA, T., MIYAMOTO, A., FURUSAWA, T., UMEZU,M. and MASAKI, J. (1988). Endocrine profiles and embryo quality in superovulated Japanese Black cattle. Theriogenology 29, 615-629.

GRACE, N.D. and BODY, D.R. (1981). The possible use of long chain (C19-C33) fatty acids in herbage as indigestible faecal marker. J. Agric. Sci. 97, 743-745.

GRASSO, F., GUILBAULT, L.A., ROY, G.L., MATTON, P. and LUSSIER, J.G. (1988). The influence of the presence of a dominant follicle at the time of initation of a superovulatory treatment on superovulatory responses in cattle. Theriogenology 31, 199 abstr.

GREENHALGH, J.F.D. (1982). An introduction to herbage intake measurements. In: Herbage Intake Handbook (Ed. Leaver, J.D.) The British Grassland Soc., Hurley. pp 1-10.

GUAY, P., REIGER, D. and ROBERGE, S. (1988). Superovulatory and endocrine responses in Holstein heifers treated with either PGF2α, closprostenol or fenoprostalene. Theriogenology 29, 1193-1199.

HACKETT, A.J. and McALLISTER, A.T. (1984). Onset of oestrus in dairy cows maintained indoors year-round. J. Dairy Sci. 67, 1793-1797.

HAFEZ, E.S.E., SUGIE, T. and GORDON, J. (1963). Superovulation and related phenomena in the beef cow. I. Superovulatory responses following PMS and HCG injection. J. Reprod. Fert. 5, 359-379.

HAHN, J. and HAHN, R. (1976). Experiences with non-surgical transfer techniques. In Egg Transfer in Cattle (Ed. Rowson, L.E.A.) pp 199-204. C.E.C. Brussels.

HALLIDAY, J.L. (1985). Studies of rumen degradation in relation to the utilization of forage. Ph.D. thesis. University of Wales.

HANSEN, P.J. andHAUSER, E.R. (1983). Genotype x environmental interactions on reproducive traits of bovine females. 3. Seasonal variation in post partum reproduction as influenced by genotype, suckling and dietary regimen. J. Anim. Sci. 56, 1362-1369.

HARPER, M.J.K., BENNETT, J.P. and ROWSON, L.E.A. (1961). A possible explanation for the failure of non-surgical ovum transfers in the cow. Nature 190, 789-790.

HASLER, J.F., McCAULEY, A.D., SCHERMERHORN, E.C., FOOTE, R.H. (1983). Superovulation responses of Holstein cows. Theriogenology 19, 83-93.

HASLER, J.F., McCAULEY, A.D., LATHROP, W.F. and FOOTE, R.H. (1987). Effect of donor-embryo-recipient interaction on pregnancy rate in a large scale bovine embryo transfer program. Theriogenology 27, 139-168.

HECKMAN, G.S., KATZ, L.S., FOOTE, R.H., OLTENACU, E.A.B., SCOTT, N.R. and MARSHALL, R.A. (1979). Estrus cycle patterns in milk monitored by electrical resistance and milk progesterone. J. Dairy Sci. 62, 64-78.

HELMER, S.D. and BRITT, J.H. (1987). Hormone secretion and characteristics of oestrous cycles after treatment of heifers with hCG or PGF2 during corpus luteum formation. J. Anim. Sci. 64, 782-789.

HERRLER, A., ELSAESSER, F. and NIEMANN, H. (1990). Rapid milk progesterone assay as a tool for the selection of potential donor cows prior to superovulation. Theriogenology 33, 415-422.

HODGSON, J. (1982). Influence of sward characteristics on diet selection and herbage intake by grazing animals. In: Nutritional limits to Animal Production from Pastures (Ed. Hacker, J.B.) C.A.B. pp 153-166.

HOLDSWORTH, R.J., BOOTH, J.M., SHARMAN, G.A.M. and RATTRAY, E.A.S. (1980). Measurement of progesterone levels in whole and fore milk from dairy cows. Br. Vet. j., 136, 546-554.

HOLLOWAY, J.W., BUTTS, W.T., Jr., McCURLEY, J.R., PEELER, H.L., BEAVER, E.E. and BACKUS, W.L. (1985). Breed x nutritional environment interactions for intake and digestibility of forage grazed by lactating beef females. J. Anim. Sci. 61, 1345-1353.

HOPPER, J.T., HOLLOWAY, J.W. and BUTTS, W.T., Jr. (1978). Animal variation in chromium sesquioxide excretion patterns of grazing cows. J. Anim. Sci. 46, 1096-1102.

HUMPHREY, W.D., KALTENBACH, C.C., DUNN, T.G., KORNTNIK, D.R. and NISWENDER, G.D. (1983). Characterisation of hormonal patterns in the beef cow during postpartum anestrus. J. Anim. Sci. 56, 445-453.

HYDER, D.N., BEMENT, R.E. and NORRIS, J.J.(1968). Sampling requirements of the water-intake method of estimating forage intake by grazing cattle. J. Range Manage. 21, 392-397.

IRITANI, A. (1988). Current status of biotechnological studies in mammalian production. Fert. and Steril. 50, 543-551.

JAMES, J.W. (1977). Open nucleus breeding systems. Anim. Prod. 24, 287-305.

JANSON, L. (1980). Studies on fertility traits in Swedish dairy cattle. 1, Effects of non-genetic factors. Acta. Agric. Scand. 30, 109-124.

JUGA, J. and MAKI-TAMILA, A. (1987). Genetic change in a nucleus breeding dairy herd using embryo transfer. Acta Agric. Scand. 37, 511-519.

KANE, M.T. (1987). Culture media and the culture of early embryos. Theriogenology 27, 49-57.

KEARY, H.W., (1848). The management of cattle. J. Royal Agric. Soc. 9, 424-452.

KIBON, A. and HOLMES, W. (1987). The effect of height of pasture and concentrate composition on dairy cows grazed on continously stocked pastures. J. Agric. Sci. 109, 293-301.

KIDDY, C.A. (1976). Variations in activity as an indication of oestrus in dairy cows. J. Dairy Sci. 60, 235-243.

KILGOUR, R., SKARSHOLT, B.H., SMITH, J.F., BREMNER, K.J. and MORRISON, M.C.L. (1977). Observations on the behaviour and factors influencing the sexually active group in cattle. Proc. N.Z. Soc. Anim. Prod. 37, 128-135.

KING, K.K., SEIDEL, G.E. Jr. and ELSDEN, R.P. (1985a). Bovine Embryo Transfer Pregnancies. I. Abortion rates and characteristics of calves. J. Anim. Sci. 61 (4), 747-757.

KING, K.K., SEIDEL, G.E. Jr. and ELSDEN, R.P. (1985b). Bovine embryo transfer pregnancies. II. Lengths of gestation. J. Anim. Sci. 61 (4), 758-762.

KIRMURA, M., NAKAO, T., MORIYOSHI, M. and KAWATA, K. 1987. Luteal phase deficiency as a possible cause of repeat breeding in dairy cows. Br. Vet. j. 143, 560-566

KOTB, A.R. and LUCKEY, T.D. (1972). Markers in nutrition. Nutr. Abs. Rev. 42, 813-845.

KRAUSLICH, H. (1974). General recommendations on procedures for performance and progeny testing for beef characteristics. Livestock Prod. Sci. 1, 33-45.

LaVOIE, V., HAN, D.K., FOSTER, D.B. and MOODY, E.L. (1981). Suckling effect on estrus and blood plasma progesterone in post-partum beef cows. J. Anim. Sci. 52, 802-812.

LeDU, Y.L.P. and PENNING, P.D. (1982). Animal based techniques for estimating herbage intake. In: Herbage Intake Handbook (Ed. Leaver, J.D.)pp 37-75. British Grassland Soc. Maidenhead, Berks.

LAMMING, G.E., WATHES, C.D. and PETERS, A.R.(1981). Endocrine patterns in the post-partum cow. J. Reprod. Fert. Suppl. 30, 155-170.

LAMOND, D.R. (1970). The effect of pregnant mare's serum gonadotrophin on ovarian function of beef heifers as influenced by progestin, plane of nutrition and fasting. Aust. J. Agric. Res. 21, 153-161.

LAMOND, D.R. (1972). Hormonal induction of multiple ovulations in the bovine. J. Anim. Sci. 34, 901 Abstr.

LAND, R.B. and HILL, W.G. (1975). The possible use of superovulation and embryo transfer in cattle to increase response to selection. Anim. Prod. 21, 1-12.

LANGHOLZ, H.J., SUHREN, M. and HEVELING, G. (1984). A comparison of Friesian bulls indoors and on pasture.In Grassland Beef Prduction (Ed. Holmes, W.)pp 113-123. Martinus Nijhoff. Amsterdam.

LAWSON, R.A.S., ROWSON, L.E.A., MOOR, R.M. and TERVIT, H.R. (1975). Experiments on egg transfer in the cow and ewe : dependence of conception rate on the transfer procedure and stage of the oestrus cycle. J. Reprod. Fert. 45, 101-107.

LEIBO, S.P. (1989). Equilibrium and non-equilibrium cryopreservation of embryos. Theriogenology 31, 85-93.

LERNER, S.P., THAYNE, W.V., BAKER, R.D., HENSCHEN, T., MEREDITH, S., INSKEEP, E.K., DAILEY, R.A., LEWIS, P.E. and BUTCHER, R.L. (1986). Age, dose of FSH and other factors affecting superovulation in Holstein cows. J. Anim. Sci. 63, 176-183

LEWIS, W.H.E. and ALLEN, D.M. (1974). Performance testing for beef characteristics. 1st World Congress on Genetics Applied to Livestock Production, Madrid. pp 671-979.

LI, P.S. and WAGNER, W.C. (1981). Effect of ACTH or cortisol infusion on LH response to GnRH in intact and adrenalectomized heifers. J. Anim. Sci. 53 Suppl.1, 341 abstr.

LINFORD, E. (1974). Cervical mucus: An agent or a barrier to conception ? J. Reprod. Fert. 37, 239-250.

LOPEZ GATIUS,F., CAMON URGEL, J. and ANGULO ASENSIO, E. (1988). Effects of single deep insemination on transferable embryo recovery rates in superovulated dairy cows. Theriogenology 30, 877-885.

LUBBADEH, W.F., GRAVES, C.N. and SPAHR, S.L. (1980). Effect of repeated superovulation on ovulatory response of dairy cows. J. Anim. Sci. 50, 124-127.

LUSBY, K.S., WETTEMAN, R.P. and TURMAN, E.J. (1981). Effects of early weaning calves from first calf heifers on calf and heifer performance. J. Anim. Sci. 53, 1193-1197.

McDANIEL, B.T. and CASSELL, B.G. (1981). Effects of ET on genetic change in Dairy Cattle. J. Dairy Sci. 64, 2484-2492.

McGUIRK, B. (1989). The revelance of MOET programmes to developing countries. Theriogenology 31, 29-40.

McGOWAN, M.R., BRAITHWAITE, M., JOCHLE, W. and MAPLETOFT, R.S. (1985). Superovulation of beef heifers with pergonal (hMG): a dose response trial. Theriogenology 24, 173-184.

McNEILLY, A.S. (1987). Prolactin and the control of gonadotrophin secretion. J. Endocr. 15, 1-5.

McNEILLY, A.S., GLASIER, ANNA, JONASSEN, JULIE and HOWIE, P.W. (1982). Evidence for direct inhibition of ovarian function by prolactin. J. Reprod. Fert. 65, 559-569.

MacMILLAN, K.L., TAUFA, V.K. and DAY, A.M. (1986). Effects of an agonist gonadotrophin releasing hormone (Buserelin) in cattle. III. Pregnancy rates after a post insemination injection during metooestrus or dioestrus. Anim. Prod. Sci. 11, 1-10.

MACEY, M.J.K. and BARBER, H.N. (1970). Chemical genetics of wax formation on leaves of brassica oloacea. Phytochemistry 9, 13-23.

MAFF (1977). Technical Bulletin 33; Energy allowances and feeding systems for ruminants. H.M.S.O.

MAFF (1979). Technical Bulletin 27; Analysis of Agricultural Materials. H.M.S.O.

MAHON, G.D. and RAWLE, J.E. (1987). The export of deep frozen bovine embryos. Theriogenology 27, 21-35.

MASON, I.L. (1971). Comparative beef performance of large cattle breeds of Western Europe. Anim. Breed. Abs. 39, 1-29.

MASSEY, J.M. and ODEN, A.J. 1984. No seasonal effect on embryo donor performance in the South-West region of the U.S.A. Theriogenology 21, 196-217.

MAURER, R.R. (1976). Storage of mammalian oocytes and embryos: A review. Can. J. Anim. Sci. 56, 131-145.

MAURER, R.R. and ECHTERNKAMP, S. (1982). Hormonal asynchrony and embryonic development. Theriogenology 17, 11-22.

MAURER, R.R., ECHTERNKAMP, S.E. and WISE, T.H. (1989). Ovarian responses to intramuscular or intravenous administration of prostaglandin F2 alpha in control and FSH treated beef heifers. J. Anim. Sci. 67, 2075-2080.

MAYES, R.W. and LAMB, C.S. (1984). The possible use of n-alkanes in herbage as indigestible markers. Proc. Nutrtion Soc. 43, 39A.

MAYES, R.W., LAMB, C.S. and COSGROVE, P.M. (1986). The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. J. Agric. Sci. 107, 161-170.

MEIJS, J.A.C. (1981). Herbage intake by grazing cows. Centre for Agricultural Publishing and Documentation, Wageningen, Holland.

MENISSIER, F., BERANGER, C. and RENARD, G. (1984). Some considerations on performance testing on apasture for European beef breeds. In: Grassland Beef Production (Ed. Holmes, W.). pp 124-135. Martinus Nijhoff. Amsterdam.

MESSINIS, I.E. and TEMPLETON, A.A. (1988). The endocrine consequences of multiple folliculogenesis. J. Reprod. Fert. Suppl. 36, 27-37.

MEYERS, P.J., ELMORE, R.G., VARNER, D.D., BLANCHARD, T.L., SHELL, J.W. and TODD, J. (1988). Use of a rapid progesterone assay in a beef sychronization programme. Theriogenology 29, 1285-1294.

MILLS, A.C., TURNER, J.W. and VINCENT, C.K. (1969). Methods of estrual detection in cross bred cows. J. Anim. Sci. 28, 146 abstr.

MLC, (1987). Beef yearbook 1987 pp 87-97, Meat and Livestock Commission, Milton Keynes, U.K.

MLC, (1988). Beef yearbook 1988 pp 75-89 , Meat and Livestock Commission, Milton Keynes, U.K.

MOHR, L.R. and TROUNSON, A.O. (1980). The use of fluorescein diacetate to assess embryo viability in the mouse. J. Reprod. Fert. 58, 189-196.

MONNIAUX, B., CHUPIN, D. and SAUMENDE, J. (1983). Superovulatory responses of cattle. Theriogenology 19, 55-81.

MOOR, R.M., KRUIP, Th.A.M. and GREEN, D. (1984). Intra-ovarian control of folliculogenesis: limits to superovulation? Theriogenology 21, 103-116.

MOORE, N.W. (1975). The control of time of oestrus and ovulation and the induction of superovulation in cattle. Aust. J. Agric. Res. 26, 295-300.

MORRIS, C.A. and DAY, A.M. (1986). Potential for genetic twinning in cattle. 3rd World Congress Genet. Appl. Livestock Prod. XI, Nebraska, U.S.A. pp 14-29.

MULVEHILL, P. and SREENAN, J.M. (1977). Improvement of fertility in postpartum beef cows by treatment with PMSG and progestagen. J. Reprod. Fert. 50, 323-325.

MUNAR, C.J. and HASLER, J.F. (1989). Results of the first frozen bovine embryos exported from the U.S.A. to Argentina. Theriogenology 31, 230 abstr.

NANCARROW, C.D. and MILLER, W.J.B. (1976). Factors affecting oestrus synchronisation relative to superovulation and egg transfer. In: Egg Transfer in Cattle (Ed. Rowson, L.E.A.) C.E.C., Brussels. pp 291-299.

NELSON, C.F. and NELSON, L.D. (1988). Cryopreservation of 7- to 9- Day bovine embryos. Theriogenology 29, 281 abstr.

NEWCOMB, R.(1979). Surgical and non-surgical transfer of bovine embryos. Vet. Rec. 105, 432-444.

NEWCOMB, R. (1980). Investigation of factors affecting superovulation and non-surgical embryo recovery from lactating British Friesian cows. Vet. Rec. 106, 48-52.

NEWCOMB, R. (1982). Egg Recovery and Transfer in cattle. In Mammalian Egg Transfer (Ed. Adams, C.E.) pp 81-118. CRC Press, Baton Raton, Florida.

NEWCOMB, R. and ROWSON, L.E.A. (1975). Conception rate after uterine transfer of cow eggs, in relation to synchronization of oestrous and age of eggs. J. Reprod. Fert. 43, 539-541.

NEWCOMB, R. and ROWSON, L.E.A. (1980). Investigation of physiological factors affecting non-surgical transfer. Theriogenology 13, 41-49.

NEWCOMB, R., CHRISTIE, W.B. and ROWSON, L.E.A. (1978). Non-surgical recovery of bovine embryos. Vet. Rec. 102, 414-417.

NEWCOMB, R., CHRISTIE, W.B., ROWSON, L.E.A., WALTERS, D.E. and BONSFIELD, W.E.D. (1979). Influence of dose, repeated treatment and batch of hormone on ovarian response in heifers treated with PMSG. J. Reprod. Fert. 56, 113-118.

NEWCOMB, R., ROWSON, L.E.A. and TROUNSON, A.O. (1978). Sacrewel project : An on farm demonstration of the potential of egg transfer. Vet. Rec. 103, 415-418.

NICHOLAS, F.W. (1985). The implications of developments in reproductive biology for livestock improvement programmes. Proc. Aus. Assoc. Anim. Breed. Genet., 5th Conf. Sydney, 1985, pp 77-82.

NICHOLAS, F.W. and SMITH, C. (1983). Increased rates of genetic change in dairy cattle by embryo transfer and splitting. Anim. Prod. 36, 341-353.

NOLAN, C.J., BULL, R.C., SASSER, R.G., RINDER, C.A., PANLASIGIN, P.M., SCHOENEMAN, H.M., REEVES, J.J. (1988). Postpartum performance in crude protein-restricted beef cows: effect on the hypothalamic-pituitary-ovarian axis. J. Anim. Sci. 66, 3208-3217.

NOTTER, D.R. (1986). Feed intake specifications in defining breeding objectives. Proc. 3rd Wld. Congr. Genet. Appl. Livest. Prod. IX, pp 233-243. Nebraska, U.S.A.

O'FARREL, R.J. (1978). Heat detection- an observation problem. Food and Farm Research 9, 95-97.

ODDE, K.G., WARD, H.S., KIRACOFE, G.H., McKEE, R.M. and KITTOCK, R.J. (1980). Short oestrous cycles and associated serum progesterone levels in beef cows. Theriogenology 14, 105-112.

OMED, H. (1986). Studies of the relationships between pasture type and quality and the feed intake of grazing sheep. PhD thesis, University of Wales.

OWEN, J.B. (1986). The Welsh Black cattle group breeding scheme. Welsh Black Cattle Soc. J. 2, 41-46.

PARKES, A.S. (1957). The development in Great Britain, 1949-52, of the technique of preserving bull semen in the frozen state. Vet. Rec. 69, 463-464.

PARNELL, P.F., BAKER, R.L. and FOULLEY, J.L. (1986). The efficiency and place of multiherd animal evaluation procedures for beef cattle. Proc. Wld. Congr. Genet. Appl. Livest. Prod. IX, 373-388. Nebraska, U.S.A.

PENNING, P.D. and HOOPER, G.E. (1985). An evaluation of the short-term weight changes in grazing in grazing sheep for estimating herbage intake. Grass and Forage Sci. 40, 79-84.

PERRY, B. and DONALDSON, L. (1984). The use of closprostenol, fenprostalene and PGF2 alpha in the superovulation of cows. Theriogenology 21, 250 abstr.

PETERS, A.R. (1989). Changes in electrical resistance of the vaginal mucosa in prostaglandin-treated cows. Vet. Rec. 124, 505-507.

PETERS, A.R. and BALL, P.J.H. (1987). Reproduction in cattle. Butterworths, London. pp 40-51.

PETERS, A.R. and LAMMING, G.E. (1984). Reproductive activity of the cow in the postpartum period. II. Endocrine patterns and the induction of ovulation. Br. Vet. J. 140, 269-280.

PETERS, A.R., PIMENTAL, M.G. and LAMMING, G.E. (1985). Hormone responses to exogeous GnRH pulses in postpartum dairy cows. J. Reprod. Fert. 75, 557-565.

PETERS, A.R. and RILEY, G.M. (1982a). Is the cow a seasonal breeder ? Br. Vet. j. 138, 533-537.

PETERS, A.R. and RILEY, G.M. (1982b). Milk progesterone profiles and factors affecting the postpartum ovarian activity in beef cows. Anim. Prod. 34, 145-153.

PHILLIPS, C.J.C. and DENNE, S.K.P.J. (1988). Variation in the grazing behaviour of dairy cows measured by a Vibrarecorder and a bite count monitor. Appl. Anim. Behaviour Sci. 21, 329-335.

PICKARD, D.W. (1986). Minerals and vitamins. In: Principles and practice of feeding dairy cows (Eds. Broster, W.H., Phipps, R.H. and Johnson, C.L.). Technical Bulletin 8, N.I.R.D. pp 73-94.

PRADO DELGADO, A.R., ELSDEN, R.D. and SEIDEL, G.E. Jr. (1989). Effects of GnRH on superovulated cattle. Theriogenology 31, 317-321.

PRATHER, R.S., SPIRE, M.F. and SCHALLES, R.R. (1987). Evaluation of cryopreservation techniques for bovine embryos. Theriogenology 28, 195-204.

PRESTON, T.R. and WILLIS, M.B. 1974. Intensive beef production. Pergamon Press, Oxford.

PUTNEY, D.I., DROST, M. and THATCHER, W.W. (1988). Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between days 1-7 post insemination. Theriogenology 30, 195-209.

RALL, W.F. and FAHY, G.M. (1985). Ice-free cryopreservation of mouse embryos at -19°C by vitrification. Nature, London. 313, 573-575.

RASBECH, N.O. (1979). Instruments for non-surgical collection and transfer of bovine embryos. Br. Vet. j. 135, 185.

REEVES, J.J. (1987). Endocrinology of Reproduction. In: Reproduction in Farm Animals (Ed. Hafez, E.S.E.). Lea and Febiger, Philadelphia, U.S.A. pp 85-106.

REIGER, D.,DESAULNIER, D. and GOFF, A.F. (1988). Ovulatory responses and embryo yield in superovulated Holstein heifers given a priming dose of FSH-P at day 2 of the oestrous cycle. Theriogenology 30, 695-699.

RHIND, S.M., DONEY, J.M., GUNN, R.G. and LESLIE, I.D. (1984). Effects of body condition and environmental stress on ovulation rate, embryo survival and associated plasma follicle stimulating horone, prolactin and progesterone profiles in Scottish Blackface ewes. Anim. Prod. 38, 201-209.

ROBERTSON, A. and RENDEL, J.M. (1950). The use of progeny testing with artifical insemination in dairy cattle. J. Genet. 50, 21-31.

ROBINSON, J.J. (1986). Nutrition and embryo loss in farm animals. In: Embryonic mortality in Farm Animals (Eds. Sreenan, J.M. and Diskin, M.G.) C.E.C., Brussels. pp 235-248.

ROCHE, J.F. (1979). Control of oestrus in cattle. World Rev. Anim. Production. 15, 49-56.

ROGERS, J.A., ODWONGO, W.O.and CONARD, H.R. (1985). Estimation of forage dry matter intake in lactating dairy cows using a deuterium oxide dilution technique. J. Dairy Sci. 68, 2596-2601.

ROWSON, L.E.A. and DOWLING, D.F. (1949). An apparatus for the extraction of fertilized eggs from the living cow. Vet. Rec. 61, 191.

ROWSON, L.E.A. and MOOR, R.M. (1966). Non-surgical transfer of cow eggs. J. Reprod. Fert. 11, 311-312.

ROWSON, L.E.A., MOOR, R.M. and LAWSON, R.A.S. (1969). Fertility following egg transfer in cow, effect of method, medium and synchronization of oestrus.

RUANE, J. (1988). Review of the use of embryo transfer in the genetic improvement of dairy cattle. Anim. Breed. Abs. 56, 437-446.

SCANLON, P., SREENAN, J. and GORDON, I. (1968). Hormonal induction of superovulation in cattle. J. Agric. Sci. 70, 179-182.

SCHIEWE, M.C., LOONEY, C.R., HILL, K.G., JOHNSON, C.A. and GODKE, R.A. (1983). Transferable embryo recovery rates following different AI schedules in beef donor cattle. Theriogenology 19, 147 abstr.

SCHOFIELD, S.A. 1988. Oestrus detection and oestrous behaviour of dairy cows in different environments. Ph.D. thesis, University of Wales.

SEIDEL, G.E. Jr. (1981). Superovulation and embryo transfer in cattle. Science 211, 351-358.

SEIDEL, G.E.Jr. and SEIDEL, S.M. (1988). Analysis of applications of ET in developing countries. Theriogenology 31, 3-16.

SNEDECOR, G.W. and COCHRAN, W.G. (1980). Statistical methods. Iowa State University Press. U.S.A. pp 215-236.

SHEA, R.F. (1981). Evaluating the bovine embryo. Theriogenology 15, 31-42.

SHEA, R.F., JANZEN, R.E. and McDORMAND, D.P. (1984). Seasonal variation to stimulation and related embryo transfer procedures in Alberta over a 9 year period. Theriogenology 21, 186-195.

SHORT, R.E., BROOKS, A.N., PETERS, A.R. and LAMMING, G.E. (1987). Opoid modulation of LH secretion during the oestrous cycle of heifers. J. Reprod. Fert. 80, 213-219.

SHELTON, J. (1984). Techniques involving embryos and oocytes. Animal Production in Australia. 15, 31-34.

SIMM, G. (1983). The use of ultrasound to predict the carcass composition of live cattle: A review. Anim. Breeding Abstr. 51, 853-875.

SIMM, G., SMITH, C. and PRESCOTT, J.H.D. (1985). Environmental effects on bull performance test results. Anim. Prod. 41, 177-185.

SINGH, E.L. (1987). The disease potential of embryos. Theriogenology 27, 9-20.

SMITH, C. (1986). Use of embryo transfer in genetic improvement of sheep. Anim. Prod. 42, 81-88.

SMITH, C. (1988). Applications of embryo transfer in animal breeding. Theriogenology 29, 202-212.

SMITH, J.F., FAIRCLOUGH, R.J., PAYNE, E. and PETERSON, A.S. (1975). Plasma hormone levels in the cow. I. Changes in progesterone and oestrogen during normal oestrous cycles. N.Z.J. Agrc. Res. 18, 123.

SMITH, J.F., PAYNE, E., TERVIT, H.R., McMGOWAN, L.T., FAIRCLOUGH, R., KILGOUR, R. and GOULD, P.G. (1981). The effect of suckling upon endocrine changes associated with anoestrus in identical cattle. J. Reprod. Fert. Suppl. 30, 241-249.

SOUTHGATE, J.R., COOK, G.L. and KEMPSTER, A.J. (1982). A comparison of the different breeds and crosses from the suckler herd. I. Liveweight growth and efficiency of food utilisation. Anim. Prod. 35, 87-98.

SPITZER, J.C., NISWENDER, G.D., SEIDEL, G.E., Jr. and WILTBANK, J.N. (1978). Fertilization and blood levels of progesterone and LH in beef heifers on a restricted energy diet. J. Anim. Sci. 46, 1071-1077.

SREENAN, J.M. (1975). Successful non-surgical transfer of fertilized cow eggs. Vet. Rec. 96, 490-491.

SREENAN, J.M. (1978). Non-surgical egg recovery and transfer in the cow. Vet. Rec., 102, 58-60.

SREENAN, J.M. (1983). Embryo transfer procedure and its use as a research technique. Vet. Rec. 112, 494-500.

SREENAN, J.M. and BEEHAN, D.(1976a). Methods of induction of superovulation in the cow and transfer results. In Egg Transfer in Cattle (Ed. Rowson, L.E.A.) pp 19-34. C.E.C. Brussels.

SREENAN, J.M., BEEHAN, D. (1976b). Effect of site of transfer on pregnancy and twinning rates following bilateral egg transfer in the cow. J. Reprod. Fert. 48, 223-224.

SREENAN, J.M. and DISKIN, M.G. (1983) Early embryonic mortality in the cow: its relationship with progesterone concentration. Vet. Rec., 112, 517-521.

SREENAN, J.M. and DISKIN, M.G. (1987). Factors affecting pregnancy rate following embryo transfer in the cow. Theriogenology 27, 99-113.

SREENAN, J.M. and GOSLING, J.P. (1977). The effect of cycle stage and plasma progesterone level on the induction of multiple ovulations in heifers. J. Reprod. Fert. 50, 367-369.

STANLEY, C.J., PARIS, F., WEBB, A.E., HEAP, R.B., ELLIS, S.T., HAMON, M., WORSFOLD, A. and BOOTH. J.M. (1986). Use of a new and rapid milk progesterone assay to moniyor reproductive activity in the cow. Vet. Rec. 118, 664-667.

STEANE, D.E., GUY, D.R. and SMITH, C. undated. Group breeding schemes for beef cattle. M.L.C.

STEWART, F., ALLEN, W.R. and MOOR, R.M. (1976). Pregnant mares serum gonadotrophin : ratio of follicle stimulating hormone and lutenizing hormone activities measured by radio-receptor assay. J. Endocrinol. 71, 371-382.

STEWART, R.E., MEE, M.O. and STEVENSON, J.S. (1987). Concentrations of progesterone and conception rates in Holstein heifers at hCG administrationduring the first 3 weeks after oestrus. J. Anim. Sci. Suppl. 65, 376 abstr.

STOBBS, T.H. (1973). The effect of plant sturcture on intake of tropical pastures. 1. Variation in the bite size of grazing cattle. Aus. J. Agric. Res. 24, 821-829.

STOBBS, T.H. and COWPER, L.J. (1972). Automatic measurement of jaw movements of dairy cows during grazing and rumination. Tropical Grasslands 6, 107-112.

SUGIE, T. (1965). Successful transfer of a fertilised bovine egg by non-surgical procedures. J. Reprod. Fert. 10, 197-201.

TANAKA, H., FUINKAWA, T. and YAMADA, Y. (1983). Breeding plan for beef cattle by embryo transfer. Proc. 5th World Conf. Anim. Prod. 2, 39-40.

TERVIT, H.R., SMITH, J.F. and KALTENBACH, C.C. (1977). Post-partum anoestrus in beef cattle: a review. Proc. N.Z. Soc. Anim. Prod. 37, 109-199.

THEISSEN, R.B. and TAYLOR, St.C.S. (1986). Interage correlations of food conversion efficiency with food intake within and between British breeds of cattle. Anim. Prod. 43, 91-100.

THEISSEN, R.B., TAYLOR. St.C.S. and MURRAY, J. (1985). Multibreed comparisions of British breeds of cattle, variation in relative growth rate, relative food intake and food conversion efficiency. Anim. Prod. 41,193-199.

THIBIER, M. and NIBART, M. (1987). Disease control and embryo importations. Theriogenology 27, 37-47.

THOMPSON, J.M. and BARLOW, R. (1986). The relationship between feeding and growth parameters and biological efficiency in cattle and sheep. 3rd World Congress Genet. Appl. Livest. Prod. XI pp 271-282.

THOMSON, M.S., BIRD, R.C., STRINGFELLOW, D.A., ROSSI, C.R. and LAURERMAN, L.H. (1989). Chemically induced viral resistance in preimplantation bovine embryos. Theriogenology 31, 267 abstr.

TOPPS, J.H. (1977). The relationship between reproduction and undernutrition in beef cattle. World Rev. Anim. Prod. 13, (2), 43-49.

TULLOCH, A.P. (1981). Composition of epicuticular waxes from 28 genera of Graminae : differences between sub-families. Can. J. Bot. 59, 1213-1221.

TULLOCH, A.P. and WEENIK, R.D. (1969). Composition of the leaf wax of Little Club wheat. Can. J. Chem. 47, 3119-3126.

VINCENT, C.K., MILLS, A.C., RUNDELL, J.W. (1965). Non-surgical transfer of embryos in beef cattle. J. Anim. Sci. 28, Abstr. No. 90.

VAN der ZWALMEN, P., TOUTAI, K., ECTORS, F.J., MASSIP, A., BECKERS, J.F. and ECTORS, F. (1989). Vitrification of bovine blastocysts. Theriogenology 31, 270 abstr.

VOSS, H.J., ALLEN, S.E., FOOTE, R.H., IM, P., KIM, G.K. and AQUADO, P. (1988). Buserelin in a superovulatory regimen in commercial herds. 1. pituitary and ovarian hormone responses. Theriogenology 31, 371-384.

WARE, C.B., NORTHEY, D.L. and FIRST, N.L. (1987). Effect of administration of FSH at the beginning of the cycle on the subsequent response to superovulation treatment in heifers. Theriogenology 27, 292.

WAGNER, H.-G.R. (1987). Present status of embryo transfer in cattle. World Animal Review 64, 2-11.

WEBSTER,A.J.F. (1985) Differences in the energetic efficiency of animal growth. J. Anim. Sci. 61, Suppl. 2., 92-103.

WHITTINGHAM, D.G. (1971). Survival of mouse embryos after freezing and thawing. Nature, London. 233, 125-126.

WIEBOLD, J.L. (1988). Embryonic mortality and the uterine enviroment in first service lactating dairy cows. J. Reprod. Fert. 84, 393-399.

WILLETT, E.L., BLACK, W.G., CASIDA, L.E. STONE, W.H. and BUCKNER, P.J. (1951). Successful transplantation of a fertilized bovine ovum. Science 113, 247.

WILMUT, I. and HUME, A. (1978). The value of embryo transfer to cattle breeding in Britain. Vet. Rec. 103, 107-110.

WILMUT, I. and ROWSON, L.E.A. (1973). Experiments on the low temperature preservation of cow embryos. Vet. Rec. 103, 107-110.

WILKINSON, S.C. and MACKIE, C.K. (1988). The use of long chain n-alkanes for estimating intake and digestibility of herbage in growing cattle. Research meeting No. 1, British Grassland Soc., Sept 1988.

WOOLIAMS, J.A. and WILMUT, I. (1989). Embryo manipulation in cattle breeding and production. Anim. Prod. 48, 3-30.

WRIGHT, I.A. and WHYTE, T.K. (1989). Effect of sward height on the performance of continuously stocked spring calving beef cows and their calves. Grass and Forage Sci. 44, 259-266.