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ASPECTS OF THE SURVIVAL AND WELFARE OF NEONATAL CALVES:

A thesis presented in partial fulfillment of the requirements for the degree of

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ABSTRACT

Neonatal calves form the basis of all dairy and beef herds. There are many factors that influence calf survival and welfare and therefore the economic viability of the farm. This thesis examines four factors that have the potential to affect the survival and welfare of neonatal calves.

1. Induction of premature parturition in dairy cows results in the birth of calves that have a higher mortality rate than those born at full-term. The mortality rate of these calves decreases as their gestational age approaches full-term. The aims of the study described in chapter two were to assess the physical and physiological state of calves born at varying gestational ages. Sixty-eight induced calves that were born up to five weeks prematurely were examined and the following observations and measurements were recorded: body weight, crown rump length, rectal temperature, the number of deciduous incisors erupted and palpable, breathing rate, heart rate, gum colour, hoof membranes, serum gamma glutamyl transferase activity and a general assessment of coordination. It was found that calves born prematurely had lighter body weights, shorter crown rump lengths, lower rectal temperatures, fewer deciduous incisor teeth erupted and that they breathe more slowly. It was concluded that welfare of calves born more than 3 weeks prematurely (<259 days gestational age) were more likely to die or become ill because of a reduced ability to maintain their body temperature and difficulty breathing and feeding effectively.

2. Although many premature calves die on the farm a few are kept for rearing and the remainder are sent for slaughter. While the calves remain on the farm their welfare and survival depends on them being fed colostrum or milk at appropriate volumes and intervals. Calves that are sent for slaughter may go without food for up to 30 hours. Although the metabolic responses of full-term calves to feeding and fasting have been examined those of premature calves have not. In chapter three the responses of premature calves to different feeding frequencies (100-120 ml colostrum /kg bodyweight split into either 2 or 4 feeds of equal volume over ten hour period) and to 30

hours fasting were examined. The calves were either four or ten days old at the start of the experiment and varied in gestational age at birth. The effects of feeding frequency and fasting were assessed using the changes in plasma glucose, β-hydroxybutyrate, triglycerides, urea, total plasma protein concentration, packed cell volume and the rectal temperature.

It was found that premature calves tended to have lower plasma glucose and higher β-hydroxybutyrate concentrations than those born at full term indicating a greater dependence on lipids to provide their energetic requirements. The wide variation in these parameters was primarily determined by the gestational age of the calves at birth. Calves born further from full-term apparently depend on lipid catabolism to a greater degree.

The premature calves gained no apparent energetic advantage when fed a similar volume of milk in four feeds as compared with twice within ten hours.

There was considerable variation in the ability of the calves to tolerate 30 hours of fasting. The availability of energy in fasted calves (as indicated by plasma glucose concentration) was greater in those fed 50 ml colostrum/kg bodyweight rather than 25 ml/kg at the onset of fasting. Calves born at an earlier gestational age had a reduced ability to tolerate 30 hours without food. A decrease in rectal temperature during fasting was seen in some of the more premature calves. This period of fasting did not cause dehydration as indicated by the absence of significant increases in packed cell volume or total plasma protein concentration. The calves' postnatal age did not affect their responses to either feeding frequency or fasting.

3. The fetal membranes of twin calves often fuse where they abut resulting in varying degrees of placental vascular anastomosis. The gross anatomy of placentas from 18 twin-bearing cows, and the PCV changes of their calves between birth and 24 hours

were examined. Six classes of vascular anastomosis were identified. The extent of anastomosis varied from none, to extensive. The PCV decrease over the 24 hours following birth differed to a greater extent between twins from placentas exhibiting a high degree of anastomosis. It was concluded that these differences were likely to have been due to blood transfer within the joined placental vessels of the calves during birth.

4. The intake of colostral immunoglobulins gives neonatal calves the ability to resist disease. However, the capacity to absorb immunoglobulins decreases rapidly between 6 and 24 hours after birth. In the study described in chapter five it was found that failure of passive transfer of immunoglobulins, as indicated by low gamma glutamyl transferase activity, occurred in approximately 45% of the 74 dairy calves from which blood samples were taken. Calves that had not received colostrum were not easily distinguished from those that had on the basis of obvious physical or behavioural features.

The behaviour of 21 cows and their calves was observed between birth and the time the calves were separated from their dams. The amount of time the calves spent with their dams ranged from 74 minutes to 1492. Thirty-three percent of calves had not sucked within this time. Of those calves that did suck, 79% did so within 6 hours of their birth. There were no obvious behaviours on the part of the cows or their calves that influenced the time between birth and first sucking or the amount of time spent sucking by each calf.

Animal Ethics

Ethics approval was obtained from the Massey University Animal Ethics Committee for all experiments described in this thesis.

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Chapter 1: Introduction

Neonatal calves are vulnerable, and tend to evoke empathy in humans. This promotes good husbandry practices supported by the awareness that calves form the basis of the future herd and profitability of the farm. However, in New Zealand only 36% of full-term dairy calves are reared (Bunny,1992) as replacement heifers or dairy beef. The remainder are a 'by-product' of the dairy industry. These calves which are not reared are of little economic value and after being kept for four days are usually sent for slaughter as 'bobby' calves (AWAC,1993; 1994; 1997). Thus a conflict is created between the natural tendency to care for these animals and the practical realisation that there is little economic benefit in doing so.

Beef calves are born outdoors and are reared by their mothers and there is consequently a high rate of calf survival provided that the cow and calf are well nourished and kept in an environment conducive to their health (Webster,1984). The mortality rate in suckler beef calves one day to six months of age has been reported as 0.5% to 3.6% (Webster,1984). In contrast the mortality rate of dairy calves is higher ranging between 6.3% and 14.3% (Webster,1984; Bunny,1992). Dairy calves are born on farms where the primary product is milk. Maximising milk production depends on the cow and calf being separated soon after birth. In New Zealand dairy calves are separated from their mothers within a day or two of birth and then reared by farm staff. The management of dairy calves by the farm staff results in considerable variation in their survival rates and welfare (Webster,1984).

Economically, the survival of beef calves and replacement dairy heifer calves and their subsequent growth into adult cattle is of primary importance. The economic viability of dairy and beef farms depends on the efficient production and sale of milk, beef and replacement stock, none of which would be possible if neonatal calves did not survive, grow and then reproduce.

The low economic value of dairy calves is reflected in the practice of inducing premature parturition in dairy cattle. Induction is a management procedure that allows all cows within a herd to be maintained at a similar stage of lactation, however it results in the birth of a large number of premature calves each year (MacDiarmid,1983ab). The mortality rate of induced calves is generally greater than that in full-term calves (Welch *et al.*,1973; O'Farrell and Crowley,1974; O'Farrell and Langley, 1975; Thomas,1975; MacDiarmid,1980; Bunny,1992). Bunny (1992) found that the mortality rate of induced dairy calves was 61%, significantly higher than the 7% mortality rate found for full-term dairy calves in the same study. Clearly the ability of the induced calves to survive is adversely affected by their premature birth.

Although cattle have been domesticated and used to benefit humanity for thousands of years it is only relatively recently that society has begun to examine our ethical responsibility to domestic animals. In 1965 the Brambell Committee accepted that rather than being unconscious, insentient, automata some animals (such as the higher vertebrates) can experience pain and suffering as well as stress and emotions (Monamy,1996). Acknowledgment of these traits in non-human animals suggests that we have sufficient reason to be concerned for their welfare (Monamy,1996).

The increasing concern in society for the welfare of animals, rather than just their survival, is reflected by the development of new legislation relating to animal welfare, an increase in vegetarianism (with concern about animal cruelty cited as the primary reason for not eating meat) and the recognition of potential international trade sanctions as the result of real or perceived inhumane practices (Mellor, 1992a; Gregory,1996; Orr,1996). Whether motivated by a personal ethical commitment to 'right' action, social pressure, practical benefits, potential economic gain or the threat of legal sanctions, the promotion of calf welfare is likely to improve or maintain their quality of life (Mellor, 1992a; Gregory,1996).

Animal welfare can only be maintained or improved if there is an agreed definition of what it is. Many different definitions have been proposed (Brambell Committee, 1965; van Putten, 1973; Dawkins,1980; Curtis and Stricklin,1991; Broom, 1993; Hutson,1994a). That given by Hutson (1994a), although general, encompasses many of these and asserts that "welfare is a state that ...is the result of interaction with the environment, ... (that) has physical and mental components". An animal's state of welfare may be seen as a reflection of the quality of its environment. That is, it reflects the way in which its physical, physiological, behavioural and psychological needs are being affected by external variables. A high quality environment (one that promotes good welfare) would be likely to include factors that allow an animal to maintain homeostasis, and achieve psychological and physical satisfaction.

A calf's state of being may be described as somewhere along a continuum between the negative state of suffering and the positive state of good welfare (Mellor and Reid,1994; Hutson,1994a). Suffering is characterised by the experience of anxiety, fear, pain and distress. Good welfare is the state being manifest in an animal when its nutritional, environmental, health, behavioural and mental needs are met (Mellor and Reid,1994). These categories are based on the five freedoms formulated by the Farm Animal Welfare Council of the UK (FAWC,1992). They are as follows:

- Freedom from thirst, hunger and malnutrition
- Freedom from discomfort
- Freedom from disease, injury and pain
- Freedom to display normal patterns of behaviour
- Freedom from distress

It must be recognised however, that it is likely that no system, natural or otherwise exists in which none of these areas are compromised (Mellor and Reid,1994). Taking this into consideration it is perhaps more meaningful and practical to assess the degree of compromise in each of these five areas. Hence, the 'five freedoms' were converted by Mellor and Reid (1994) into the five domains of potential welfare compromise.

These are as follows:

Domain 1:	Nutritional	Dehydration/ Undernutrition/ Malnutrition
Domain 2:	Environmental	Environmental Challenge
Domain 3:	Health	Disease/ Injury/ Functional Impairment
Domain 4:	Behavioural	Behavioural Restriction/ Interactive Restriction
Domain 5:	Mental	Anxiety/ Fear/ Pain/ Distress

Compromise in domains one, two, three or four will usually also register as mental compromise in domain five which represents the components of suffering (Mellor and Reid, 1994)

When the physiological, physical and behavioural capabilities of calves up to two weeks of age are taken into consideration the potential for welfare compromise in each of the five domains can be considered. The potential for compromise will vary depending on the internal and external environment of the calves in question. These will be affected by many factors including the production system they are born into (beef or dairy), their treatment by farm staff and the maturity of the calf at birth.

In Table 1.1 the potential for compromise in the case of the full-term beef calf, full-term dairy calf and premature induced dairy calf is considered. During the first two weeks of life the beef calf is kept in a near natural state. This provides a good comparison for the dairy calf that is separated from its mother soon after birth and reared artificially.

Although we are familiar with the capabilities of beef and dairy calves born at full-term and have a basic awareness of the ways in which their welfare may be compromised, there has been little research into the physiological state of calves that are born prematurely. Immaturity of the physiological systems necessary for survival is likely to cause a greater potential for welfare compromise in induced calves. The degree of immaturity and therefore the potential for compromise would be likely to increase the earlier an induced calf is born.

Table 1.1: The ways in which the welfare of full-term beef, full-term dairy and premature induced dairy calves may be compromised between birth and two weeks of age.

Domain	Full-term Beef Calf	Full-term Dairy Calf	Premature Induced Dairy Calf
1: Nutritional			
Water Deprivation (Thirst)	-/√	-/√	√
Undernutrition (Hunger)	-/√	-/√	√
Malnutrition	-	-/√	√
2: Environmental			
Environmental Challenge	-/√	-/√	√√
3: Health			
Disease	√	√√	√√
Injury	√	√	√
Functional Impairment	-	-	√√
4: Behavioural			
Behavioural Restriction	-	√	√
Interactive Restriction	-	√	√
5: Mental			
Anxiety	-/√	-/√	-/√
Fear	√	√	-/√
Pain	√	√	-/√
Distress	√	√	√

- = Compromise unlikely; √ = Potential for compromise;

√√ = Strong potential for compromise

1.1 Domain One: Nutritional

During the first two weeks of life the natural sources of liquid and nutrients for all calves are colostrum and milk (Webster,1984). The constituents of cow's milk are easily digested and usually balanced to meet a calf's nutritional requirements (Webster,1984). A calf fed on sufficient colostrum and cow's milk is therefore unlikely to experience welfare compromise through malnutrition (Webster,1984).

1.1.1 Beef Calves

Beef calves are normally left with their dams and have ready access to milk *ad libitum* thereby reducing the likelihood of undernutrition or dehydration through intake of insufficient volumes of milk. However, if sucking is prevented by rejection of the calf by the cow or weakness of the calf or cow, or if sucking is allowed but lactation is impaired making insufficient milk available, a calf's welfare may be compromised through the experience of undernutrition and dehydration.

1.1.2 Full-term Dairy Calves

Dairy calves are usually left with their dams for up to two days following their birth after which time they are separated. Before separation, initiation of sucking depends on facilitative interaction between the cow and calf. However, the long-term selection of dairy cows for physical and behavioural characteristics that increase milk production and ease of handling have adversely affected suckling behaviour in dairy cows and sucking behaviour by their calves (Edwards,1982; Edwards and Broom,1982; Edwards and Broom;1983; Ventorp and Michanek,1992). Dairy calves are more likely than beef calves to experience welfare compromise through undernutrition or dehydration because of failure to suck or a delay between birth and first sucking during the time they remain with their mother.

After dairy calves are separated from their dams they are dependent on farm staff to provide them with appropriate volumes of milk or milk-replacer at correct intervals. While whole cow's milk is nutritionally balanced, liquid milk-replacers may not be, thereby presenting the potential for compromise through malnutrition in calves fed on these artificial diets (Webster,1984).

Dairy calves may have access to milk or milk-replacer from feeders *ad libitum* or be fed at regular intervals, usually twice daily (AWAC,1993). They must be taught to feed from a calf feeder (Webster,1984). If they do not learn to feed from the feeder or if access to the feeder is restricted through competition with other calves they may experience undernutrition and/or dehydration.

During the first week of life, calves left with their mother will normally feed 7 to 10 times a day and will seldom consume more than one litre in a feeding session (Webster,1984). Feeding dairy calves larger volumes of milk with long intervals between feeds may overload the abomasum altering the normal flow of digesta into the small intestine and increase the risk of enteritis and enterotoxaemia (Webster,1984).

1.1.3 Induced Calves

An induced calf's ability to feed effectively and digest milk may be compromised through weakness or an impaired ability to feed (Adams,1969; Welch *et al.*,1973; Bailey *et al.*,1973; Aldridge *et al.*,1992). Like full-term calves, compromise through undernutrition, dehydration or enteritis is possible if the volume of milk consumed is insufficient or if feeding occurs at inappropriate intervals. Depending on the degree of prematurity of an induced calf its nutritional requirements in terms of frequency of feeding and volume consumed may differ from those of full-term calves. As with full-term calves malnutrition would be a source of welfare compromise only if the calves were not fed cow's milk or nutritionally balanced milk replacer.

1.2 Domain Two: Environmental

The environmental needs of a young calf can be summarised as being thermal comfort, physical comfort and space (Webster,1984). Thermal comfort is derived from the external environment being neither too hot nor too cold, or too wet or dry so as to cause distress (Webster,1984). The calf must be able to maintain its body temperature by balancing heat loss to the environment with heat production. Physical comfort is derived from contact with surfaces that do not cause irritation, injury or discomfort.

According to the Brambell Committee Report (1965) a calf should have at least enough space to stand up, lie down, turn round, stretch its limbs and groom itself.

At the time of their birth calves go from the thermoneutral, sheltered environment *in utero* into one that is changeable. Being precocial species, the neonates of cattle and other ungulates can maintain their deep body temperature over a wide range of environmental temperatures within hours of birth (Randall,1978). In New Zealand calves are usually born outdoors on pasture into an environment in which the air temperature is likely to be cooler than that of their body. They will also be exposed to varying amounts of sunshine, wind, rain and in some cases snow. Therefore, the main potential for welfare compromise is likely to come from hypothermia.

Hypothermia is the result of the rate of heat loss being greater than heat production. It may be due to excessive heat loss or insufficient heat production (Mellor and Cockburn,1986; Carstens,1994). The risk of a neonatal calf becoming hypothermic depends on a number of factors including the maturity of thermogenic and thermoregulatory systems, the amount of body energy reserves (lipids and glycogen), the environmental conditions and the time between birth and first sucking (Alexander *et al.*,1973; Randall, 1978; Mellor and Cockburn,1986; Carstens,1994). Lipids and glycogen are the energy substrates used during shivering and non-shivering thermogenesis (Alexander *et al.*,1973; Mellor and Cockburn,1986). The rate with which they are used depends on the environmental temperature. The rate of thermogenesis required at cooler air temperatures is greater and therefore under these conditions energy reserves are used faster (Mellor and Cockburn,1986). Sucking provides an external source of energy and replenishes body energy reserves allowing heat production to be continued. For this reason the time between birth and first sucking strongly influences the duration of time that heat production and normal body temperature can be maintained.

1.2.1 Beef Calves

Given that beef calves are quick to stand and suck after birth (Selman *et al.*,1970b) they are unlikely to suffer from hypothermia as a result of depletion of body energy reserves. They usually remain in their birth environment with their mother and are therefore not likely to have their access to milk restricted, or be subject to restricted space or adverse physical conditions. Under the birth conditions experienced in New Zealand they would also be unlikely to become hypothermic due to excessive heat loss if milk is available. Therefore if there are no unusual circumstances such as a difficult birth, extreme weather conditions, rejection or separation as the result of misadventure, beef calves would be unlikely to experience welfare compromise through environmental challenge.

1.2.2 Dairy Calves

The external environmental conditions experienced at birth and amount of energy reserves available to a dairy calf are likely to be similar to those experienced by beef calves. Dairy calves are slower to stand and suck than beef calves (Selman *et al.*,1970b). The weaker maternal behaviour of dairy cows is likely to increase the time between birth and first sucking (Selman *et al.*,1970a; Edwards and Broom,1982) which may make dairy calves more susceptible to hypothermia if weather conditions are adverse. Once separated from their dams, dairy calves are usually kept in a rearing facility, the structure and conditions of which will vary (Webster,1984). These facilities may increase or reduce the risk of welfare compromise through hypothermia or physical discomfort depending on the amount of shelter they provide from the weather, whether the calves can choose shelter or outdoor conditions, the amount of space provided, the housing surfaces and type of flooring provided (Webster,1984).

1.2.3 Induced Calves

Induced calves will be as susceptible to compromise through physical discomfort as full-term dairy calves but are likely to be more susceptible to hypothermia. Heat production may be impeded by immaturity of the systems used in thermogenesis and thermoregulation (Alexander *et al.*,1973). If the amount of glycogen and lipid in body reserves is less or their ability to feed is limited they would also be more likely to become hypothermic through exhaustion of energy substrates. The rate of heat loss in

premature calves may be greater because of a proportionately greater surface area and reduced tissue insulation (Randall, 1978; Carstens,1994).

1.3 Domain Three: Health

1.3.1 Disease

Immunoglobulins are necessary to help resist the disease-causing pathogens acquired from the external environment. Calves do not receive immunoglobulins through placental transfer and depend on the intake of colostral immunoglobulins (Michanek and Ventorp,1989; Wedasingha,1992; Besser and Gay,1994). The ability to absorb immunoglobulins decreases over the first 24 hours after birth. Therefore, the time between birth and first sucking will strongly influence the amount of immunoglobulins absorbed and the potential for welfare compromise through disease (Ventorp and Michanek,1991; Stott *et al.*,1979ab; Webster,1984; Edwards *et al.*,1982; Michanek and Ventorp, 1989).

Beef calves have previously been observed to suck sooner after birth than dairy calves reducing the likelihood of compromise through low immunity to pathogens in these breeds (Selman *et al.*,1970ab).

The greater length of time between birth and first sucking commonly seen in dairy calves and the weak mother-young bonding in these breeds increases the likelihood of immunocompromise as the result of insufficient and/or delayed colostrum intake (Selman *et al.*,1970ab; Edwards and Broom,1982). Rearing dairy calves indoors can allow the build up of pathogens and increased contact with other calves so that the exposure to pathogens is increased (Webster,1984). Because of this dairy calves are more likely to contract common calf diseases such as enteritis linked to diarrhoea, and pneumonia (Webster,1984).

Premature calves are kept under similar conditions to full-term dairy calves and are also susceptible to welfare compromise through disease if they do not receive sufficient

colostrum soon after birth. An impaired ability to stand, find the udder, suck or absorb colostral immunoglobulins will reduce the likelihood of adequate colostrum intake and thereby increase the risk of disease (Muller *et al.*,1975; Beardsley *et al.*,1976; Langley and O'Farrell,1976; Naylor,1986; Bunny,1992).

1.3.2 Injury

The welfare of calves may be compromised through injury. Neonates have no experience of the extrauterine world and would be unlikely to recognise dangerous situations predisposing them to injury through misadventure. This would increase their chance of injury, when compared to an adult. Calves born on to rugged terrain would be more likely to becomes injured through misadventure than those born onto flat paddocks.

Some induced calves are lethargic and unresponsive at birth. It is possible that cows may accidentally injure their newborn hypoactive calves in attempt to induce activity, thereby increasing the risk of injury in these calves. Otherwise, the risk of injury would be similar for beef, dairy and premature calves. Injury during birth itself is likely to be equally prevalent in all three types of calves. Of the three factors that make up domain three, injury is the least likely overall to be a source of welfare compromise.

1.3.3 Functional Impairment

At birth the mammalian neonate undergoes a large number of changes as it adapts to extrauterine conditions. Impairment of any of the physiological systems in which postnatal adaption is required would be likely to compromise the welfare of the neonate and perhaps result in death. The risk would be similar for beef and dairy calves.

Immaturity of physiological systems would constitute functional impairment. Therefore, welfare compromise through functional impairment is more likely in induced calves than in those born at full-term. Functional impairment would be the major source of potential welfare compromise in premature induced calves with the degree of impairment due to immaturity being greater in those born at an earlier gestational age.

1.4 Domain Four: Behavioural and Interactive Restriction

1.4.1 Beef Calves

Beef calves normally remain with their dams for the first 6 months of life and therefore are unlikely to experience welfare compromise through behavioural or interactive restriction. Under these circumstances they would be able to behave and interact freely.

1.4.2 Full-term and Induced Dairy Calves

Dairy calves born either prematurely or at full-term are usually separated from their dams within two days of their birth. From this time their lives and consequently their behaviour is different from that of a beef calf. Of the behaviours expressed by beef calves it is hard to say which may be seen as needs and therefore what constitutes a deprivation or restriction. As yet there is no accepted definition of what a behavioural need is, and thus when behaviour is sufficiently restricted to constitute welfare compromise (Jensen and Toates,1993). Behaviours that may differ in beef calves and dairy calves include feeding behaviour, play, investigative behaviour, and resting behaviour (Webster,1984).

With this acknowledged it can be said that it would be likely that separation of a calf from its dam would constitute interactive restriction. It takes only a few minutes after the birth for the cow-calf bond to be established (Albright and Arave,1997), yet calves appear to adjust to separation relatively quickly. Even so separation would prevent cow-directed interaction or behaviours and would be likely to constitute welfare compromise.

1.5 Domain Five: Mental

1.5.1 Full-term Calves

Suffering is characterised by the experience of anxiety, fear, pain and distress all of which are predominantly mental phenomena. For an animal to suffer it must be both sentient and conscious (Mellor and Reid,1994). Sentience is having the capacity to feel or perceive using the senses (Collins Concise English Dictionary,1978). An animal can be said to be conscious if it is aware of itself and its feelings in relation to its environment (Collins Concise English Dictionary,1978). Without consciousness a

sentient animal is incapable of suffering. A full-term neonatal calf is both sentient and conscious and therefore has the capacity to suffer and experience states of good welfare.

Evidence for the experience of anxiety, fear, distress and pain in calves is gained through observation of their physiology, behaviour and anatomical structure. The potential for compromise through the experience of these emotions depends on the following:

- how aware a calf is of its surroundings or of itself in relation to its surroundings,
- whether it has a concept of uncertainty or an expectation of the way things should be, and
- whether the structural and other features of the systems involved in physiological responses to anxiety, fear and distress are mature enough at the time of birth to allow a physiological response to these stimuli.

Calves are precocious, that is, they are relatively independent and mature at the time of full-term birth (Randall,1978). All of their senses are functional, therefore there is no reason to believe that a calf would not be aware of its surroundings, or its surroundings in relation to itself. The observation that calves often follow their mothers and recognise them as the source of milk is evidence that they are aware of their environment in relation to themselves. Expectation of the way things should be comes through experience and memory, but may also be innate. Although immediately after birth there may be little expectation of the way things should be, this would develop with time. Calves quickly learn to avoid noxious stimuli and also show an escape or startle reaction to the painful stimuli of a needle stick or tail twist.

The physiological systems involved in the experience of anxiety, fear, distress and pain include the central nervous system, the sympathetic-adrenomedullary system and the hypothalamic-pituitary-adrenal system (Dantzer and Mormede,1983). The central nervous system is involved in sensing the environment and the mental processing of this information. Physiological responses to sensory stimuli are generated through the

sympathetic-adrenomedullary system and hypothalamic-pituitary-adrenal systems. Full-term calves can react behaviourally in response to visual, auditory, gustatory, tactile or olfactory stimuli, secrete adrenaline in response to situations that are perceived as threatening, and secrete cortisol in response to painful stimuli or situations that cause distress, providing evidence of the responsiveness of these systems.

1.5.2 Induced Calves

The lack of knowledge regarding the state of induced calves at the time of birth makes it difficult to speculate about their ability to experience compromise within domain five. Given that anxiety, fear, pain and distress are experiences centred in the central nervous system a calf's capacity to experience these states will therefore depend on its cognitive abilities and the degree of CNS development. It is possible that if the CNS of an induced calf was comparatively immature at the time of birth its ability to process sensory input may differ from that of a full-term calf, altering its capacity to suffer.

Full-term birth comes after the development of the hypothalamic-pituitary-adrenal system (HPA axis). Induced parturition mimics the increase cortisol secretion that occurs soon before birth (MacDiarmid,1983a). It is likely that the HPA axis of premature calves is immature at the time of birth, with the degree of immaturity being greater in calves born at earlier gestational ages. Immaturity of the HPA axis of premature calves may interfere with their physiological responses to stimuli that would usually be associated with the experience of anxiety, fear, distress or pain.

1.6 Examining Some Potential Sources of Welfare Compromise in Neonatal Calves

In order to avoid welfare compromise it is necessary to develop an awareness of how the environmental variables under our control affect the animals in our care. Measurement of physiological parameters allows us to discover and define signs of normal and abnormal functioning (Mellor, 1992a). Examining the physiological effects of specific factors allows us to assist domestic animals to maintain homeostasis and thereby actively maintain or improve their welfare.

In New Zealand current knowledge regarding the appropriate treatment of animals has been compiled by the Animal Welfare Advisory Committee in the form of codes of recommendations and minimum standards. The codes containing recommendations applicable to neonatal calves are those concerning the welfare of dairy cattle (AWAC,1992), bobby calves (AWAC,1993; AWAC,1997) and animals transported within New Zealand (AWAC,1994). Scientific findings that support or increase our understanding of the effects of treating animals in the recommended way allow the codes to be strengthened or improved.

This thesis examines four issues that affect the welfare of neonatal calves. As these issues are separate each chapter is complete within itself containing its own introduction, materials and methods section, results section and discussion.

1.6.1 Induced Calves

Induction of parturition is a common practice on New Zealand dairy farms that results in the birth of a large number of premature calves each year. Nevertheless, little is known about the state of these calves at the time of their birth and the ways in which prematurity affects the potential for compromise in each of the five domains previously discussed.

A survey by Bunny (1992) found that 23% of calves from cows in which labour was induced were found dead, 30% were killed and 8% were found alive but later died. Of the 39% of induced calves that lived 36% were bobbied and 3% were reared (Bunny,1992). The comparable values in full term calves were 5% found dead, 1% killed, 1% found alive but died, 57% bobbied and 36% reared (Bunny,1992). From these statistics it can be seen that the mortality rate of induced calves is considerably greater than that in full-term calves and that there is likely to be greater potential for welfare compromise in calves born prematurely. Awareness of the physical and physiological states of calves born prematurely allows us to treat them in ways that maintain or improve their quality of life. The aims of chapters two and three are to

increase our knowledge of induced calves that were born alive at varying gestational ages.

The physical states of premature induced calves are described in chapter two. This is set within the context of estimating their gestational ages based on simple physical measurements. A large proportion of induced calves that are born alive are kept for a minimum of four days then sent for slaughter (bobbied) rather than reared. During the time these calves are kept they must be fed appropriately. The metabolic responses of induced calves to two feeding frequencies (twice or four times daily) are described in chapter three. When calves are sent for slaughter they may be without food for up to 30 hours. The metabolic response of premature calves to fasting for 30 hours and the effects of postnatal age on the metabolic responses to the two feeding frequencies and to fasting are also examined in chapter three.

1.6.2 Full-term Twin Beef Calves

In order to increase calf numbers, twinning may be induced in beef cows through embryo transfer (Sakakibara *et al.*,1996). Twinning in cattle often results in variable degrees of fusion of the placentas and fetal membranes of co-twins (Lillie,1922; Williams *et al.*,1963; Mellor,1969a; Sloss and Dufty,1980). Unlike other species the large placental blood vessels of twin calf fetuses may join (anastomose) thereby altering the haemodynamics of the fused feto-placental circulations (Lillie,1922). Moreover, the circulatory changes that occur in calves during birth may be altered by placental vascular anastomosis. The effects of different degrees of placental vascular anastomosis on postnatal haemodilution within and between twin pairs are estimated and discussed in chapter four.

1.6.3 Full-term Dairy Calves

Immunoglobulin transfer in full-term dairy calves before they are separated from their dams is examined in chapter five. Although this subject has been the focus of extensive research overseas (Selman *et al.*,1970ab; Edwards,1979; Edwards,1982; Edwards,1983; Edwards and Broom, 1982; Ventorp and Michanek,1991), calving conditions in many of these overseas studies are considerably different from those in this country. The present

study focuses on the suckling-related behaviour of cows and sucking behaviour by their calves born under normal farming conditions in New Zealand.

Chapter Two: Aging of Premature Calves

Abstract

The present study has shown that calves born prematurely as the result of induction tend to have shorter crown rump lengths, lighter body weights, lower rectal temperatures, fewer deciduous incisor teeth erupted and breathe more slowly than calves born at full-term. The welfare of calves born more than 3 weeks prematurely is more likely to be compromised through a reduced ability to maintain their body temperature and difficulty breathing and feeding. An induced calf that weighs less than 25 kg, has a crown rump length of less than 74 cm, a rectal temperature lower than 37.8°C, less than 4 incisors erupted and that takes 30 breaths per minute or less, is likely to be greater than 3 weeks premature (< 259 days gestational age). The present study has also shown that a premature calf's postnatal age cannot be accurately estimated through examination of the state of the umbilical cord remnant or the number of deciduous incisors that have erupted or are palpable.

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Chapter 2: Aging of Premature Calves

2.1 Introduction

Many New Zealand dairy farms aim to have the maximum milk production of all cows within a herd coincide with maximum grass growth (MacDiarmid,1983a). The onset of production of large volumes of milk is stimulated by the hormonal changes associated with calving. This means that all cows must give birth within a 6-8 week period (Chesterton and Marchant,1985), and therefore also conceive within two cycles of calving. Cows that do not conceive within two cycles are likely to remain out of synchrony with the rest of the herd. Lactational synchrony can be regained at the other end of the pregnancy if the cow is induced to give birth prematurely through an injection of synthetic corticosteroid analogue.

2.1.1 Induction of Parturition

In a normal bovine pregnancy, the increased activity of the fetal hypothalamic-pituitary-adrenal axis toward the later part of gestation causes an increase in fetal cortisol concentrations (MacDiarmid,1983a; Liggins, 1989). This causes a change in the placental metabolism of steroids; the rate of oestrogen secretion rises and that of progesterone falls (MacDiarmid,1983a; Liggins, 1989). The resulting change in oestrogen:progesterone ratio serves as the trigger for the release of prostaglandin F2α by the uterine cotyledons (MacDiarmid,1983a). As oestrogen concentrations continue to rise the responsiveness of the uterine muscle to oxytocin and prostaglandins increases thereby initiating labour (MacDiarmid,1983a). Injected synthetic corticosteroid analogues readily cross the placenta from the maternal circulation and presumably activate the changes in placental enzymes that are normally the target of endogenous fetal cortisol (MacDiarmid,1983a).

Parturition may be induced using a variety of drugs and regimes (MacDiarmid,1980; MacDiarmid,1983a). If a cow is within two to three weeks of full-term a single injection of short-acting corticosteroid (commonly dexamethasone trimethyl acetate) is usually given. Earlier in pregnancy, the short-acting corticosteroids are less effective and long-acting corticosteroids (such as dexamethasone, betamethasone, flumethasone

and triamcinolone acetonide) have been found to be more reliable (MacDiarmid,1983a). Improved precision of calving date is gained by administering an initial priming dose of long-acting corticosteroid, followed 6 to 12 days later by an injection of a short-acting formulation (MacDiarmid,1983ab).

Induction of parturition is commonly practiced in New Zealand and results in the birth of a large number of premature calves (Bunny,1992). Although studies have addressed the postnatal effect of induction on cows (Adams,1969; O'Farrell and Crowley, 1974; Thomas, 1975; Welch *et al.*,1979; MacDiarmid,1983a,b; Bunny,1992), little attention has been paid to the physiological status of the resulting premature calves.

2.1.2 Premature Induced Calves

Bunny (1992) found that 61% of induced calves die on the farm compared to 7% of calves born to non-induced cows. Of the total number of induced calves only 3% were reared with the remainder (36 % of the total number of induced calves) being sent for slaughter (Bunny,1992). The mortality rates of induced calves vary according to many factors with the most important being the degree of prematurity at birth and the drug regime used to induce parturition (Adams,1969; MacDiarmid,1983b; Bunny,1992). These calves may be born dead, die soon after birth because of an inability to adapt to extra-uterine conditions, or be killed by farm staff (MacDiarmid,1980; Bunny,1992).

The results of a survey of Taranaki dairy farmers conducted by Bunny (1992) indicated that the main basis on which the decision whether to kill or keep an induced calf alive was made was the size and vigour of the calf. Its ability to stand and suck were also major considerations. These indices as well as others that reflect the viability of an induced calf will be dependent on the maturity of the physiological systems necessary for extra-uterine survival. Maturity and therefore viability of preterm calves increases as the gestational age approaches full-term. This is seen as an increase in neonatal mortality as the degree of prematurity increases (MacDiarmid,1980; Welch and Kaltenbach,1977; Welch *et al.*,1979). Welch and Kaltenbach (1977) reported an increase of 2.4% in the mortality rate for each additional week of prematurity of the calf.

In this chapter a pilot study of physical and physiological state of induced calves of various gestational ages is described. The exact due date of the cow in which labour was induced and the gestational age of the calf was not known for many of the animals. Measurement of physical parameters that change as the fetus grows and matures allow estimation of the gestational age of induced calves at the time of their birth.

The growing fetus increases in both curved crown rump length (CRL) and weight during gestation (Mellor,1983). These parameters have been used in a number of studies for estimating the gestational age of ruminant fetuses (Nichols, 1944; Committee on Bovine Reproductive Nomenclature, 1972; Evans and Sack, 1973; Richardson *et al.*,1990; Sivachelvan *et al.*, 1996). Welch and Kaltenbach (1977) found that induced calves that were more than 10 kg lighter than the mean weight of naturally born calves had a higher mortality rate. Additional parameters measured or assessed in the present study include rectal temperature, number of deciduous incisor teeth erupted and palpable, breathing rate, state of the hoof membranes, state of the umbilical cord remnant, heart rate and gum colour.

2.1.3 Eruption of Deciduous Incisors

Cattle have eight incisors (four pairs) on the mandible (Sisson and Grossman,1953). The deciduous incisors of calves erupt during the perinatal period (Sisson and Grossman, 1953). Most full-term calves have either six or eight of these teeth (3 or 4 pairs) cut through by two days of postnatal age (Todd, 1998, *unpublished data*). The eruption of incisors in fetal calves may occur at specific times during the perinatal period. Noting the number of incisors that have erupted and the number palpable in an induced calf may therefore aid in estimation of its gestational age. When Sivachalen *et al.* (1996) used tooth eruption to estimate the gestational age in caprine and ovine fetuses they found that the teeth buds become prominent one to four weeks before birth and that at birth 1 to 3 incisors had erupted.

2.1.4 Maintenance of Body Temperature

Calves and other ungulates are relatively mature at birth (Randall,1978). When born at full-term they can maintain deep body temperature over a wide range of ambient temperatures (Randall,1978). Body temperature represents the balance between heat loss and heat production both of which are affected by a number of factors

(Carstens,1994). A calf's ability to maintain its body temperature within the normal range depends on the maturity of its thermoregulatory and thermogenic systems, as well as the amount of muscle glycogen and brown adipose tissue (BAT) within its body (Mellor and Cockburn,1986; Schoonderwoerd *et al.*,1986; Carstens,1994). Rectal temperatures were therefore measured here as an overall measure of the success of thermoregulation in the calves.

Heat Loss

The rate of heat loss is influenced by the insulation provided by an animal's tissues and coat, the amount of surface area exposed to the surrounding environment, the degree of behavioural modification it exhibits as well as the conditions (temperature, wind, rain, sunshine) of the external environment (Mellor and Cockburn,1986; Carstens,1994).

Piloerection of the hair coat increases the layer of air entrapped between the hair and the calf's skin (Carstens, 1994). This layer decreases the rate of conductive and convective heat loss (Carstens,1994). Normally the hair coat covering the whole body develops between 210 and 245 days gestation (Gjesdal,1969; Evans and Sack,1973).

The thickness of cutaneous tissues and ability to induce vasoconstriction of cutaneous blood vessels affect the rate of heat loss. Tissue insulation of full-term neonatal calves is low at birth and increases by 37% during the first 14 days of postnatal life (Carstens,1994) with the intake of lipid-rich colostrum. The ability to reduce heat loss through vasoconstriction of cutaneous vessels also increases with age (Carstens,1994).

Induced calves weigh less than full-term calves and have a higher surface area to body weight ratio. Thus the surface area over which heat is lost is greater in proportion to the amount of tissue that produces heat (Randall,1978; Carstens, 1994). The presence of adverse environmental conditions such as strong wind, cold environmental temperatures, rain or snow increase the rate with which heat is lost to the environment from the exposed surfaces of the animal.

The combination of these factors suggests that premature induced calves are likely to lose heat to the environment at a greater rate than full-term calves. If so, premature

calves would require a greater rate of thermogenesis per unit body weight than full-term calves in order to maintain their body temperature.

Thermogenesis

The capacity for heat production is a function of the extent of BAT and muscle glycogen reserves as well as the developmental maturity of the systems required to produce heat. It is also affected by whether the neonate has fed (Mellor & Cockburn,1986). Heat is produced through basal metabolism, non-shivering thermogenesis, shivering thermogenesis, physical activity and during digestion (Carstens,1994). It is influenced by the extent of body reserves, colostrum intake, breed/genotype, dystocia and environmental factors (Mellor and Cockburn,1986; Carstens,1994).

BAT is specialised for heat production through non-shivering thermogenesis (Schoonderwoerd *et al.*,1986). In fetal calves growth of BAT occurs rapidly during mid-gestation (Carstens,1994). The functional maturation of BAT occurs during late gestation (Carstens,1994). During this time increased production of mitochondria and the uncoupling protein in BAT increase the ability of a calf to generate heat through non-shivering thermogenesis (Carstens,1994).

Movement of the muscles as in shivering (shivering thermogenesis) or physical activity allows the mobilisation of glucose from muscle glycogen to produce heat (Mellor and Cockburn,1986; Carstens,1994). Muscle glycogen deposition increases progressively before full-term birth (Mellor and Cockburn,1986). This does not appear to be controlled by the pre-partum plasma cortisol surge, although a parallel rapid increase in liver glycogen is (Mellor,1993).

At the environmental temperatures encountered during winter and spring in New Zealand, calves are likely to require a rate of heat production above that of basal metabolism in order to maintain their body temperature (Gonzalez-Jimenez and Blaxter,1962; Dirks,1995). Their capacity to do so is dependent on the functional maturity of physiological systems involved in thermogenesis and thermoregulation, as well as the extent of thermogenic energy substrates (Carstens,1994). All of these factors

may be impaired in premature calves with the extent of impairment being greater in calves born at an earlier gestational age.

Alexander *et al.* (1973) showed that although the basal metabolism of premature lambs is similar to that of full-term lambs, their summit metabolic rate is lower. The degree to which maximum thermogenesis was impaired became less as the lambs neared full-term due to an increase in the ability to produce heat by shivering (Alexander *et al.*,1973). It is likely that these phenomena also apply to premature calves.

Light-weight neonates (such as premature calves) have a lower summit metabolic rate and a greater surface area to bodyweight ratio (Carstens,1994). Alexander *et al.* (1973) found that once a lamb is born, the replacement of BAT with white adipose tissue begins regardless of gestational age and the ability to produce heat through non-shivering thermogenesis increases no further (Alexander *et al.*,1973). Given that calves are similar to lamb in terms of energy reserves, premature calves would be likely to have a reduced quantity of BAT and a limited ability to produce heat through non-shivering thermogenesis.

Thermogenesis depends on the availability of energy substrates. As with BAT, the deposition of the muscle glycogen necessary for shivering is also likely to be interrupted by premature birth. Before birth the accumulation of glycogen and triglycerides within the liver, muscles and adipose tissue is promoted by glucocorticoids and the high plasma insulin:glucagon ratio of the fetus (Mayor and Cuezva,1985; Ktorza *et al.*,1985). The postnatal increase in plasma catecholamine concentration and decrease in the insulin:glucagon ratio triggers liver glycogenolysis and gluconeogenesis, thereby inhibiting or reducing continued deposition of these substrates (Mayor and Cuezva,1985, Ktorza *et al.*,1985). Smaller quantities of glycogen and BAT would reduce the period of time for which a calf could maintain the raised metabolic rate necessary for increased heat production (Mellor and Cockburn, 1986).

Thermoregulation - Balancing Heat Production and Heat Loss

Balancing the rate of heat loss with that of heat production and mobilisation of energy substrates used for thermogenesis depends on the integration of the physiological

processes involving the sympathetic nervous system and pancreatic, adrenal and thyroid glands (Carstens,1994). In a study of hypothermic lambs, Eales *et al.*(1982) found that parts of the central nervous system (CNS) of premature hypothermic lambs were unmyelinated. Myelin functions as an insulator to increase the velocity of stimuli being transmitted between a nerve cell body and its target (Urban *et al.*,1997) It is possible that the parts of the nervous system necessary for the maintenance of body temperature such as the hypothalamus may be functionally immature in premature calves. Myelination of a calf's CNS continues until it is 3 months of post-natal age (Urban *et al.*,1997). Thus, the degree of CNS myelination in a calf born prematurely is less than that in those born at full-term (Urban *et al.*,1997). Reduced myelination in the more premature neonates may impair their ability to thermoregulate. With advancing gestational age the degree of myelination of the parts of the CNS involved in thermoregulation is likely to increase, thereby improving the ability of the neonate to maintain its body temperature as its gestational age nears full-term. Impaired thermoregulatory ability in premature calves would result in them being prone to hypothermia. They may also exhibit greater fluctuations in rectal temperature.

2.1.5 Respiratory Adaptation in Induced Calves

At birth, the placental circulation is rapidly restricted and lost cutting off the calf's umbilical supply of oxygen. Extra-uterine survival is dependent on an effective transition to air breathing. (Randall,1978). Efficient pulmonary exchange relies on the maturity of lung structure and function. Maturation of the fetal lung involves structural changes, improved distensability with air and the onset of surfactant production (Liggins,1969). It occurs late in gestation and is usually closely correlated with the rising plasma cortisol concentrations before birth (Liggins,1969). Fetal ACTH or cortisol infusions induce premature maturation of the lungs (Liggins,1969). Induction using corticosteroids may accelerate lung development in the bovine fetus improving its viability despite being born prematurely. Even so, respiratory failure due to structural or functional immaturity of the lungs may be a major cause of pathophysiology in induced calves. The degree of lung development induced may vary depending on the time course and pattern of corticosteroid administration. Thus breathing rate was measured as an albeit imprecise measure of respiratory effectiveness.

2.1.6 Colostrum Absorption in Induced Calves

Although many induced calves die on the farm, some are kept for slaughter and a few are reared. If these calves are to survive they must have the physiological ability to resist environmental pathogens that is necessary to protect them against disease. Neonatal calves acquire passive immunity to disease through the absorption of colostral immunoglobulins.

Conclusions given by different authors as to whether induction affects a calf's ability to attain an adequate immune status soon after birth vary. This may be due to differences in the effects of the induction methods used and complicated by variation in the gestational age of the calves studied. Langley and O'Farrell (1976) found that the amount of immunoglobulin absorbed by a newborn calf was affected by the time from glucocorticoid analogue administration to parturition and by its degree of prematurity. The serum immunoglobulin content of calves given a similar volume of colostrum may vary depending on the colostral immunoglobulin concentration, the degree of immunoglobulin absorption and the interval between colostrum intake and blood sampling (Stott *et al.*,1979a,b; MacKenzie,1984; Ventorp and Michanek,1991).

The serum immunoglobulin concentration of calves from cows in which labour had been induced using short-acting corticosteroids has been found by several authors not to be adversely affected (Muller *et al.*,1975; Beardsley *et al.*,1976; Naylor,1986; Bunny,1992). In comparison, the effect of long-acting corticosteroids on the immune status of calves is debatable. Some authors have found that calves induced using long-acting corticosteroids had significantly lower serum immunoglobulin concentrations (Bailey *et al.*,1973; Husband *et al.*,1973) whereas others have found no effect (Hoerlin and Jones,1977).

Lowered serum immunoglobulin concentrations may have been due to inadequate levels of immunoglobulins in the available colostrum (Bailey *et al.*,1973) as the result of pre-calving milking (Welch,1972; O'Farrell and Crowley,1973; Allen,1976). Intake of a

comparatively smaller volume of colostrum or reduced absorption of immunoglobulins from the gut would also result in lower serum immunoglobulin concentrations (Bailey *et al.*,1973; Husband *et al.*,1973; Naylor, 1986). When calving is induced using long-acting corticosteroids the calves are sometimes lethargic and slow to stand and suck which would reduce the amount of colostrum absorbed (Adams,1969; Welch *et al.*,1973; Bailey *et al.*,1973; MacDiarmid,1983b; Bunny,1992).

Even so, although absorption of immunoglobulins may be lower in induced calves than in those born at full-term, it is greater in premature induced calves than in those of the same gestational age born by caesarian section (Johnson and Stewart,1986). Thus, it has been suggested that prematurity may be the main factor responsible for slower or less effective uptake of colostral immunoglobulins in induced calves, and that glucocorticoids may actually enhance immunoglobulin uptake (Johnson and Stewart,1986). This is consistent with the known actions of the pre-partum cortisol surge on maturation of the intestinal epithelium in fetal lambs (Trahair *et al.*,1984; Trahair *et al.*,1987ab; Mellor,1992b). Gamma glutamyl transferase activity in plasma was therefore measured as an index of successful immunoglobulin uptake.

2.1.7 Circulation in Induced Calves

Heart rate and membrane colour are clinical parameters commonly used to assess the condition of the circulatory system, and were used here in calves. A heart rate outside the normal range may be an indication of pathology. The mucous membranes of the mouth are highly vascular. Variation in gum colour is a parameter that may indicate circulatory efficacy. Gums may vary in colour from white to purple, with most being a pink or red colour. Purple gums may indicate low oxygen content of the blood

2.1.8 Hoof Membranes

Before standing, a newborn calf has a thick membrane covering the sole of each hoof. When attempts to stand are made the hoof membrane is easily worn off, so that the presence or absence of the hoof membranes indicates whether the calves has been able to stand and walk or has attempted to stand. The integrity of hoof membranes in the present calves was therefore recorded.

2.1.9 State of the Umbilical Cord Remnant

A calf's umbilical cord breaks at the time of birth. In most full-term calves the cord dries, shrivels and then falls off over the following few days. Rapid drying of the umbilical cord remnant is advantageous as a wet remnant can predispose the calf to infection (Webster,1984). The rate with which the cord remnant dries may vary in calves of differing gestational ages. Its state at the time a calf is collected may give an indication of the calf's postnatal age and was therefore recorded.

2.1.10 Estimation of the Gestational Age and Physiological Status of Induced Calves

Currently little is known about the status of premature induced calves. Any one of the parameters (Table 2.1) alone may not indicate much about the physical or physiological state of an induced calf. However, the combination of these measurements and observations may allow the gestational age of premature induced calves to be estimated and their physiological state assessed. The purpose of the work described in this chapter was to evaluate this possibility.

Table 2.1: Summary of the parameters used to estimate the gestational ages of premature induced calves

Parameter	**Use**
Weight	Increases with gestational age. Aids in estimation of gestational age
Crown Rump Length	Increases with gestational age. Aids in estimation of gestational age
Rectal Temperature	Maintenance of body temperature is necessary for extra-uterine survival. Represents the balance between heat loss and heat production. In similar environments it could be a relative indicator of maturity.
Tooth Code	Incisors erupt in perinatal period. Aids in estimation of gestational age.
Breathing Rate	Adequate respiratory competence necessary for survival. In an unstressed state it may be an indicator of maturity.
Heart Rate	Commonly used clinical parameter. Indicator of the condition of the circulatory system
Gum Colour	Variation in gum colour may indicate circulatory efficiency.
Hoof Membranes	Wearing of hoof membranes indicates whether the calf has been physically able to stand and walk.
GGT activity	Indicates whether a calf has sucked and immune status.

2.2 Materials and Methods

2.2.1 Animals

Records from the Massey University Veterinary Clinic were used to obtain a list of local farms on which cows had recently been induced and may therefore have calves available. Sixty-eight induced calves were collected from around the Manawatu district. Most were between 0 and 2.5 days old at the time they were collected, although one was 4 days old and two were 6 days of age.

The calves were obtained from different farms and their breed varied. The number of calves of each breed and breed cross were as follows: twenty-eight Friesian, three Jersey, eleven Jersey x Friesian, two Jersey x Hereford, fourteen Hereford x Friesian and ten Murray Grey x Friesian calves. Both male and female calves were used. At the time the calves were collected they were given a numbered identification ear tag (Allflex, medium yellow tag) and the following measurements and observations were made.

2.2.2 Birth Date and Due Date

The difference between the induced birth date and due date of birth indicated the degree of prematurity of the calf in terms of gestational age. Although the date of birth was known in all cases, the due date of birth was known for only eleven of the calves. In New Zealand a bull is used to breed cows that do not conceive to artificial insemination within their first two cycles and as a result the date of conception and due date are often not known.

Weight

Calves were restrained on electronic scales and their weights recorded.

Crown Rump Length

The curved crown rump length (CRL) was measured. This is the distance following the contours of the spine from the base of the tail to the top of the head between the horn buds.

Rectal Temperature

The rectal temperature of each calf was measured using a clinical thermometer.

Tooth Code

The number of incisors that had erupted and the number which were palpable was recorded for each calf. Erupted teeth were those that had cut through the gum and were visible. Palpable teeth were those felt through the gum that had not yet cut through. In order to analyse these data it was necessary to transform them into a numerical format (Table 2.2). The following tooth code, with each code representing the number of erupted and palpable incisors present, was devised.

Table 2.2: The tooth codes representing the number of deciduous incisors that had erupted and the number that were palpable

Tooth code	Number of incisors erupted	Number of incisors palpable
1	0	2 - 6
2	2	0
3	2	2
4	2	4
5	2	6
6	4	0
7	4	2
8	4	4
9	6	0
10	6	2
11	8	0

The tooth code was recorded at the time the calf was collected. Postnatal changes in tooth code were followed in 31 of the calves for up to six days after collection.

Breathing Rate

The breathing rate was measured by counting the number of times the thorax and abdomen rose and fell with each breath during one minute.

Heart Rate

The heart rate was measured using a stethoscope and timer. The number of beats per minute was recorded.

State of the Umbilical Cord Remnant

At the time the calves were collected the state of the umbilical cord remnant was recorded. Wet, wet-dry, dry, shriveled and absent were the terms used to describe the state of each calf's cord remnant. These were numerically transformed to 1, 2, 3, 4 and 5 respectively. The status of the cord remnant was followed for up to six days in 31 of the calves.

Gum Colour

The gum colour of each calf was recorded at the time it was collected as being pink, pink-red, red, red-purple or purple, which were numerically transformed to 1, 2, 3, 4 and 5 respectively.

Hoof Membranes

The degree to which the hoof membranes were worn on all feet was recorded as being unworn, half worn or absent.

Gamma-Glutamyl Transferase Activity

Gamma glutamyl transferase (GGT) is a membrane protein involved in amino acid transport (Thompson and Pauli,1981). In secretion products such as colostrum and milk there is high GGT activity. Measurement of a calf's serum GGT activity has been found to be a reliable indicator of whether it has sucked given that the amount of time between sucking and blood sampling is sufficient to allow for absorption of colostrum from the digestive tract (Thompson and Pauli, 1981; Perino *et al.*, 1993; Vermunt *et al.*, 1995).

Blood samples were taken from the calves by jugular venepuncture into 10 ml plain vacutainers. They were centrifuged and the serum was then analysed for the GGT activity with a Hitachi 704 multichannel analyser (Boehringer-Mannheim,1489224) (Persijn and van der Slik,1976).

General Observations

At the time the calves were collected any extra observations that would help describe them were noted. These observations included vocalisations, movement, feeding behaviour and general coordination.

2.2.3 Data Analysis and Statistics.

Correlations between the gestational age, bodyweight, CRL, tooth code, rectal temperature, breathing rate, heart rate, gum colour, GGT activity, state of the umbilical cord remnant and post-natal age were determined using linear regression (Microsoft Excel V 5.0 (Microsoft Corporation, USA). The significance of correlations between the parameter pairs listed in Tables 2.3, 2.5 and 2.6 were determined using statistical tables (Clarke and Cooke, 1978). Except where otherwise stated, results are expressed in terms of the mean ± the standard error of the mean (mean ± SEM).

2.3 Results

Aging Premature Calves

2.3.1 Gestational Age

The eleven calves in which gestational age was known were all less than two days of age at the time they were collected. There were four Friesians, three Murray Grey-Friesian crossbreds, two Hereford-Friesian crossbred calves and two Jerseys. Gestational age showed significant positive correlations with body weight, rectal temperature and breathing rate (Table 2.3). The absence of a correlation between gestational age and CRL was probably due to the small sample size as CRL was known in only six of the calves in which gestational age was also known because its measurement was introduced after the first seventeen calves had been collected.

Table 2.3: Correlations coefficients (r) between parameters - all breeds

Parameter	Est. Gest. Age	CRL	Weight	Tooth Code	Rectal Temperature	Breathing Rate
Gestational Age	0.799**	a	0.797**	0.55	0.698**	0.717*
Estimated Gestational Age		0.737 ***	b	0.589***	0.373**	0.395**
Crown Rump Length			0.748***	0.636***	0.566***	0.257
Body weight				0.618***	0.329**	0.445***
Tooth Code					0.506***	0.232

a = insufficient data points - low degrees of freedom

b = bodyweight used to estimate gestational age therefore the correlation between these parameters not meaningful

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

Estimated Gestational Age

Bodyweight is well known to increase with gestational age (Mellor, 1983; Richardson *et al.*,1990). Therefore by using the linear equation that relates these parameters in the

eleven calves in which both were known ($y = 1.5386x + 220.31$, $r = 0.797$, $P < 0.01$), the gestational ages of the remaining fifty-seven calves were estimated based on their body weights. Dairy calves (Jersey, Friesian, Jersey-Friesian crossbred calves) made up 54% of the group of calves in which the gestational age was known, compared to 63% of the calves in which gestational age was not known. Examples of the estimated gestational ages of calves based on bodyweight are given in Table 2.4.

Table 2.4: The estimated gestational ages of calves based on their bodyweight according to the equation: $y = 1.5386x + 220.31$, where y = gestational age and x = body weight

Bodyweight (kg)	Estimated Gestational Age (days post conception)	Prematurity (weeks)
39	280	Full term
34	273	1
30	266	2
25	259	3
21	252	4
16	245	5

Estimated gestational ages were plotted against the other parameters to indicate the presence or absence of a relationship with increasing gestational age (Table 2.3). Using the estimated gestational age allowed these assessments to be made with a greater sample size and therefore higher degrees of freedom. Significant correlations were found between estimated gestational age and CRL, rectal temperature, tooth code and breathing rate (Table 2.3).

2.3.2 Body weight

The calves weighed between 16.5 and 38.5 kg with a mean weight of 25.4 ± 0.6 kg (Appendix 2.1). The range and distribution of body weights is shown in Fig 2.1. Body weight showed significant correlations with CRL, rectal temperature, tooth code, breathing rate (Table 2.3), gum colour ($r = 0.279$, $P < 0.05$) and GGT level ($r = 0.317$, $P<0.05$).

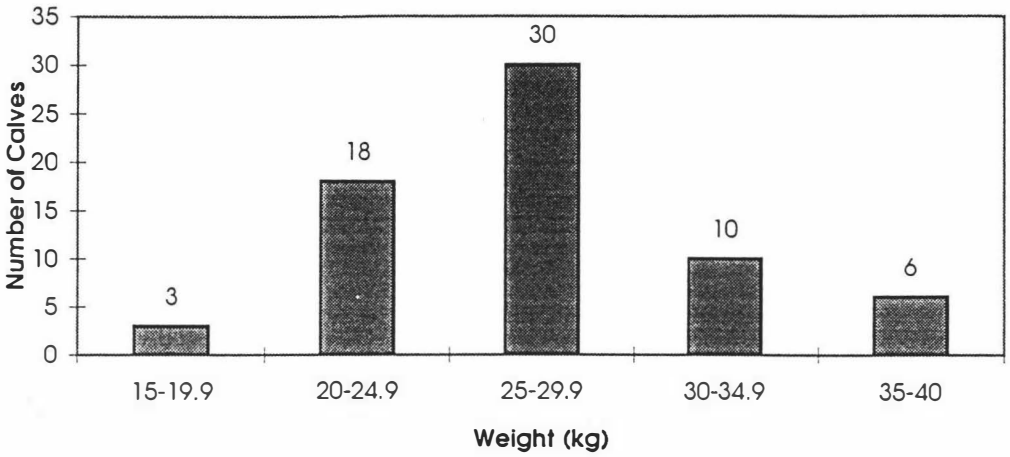

Fig 2.1: Number of calves in each weight bracket

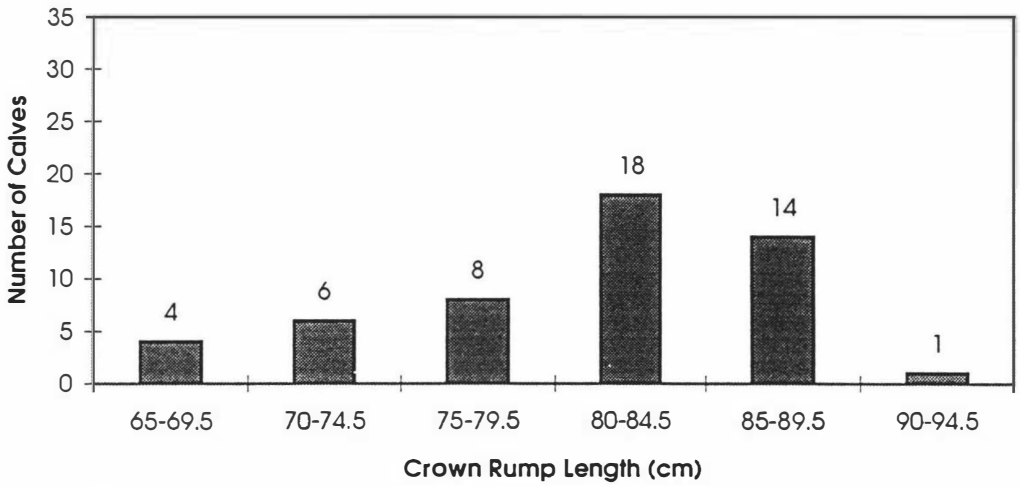

Fig 2.2: Number of calves in each crown rump length bracket

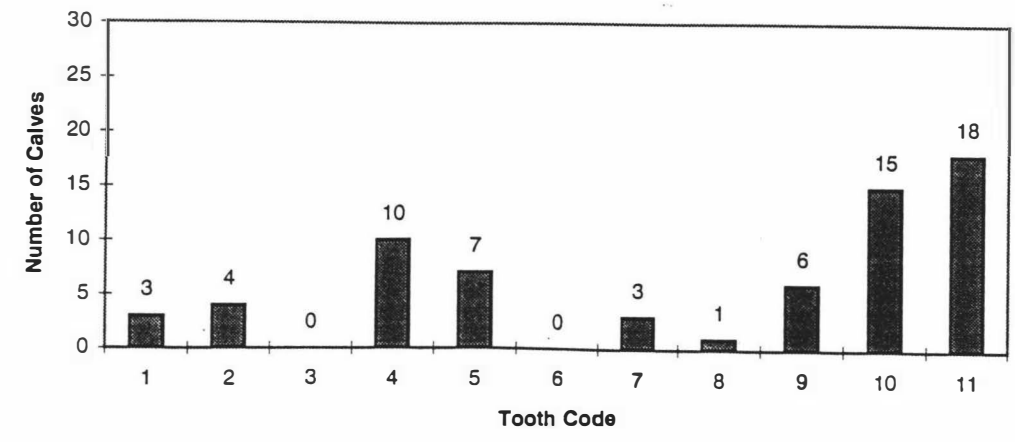

Fig 2.3 Number of calves with each tooth code

2.3.3 Crown Rump Length

The range and distribution of CRL is shown in Fig. 2.2. The mean CRL was 76 ± 1 cm with a range of 66 and 92 cm (Appendix 2.1). There were significant correlations between CRL and estimated gestational age, rectal temperature, tooth code and heart rate (Table 2.3).

Although the variation in CRL is not easily illustrated, the range in frame size of Friesian calves of differing gestational ages is illustrated by variation in back heights shown in plates 2.1-2.5. These calves had shoulder heights that ranged from approximately 52 to 78 cm.

2.3.4 Tooth Code

At the time the calves were collected most were less than 3 days old and their average tooth code was 7. This translates into four incisors that had erupted and two that were palpable. The range and distribution of tooth codes is shown in Fig 2.3. Tooth code showed significant correlations with estimated gestational age, body weight, CRL and rectal temperature (Table 2.3).

The incisors of calves appeared to erupt in a consistent order. That is, the first pair (in the centre at the front of the mouth), then the third pair, followed by the second pair and finally the fourth pair. Calves with 2, 4, 6 and 8 incisors erupted are shown in Plates 2.6-2.9.

2.3.5 Rectal Temperature

At the time the calves were collected their rectal temperatures ranged between 34.1°C and 39.8°C, with a mean value of 38.6 ± 0.1°C (Appendix 2.1). Low temperatures of 34.1°C and 36.9°C were present in two calves both of which died within 2 days. All other calves had rectal temperatures above 37°C. The range and distribution of rectal

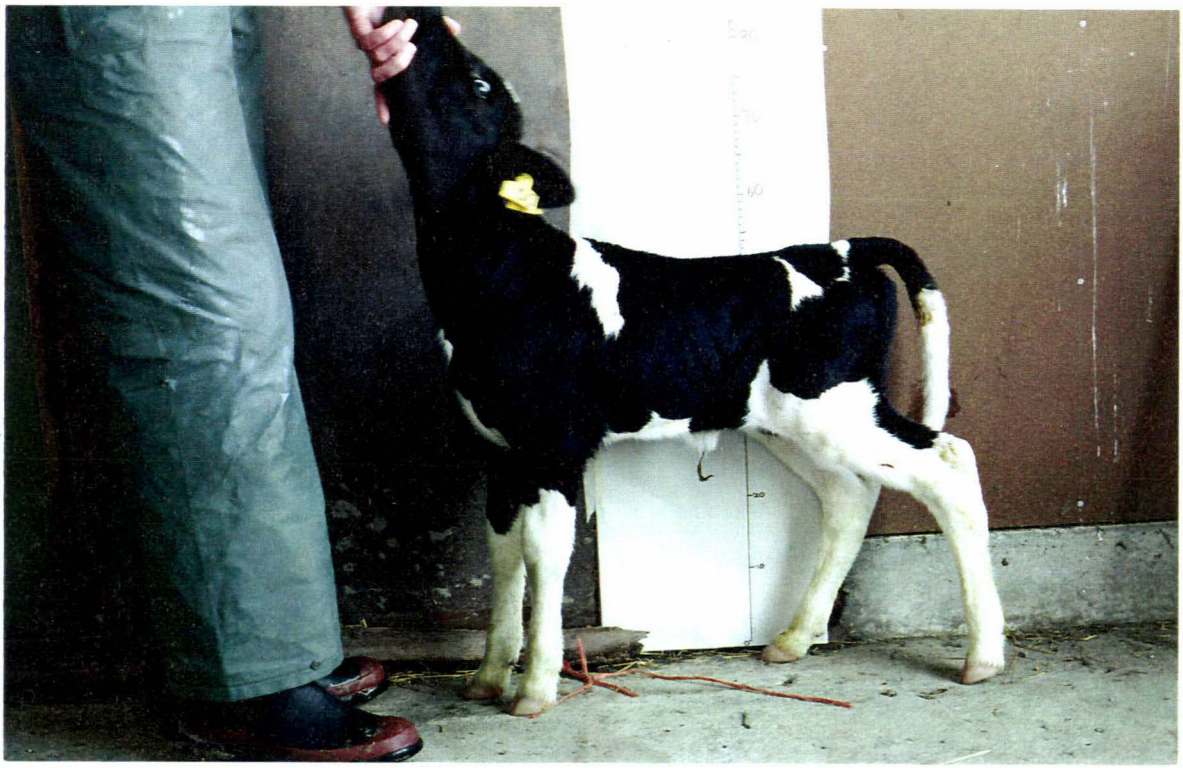

Plate 2.1 : Calf No.50 Estimated gestational age = 247 days; Approximately 52 cm tall; CRL = 68 cm; Body weight = 17 kg; Two incisors erupted, none palpable; Breathing rate = 18 breaths/ minute.

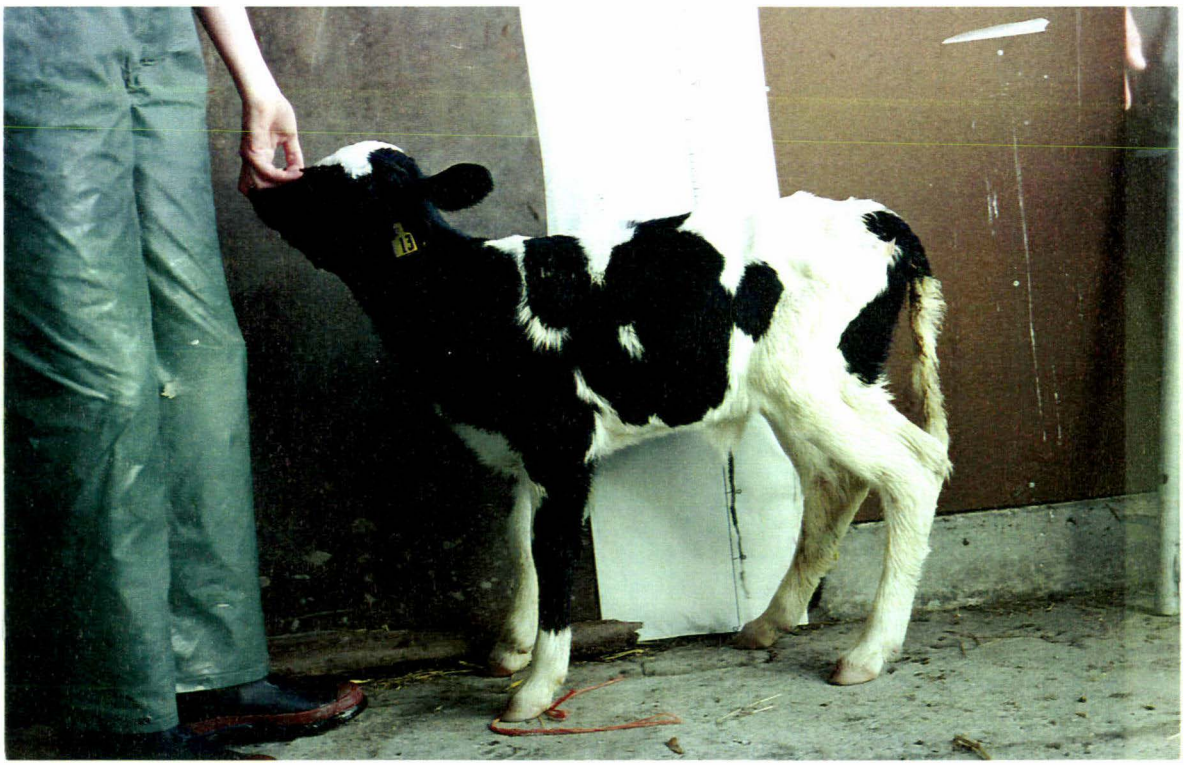

Plate 2.2: Calf No.13. Estimated gestational age = 256 days; Approximately 62 cm tall; CRL = 72 cm; Bodyweight = 23.3 kg; Six incisors erupted, two palpable; Temperature = 39.7°C; Breathing rate = 30 breaths/minute.

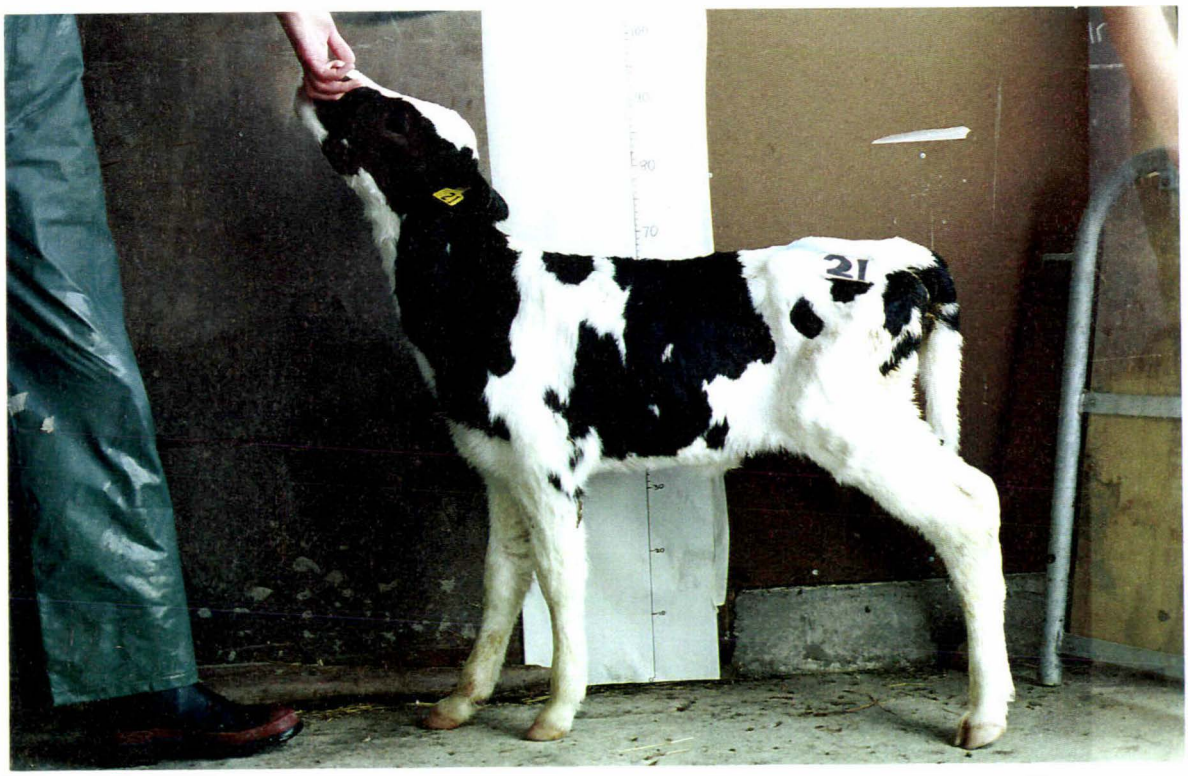

Plate 2.3 : Calf No.21 Estimated gestational age = 265 days; Approximately 66 cm tall; CRL = 83 cm; Body weight = 29 kg; Six incisors erupted, two palpable; Temperature = 39°C; Breathing rate = 30 breaths/ minute.

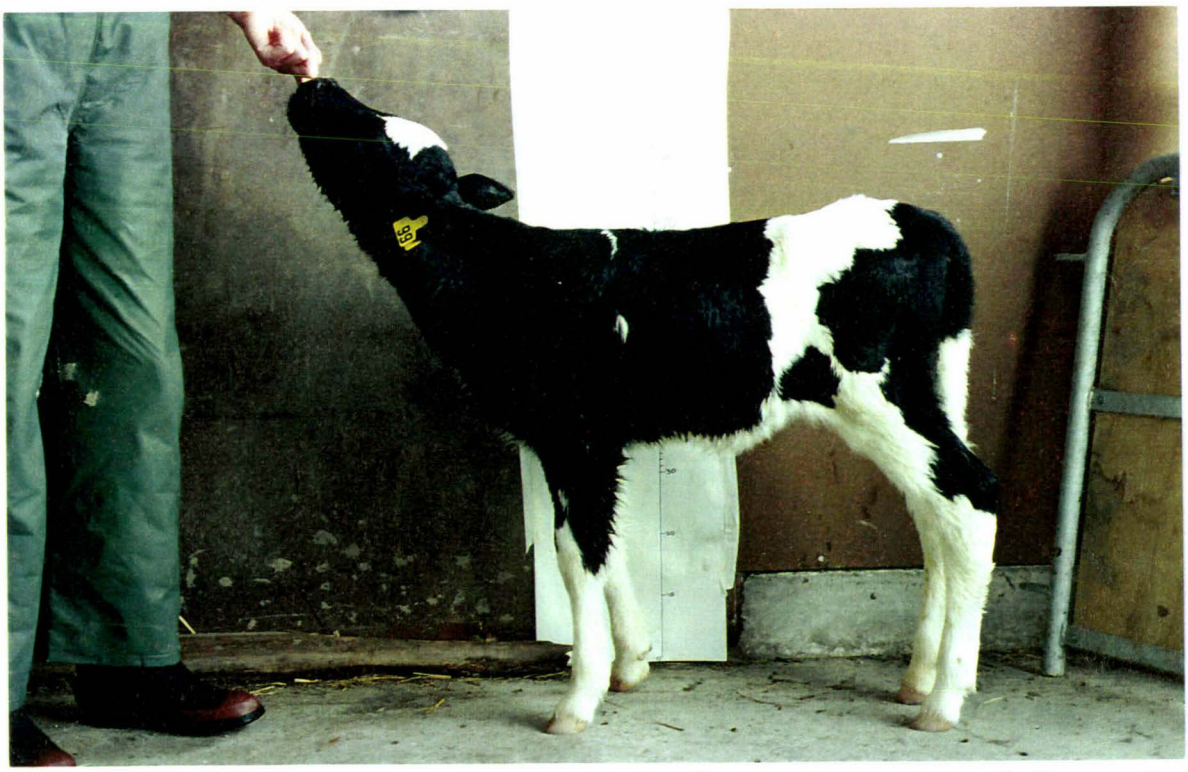

Plate 2.4: Calf No. 99. Estimated gestational age = 270 days; Approximately 69 cm tall; CRL = 79 cm; Body weight = unknown; Six incisors erupted, two palpable; Temperature = unknown; Breathing rate = unknown.

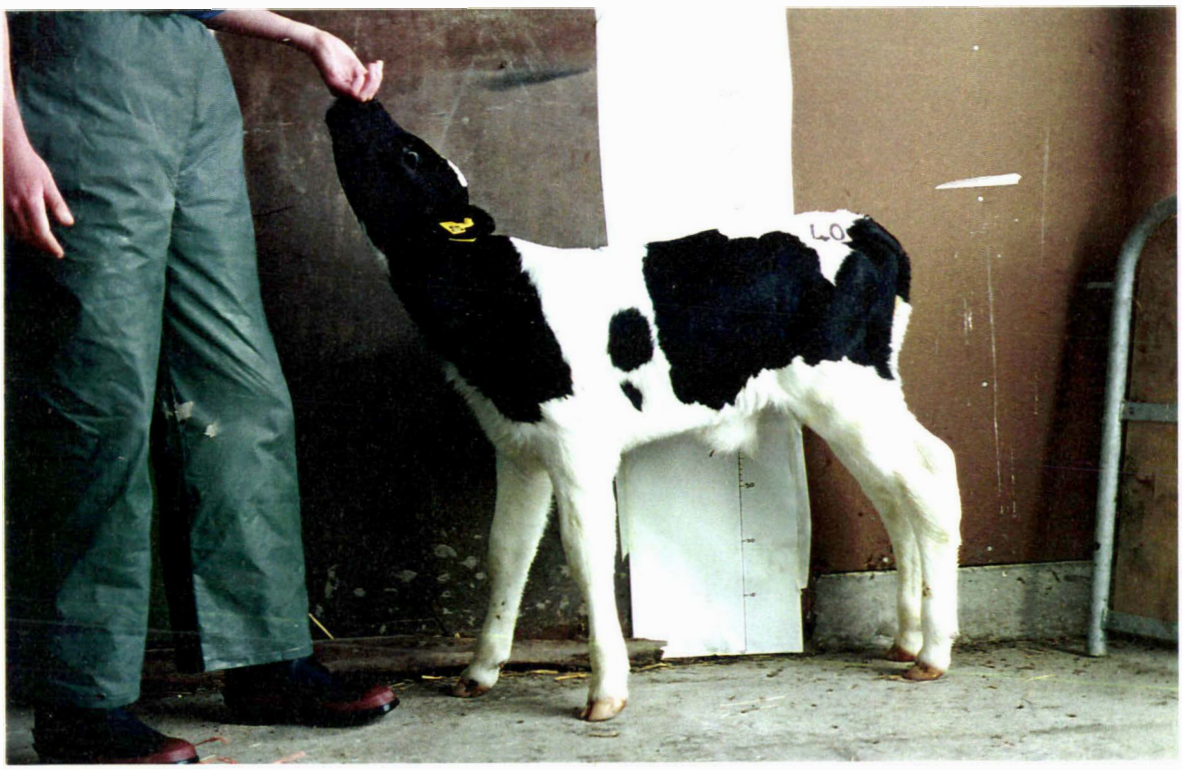

Plate 2.5 : Calf No.40 Estimated gestational age = 279 days; Approximately 78 cm tall; CRL = 92 cm; Body weight = 38 kg; Eight incisors erupted; Temperature = 39°C; Breathing rate = 48 breaths/ minute.

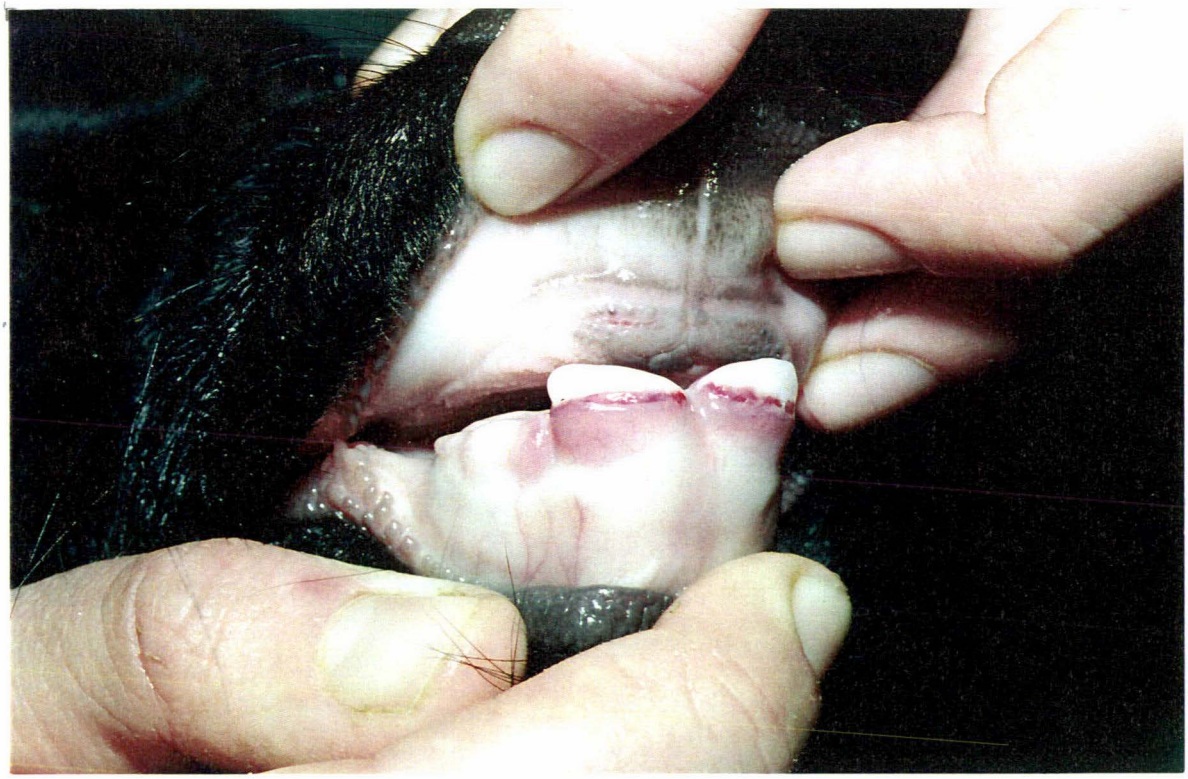

Plate 2.6 : Tooth code = 2, two incisors erupted and two palpable

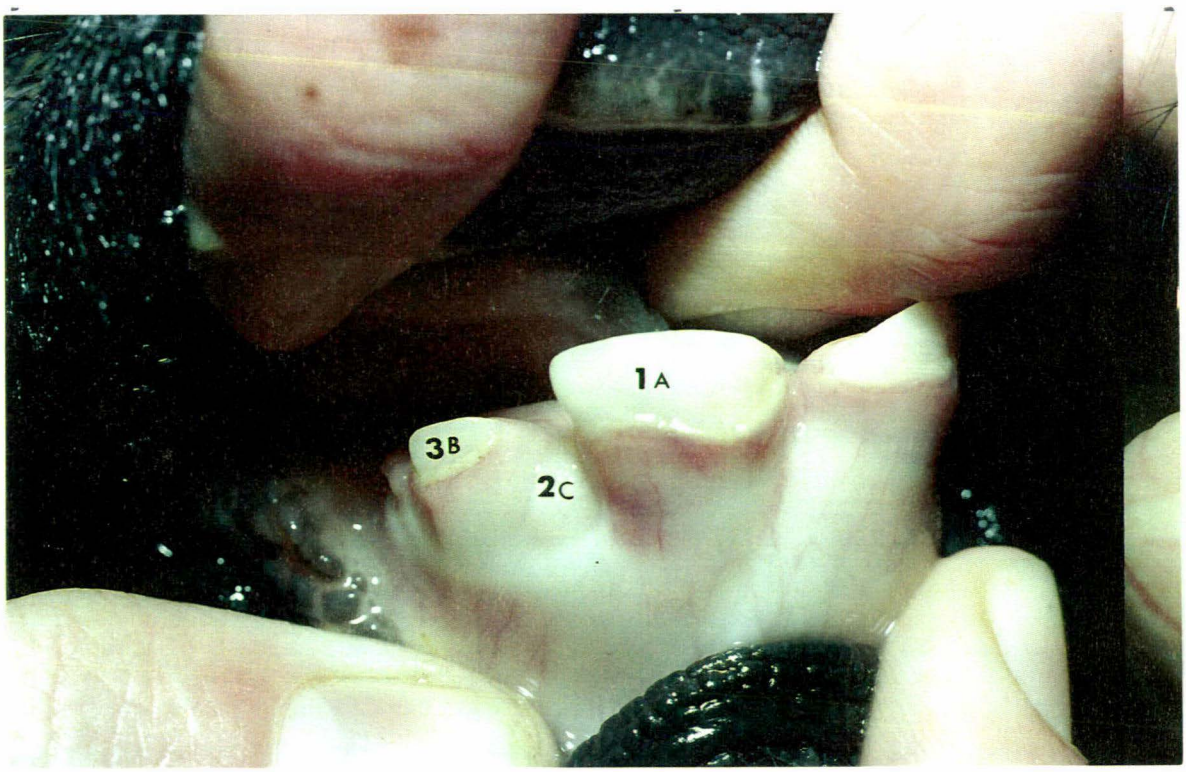

Plate 2.7: Tooth code = 7, four incisors cut, two palpable.
1 = first incisor, 2 = second incisor, 3 = third incisor,A = first incisor set erupted, B= second set erupted, C = third set erupted,

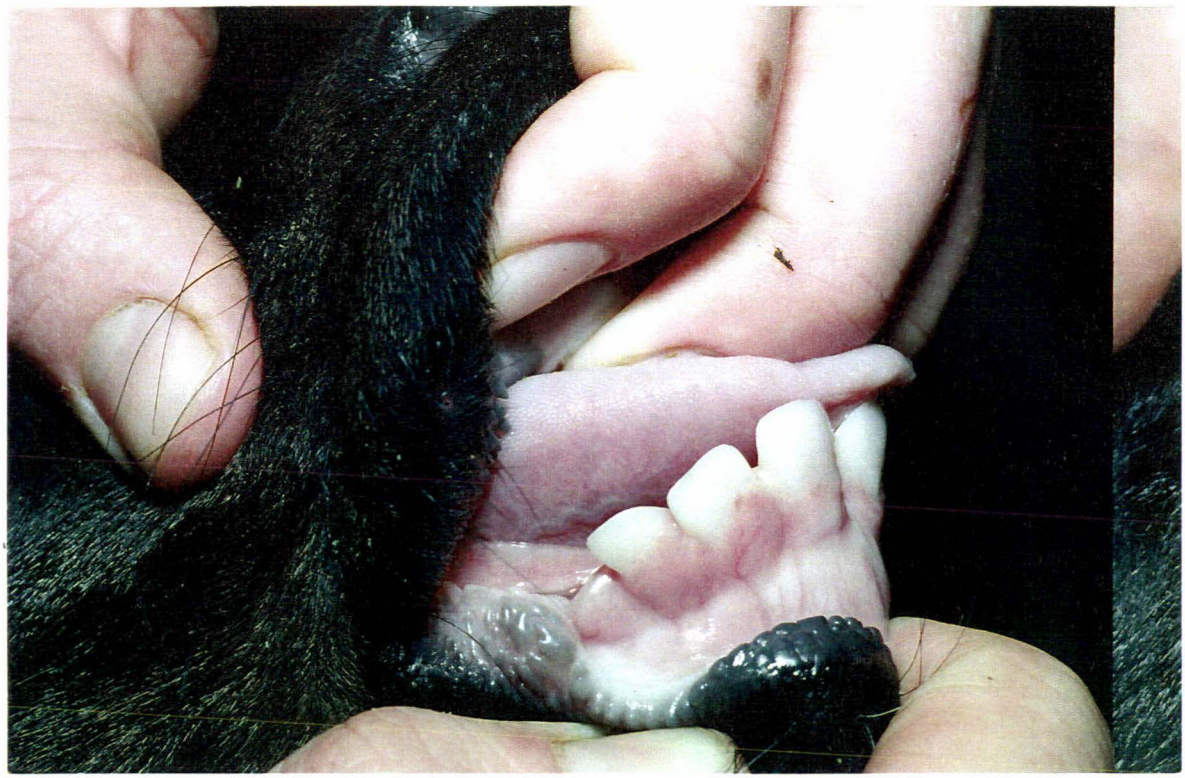

Plate 2.8 : Tooth code = 10, six incisors erupted and two palpable

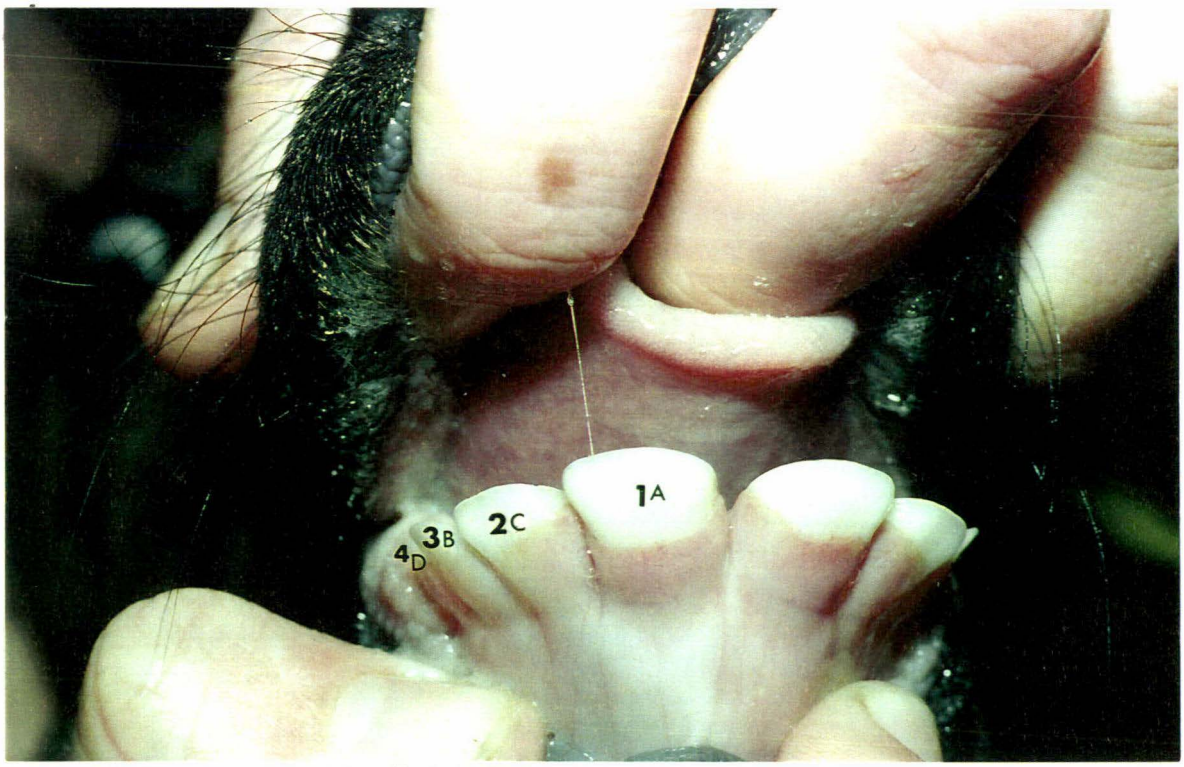

Plate 2.9: Tooth code = 11, all eight incisors cut.
1 = first incisor, 2 = second incisor, 3 = third incisor, 4 = fourth incisor,
A = first incisor set erupted, B= second set erupted, C = third set erupted, D = fourth set erupted.

temperatures are shown in Fig 2.4. Rectal temperature showed significant positive correlations with gestational age, estimated gestational age, body weight, CRL and tooth code (Table 2.3).

2.3.6 Breathing Rate

The range and distribution of breathing rates are shown in Fig 2.5. Breathing rate varied from 18 to 100 breaths/minute with a mean value of 49 ± 2 breaths/minute (Appendix 2.1). It was positively correlated with gestational age, estimated gestational age, bodyweight and CRL (Table 2.3).

2.3.7 Heart Rate

The range and distribution of heart rates are shown in Fig 2.6. Heart rate had a mean value of 128 ± 4 beats/minute and ranged between 60 and 180 beats/minute, however none of this variation could be attributed to gestational age (Appendix 2.1).

2.3.8 Gum Colour

The range and distribution of gum colours are shown in Fig. 2.7. Most calves (37%) had pink gums and in a few they were purple (7%). There was no correlation between gum colour and gestational age.

2.3.9 Hoof Membranes

At the time they were collected 59 of the 68 calves had worn hoof membranes (Appendix 2.1). Of the remaining 9 calves, the hoof membranes were unworn in 2 and half worn in 7. The two calves in which the hoof membranes were unworn also had low rectal temperatures and died within two days of these observations being made.

2.3.10 GGT Activity

GGT activity ranged from 7 to 6727 U/l (Appendix 2.1). Of the 61 calves in which GGT was measured, 28 (46%) had levels below 100 U/l, and 11 (18%) had levels above 1000 U/l. The range and distribution of GGT levels is shown in Fig. 2.8.

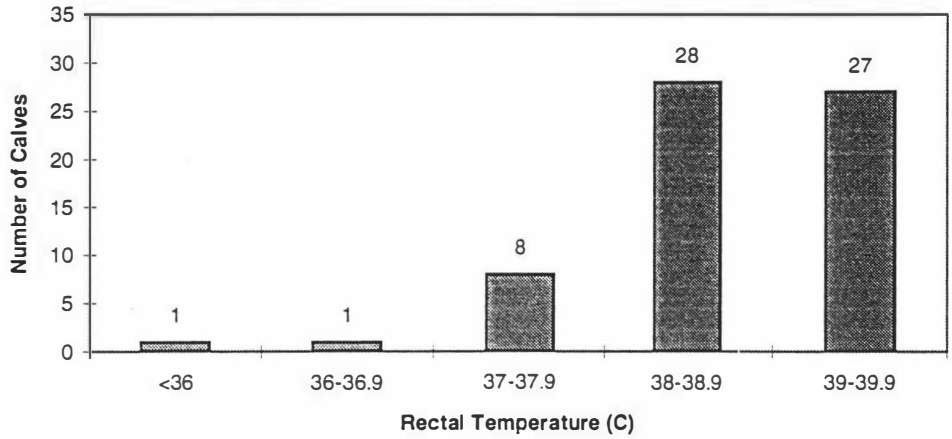

Fig 2.4: Number of calves in each temperature bracket

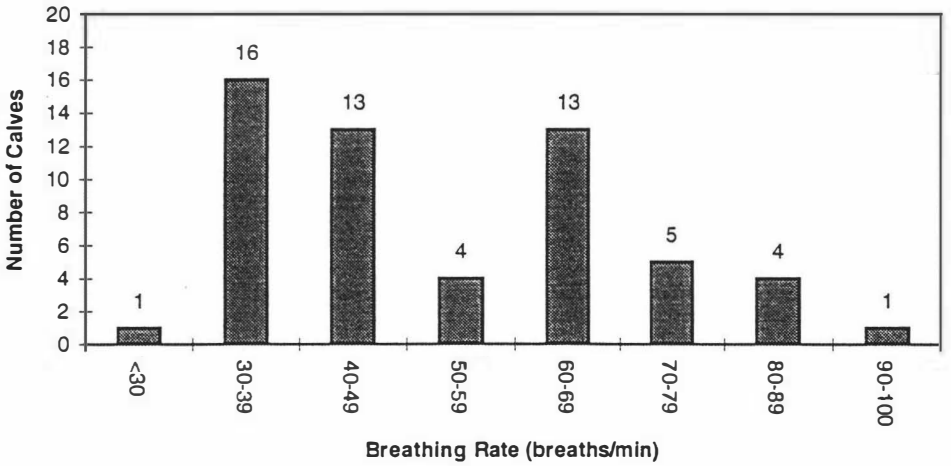

Fig 2.5: Number of calves in each breathing rate bracket

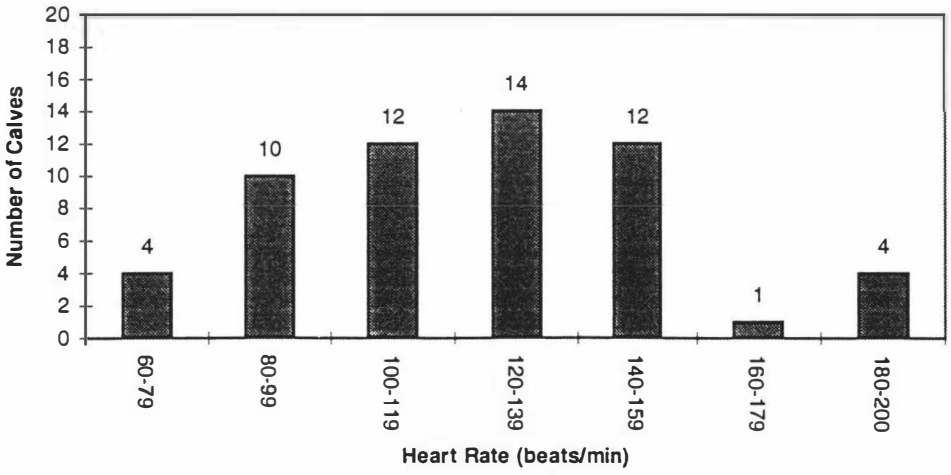

Fig 2.6: Number of calves in each heart rate bracket

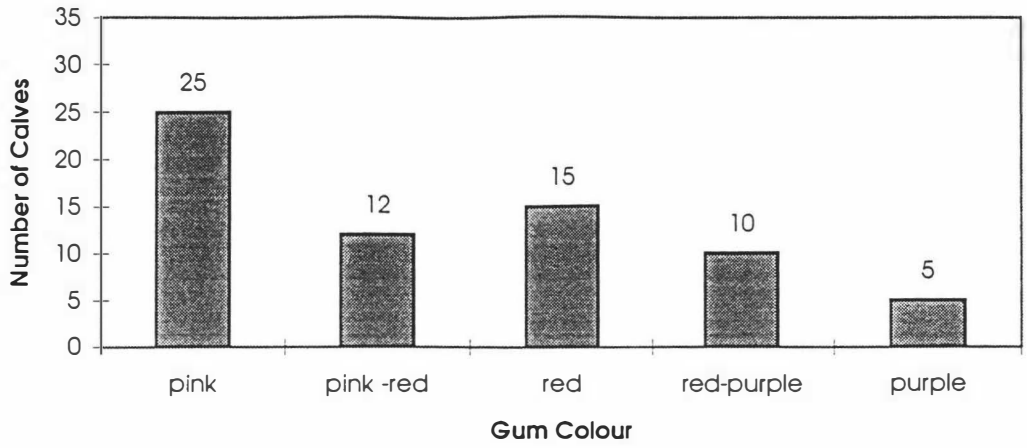

Fig 2.7: Number of calves with each gum colour

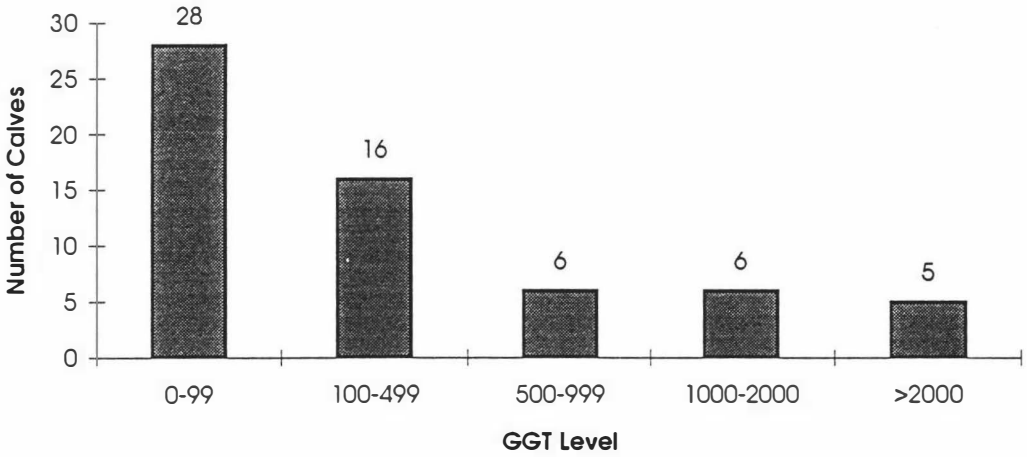

Fig 2.8: Number of calves in each GGT bracket

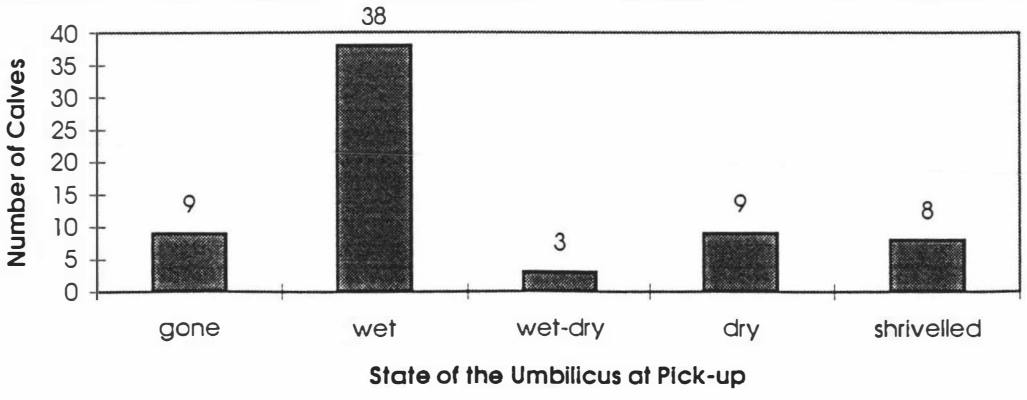

Fig 2.9: Number of calves with each umbilical state

2.3.11 State of the Umbilical Cord Remnant

Of the 67 calves in which the state of the umbilical cord remnant was recorded, 38 had wet cord remnants at the time they were collected (Appendix 2.1). The range and distribution of the state of the cord remnant at the time the calves were collected is shown in Fig. 2.9.

2.3.12 General Observations

Some of the calves of an earlier known or estimated gestational age could be differentiated from less premature calves on the basis of their vocalisations and degree of coordination (Appendix 2.1).

Vocalisations

Four of the calves estimated to be more than two weeks premature were described as 'bawlers'. These calves vocalised frequently and on some occasions for protracted periods. The sound they made was more a drawn out squeal like that of a pig or goat than the usual sound made by a calf. One of these calves was kept for approximately two weeks during which time the bawling decreased and it began to vocalise more like a full-term calf.

Coordination

A blood sample was taken from all calves at the time they were collected from the farms. Most calves tried to move their neck away from the needle but generally stayed relatively still offering only mild resistance to the procedure once restrained. However, some of the more premature calves were noted as being 'hyper-reactive'. That is, in response to jugular venepuncture these calves would flail their legs in an uncoordinated manner or lie completely still offering no resistance, rather than actively trying to move their neck to avoid the needle stick. The impression gained was of an 'all or none' response. They showed either generalised uncoordinated movement or lack of activity as opposed to the moderate coordinated response seen in the calves of greater gestational age.

All calves were able to suck and to swallow when feeding. However the co-ordination of these behaviours seemed to be impaired in some of the calves that were estimated to be more than 3 weeks premature. Some calves would suck and then not swallow (swallowing also occurred, but when there was no milk in the mouth). This resulted in their mouths filling with milk which then dribbled out of the sides of the mouth in volumes that appeared greater than seen when full-term calves fed. The ability to coordinate these behaviours and feed effectively appeared to improve with age.

Hair Coat

Some of the calves estimated to be of an earlier gestational age had shorter hair. Their body's were completely covered with hair, but the hair length was less than that of calves born closer to full-term. Hair coat length was assessed qualitatively - rather than being measured.

2.3.13 Correlations Between Physical Parameters within Breeds and Crossbreeds

There was variation in the body structure between and within the different breeds and crossbreeds. The ratio between body weight and CRL gave an approximation of the body mass in relation to frame size. Friesian calves had a significantly lower body weight to CRL ratio than Hereford-Friesian crossbred calves (0.33 ± 0.01 vs 0.37 ± 0.01; $p < 0.05$). Such variation may have affected the degree of correlation between the parameters measured in different breeds or breed crosses. For this reason the degree of correlation between the parameters measured was also determined within some of the breeds and crossbreeds.

Friesian Calves

The estimated gestational age of the 28 Friesian calves was significantly correlated with tooth code and CRL (Table 2.5). CRL also showed a strong positive correlation with body weight, as well as moderate correlation with tooth code (Table 2.5). Body weight showed a strong positive correlation with tooth code (Table 2.5).

Hereford-Friesian Crossbred Calves

The estimated gestational age of the 14 Hereford-Friesian crossbred calves was significantly correlated with tooth code, breathing rate, rectal temperature and CRL (Table 2.6).

Table 2.5: Correlation coefficients (r) between parameters for Friesian calves

Parameter	CRL	Weight	Tooth Code	Rectal Temperature	Breathing Rate
Estimated Gestational Age	0.868***	b	0.696***	0.329	0.232
Crown Rump Length		0.871***	0.550 **	0.327	0.206
Body Weight			0.704 ***	0.334	0.229
Tooth Code				0.322	0.061

b = bodyweight was used to estimate gestational age therefore the correlation between these parameters not meaningful

* = P < 0.05

** = P < 0.01

** = P < 0.001

Table 2.6: Correlation coefficient (r) between parameters in Hereford-Friesian crossbred calves

Parameters	CRL	Weight	Tooth Code	Temperature	Breathing Rate
Estimated Gestational Age	0.835***	b	0.765**	0.689**	0.660*
Crown Rump Length		0.836***	0.897***	0.942***	0.522
Body Weight			0.764**	0.684**	0.662*
Tooth Code				0.876***	0.505

b = bodyweight was used to estimate gestational age therefore the correlation between these parameters not meaningful

* = P < 0.05

** = P < 0.01

** = P < 0.001

CRL also showed strong positive correlations with rectal temperature and tooth code. Body weight was correlated with CRL, rectal temperature and tooth code (Table 2.6). Rectal temperature was also significantly correlated with tooth code and breathing rate (Table 2.6).

Murray Grey-Friesian Crossbred Calves

The body weight of the 10 Murray Grey-Friesian crossbred calves was significantly correlated with tooth code ($r = 0.846$, $P < 0.01$). There were no other correlations between physical parameters within this breed cross.

Jersey-Friesian Crossbred Calves

There were no significant correlations between parameters within the 11 calves of this crossbreed.

Generally correlations between parameters within the Hereford-Friesian and Friesian calves were stronger than when they were made accross all breeds.

2.3.14 Changes in Parameters with Postnatal Age

Changes in the state of the umbilical cord remnant

There was a significant correlation between postnatal age and remnant state ($r = 0.558$, $P < 0.05$). By four days of age the cord remnant of most calves (73%) had shriveled although a few (13%) remained wet.

Changes in the tooth code

One day after birth 42% calves had 2 incisors that had erupted, 25% had 4 and 33% of the calves had 6 or 8 incisors erupted. By 4 days of age many of the calves had had at least one more pair of incisors erupt. At this stage 24 % of calves had 2 incisors that had erupted 11% had 4 and the majority (65%) had 6 or 8 incisors.

2.4 Discussion

In summary, the findings of the present study are as follows:

- In addition to being lighter and having shorter crown rump lengths, calves born at an earlier gestational age tended to have a lower rectal temperature, fewer deciduous incisor teeth erupted, and they breathed more slowly. These parameters combined allow estimation of a calf's gestational age to within approximately 1-2 weeks.
- Generally, neonatal calves born up to 3 weeks premature (> 259 days gestational age) were able to maintain a normal body temperature over the range of environmental temperatures experienced in Spring in the Manawatu region.
- Calves born with 6-8 incisors erupted are likely to be within two weeks of full-term birth. Those with less than 4 incisors erupted are likely to be greater than 3 weeks premature.
- Heart rate, gum colour and the presence or absence of hoof membranes are of little use in estimating the gestational age of an induced calf.
- A calf with a wet umbilical cord remnant is unlikely to be any greater than two days of age, however drying can be slow making this parameter an imprecise indicator of postnatal age.
- The number of deciduous incisor teeth erupted and palpable is not a reliable indicator of postnatal age.

Aging Premature Calves

2.4.1 Estimating the Gestational Age Using Bodyweight

The anticipated strong correlation between known gestational age and bodyweight in 11 of the calves is supported by observations of ruminant fetal growth in previous studies (Committee on Bovine Reproductive Nomenclature,1972; Mellor,1983; Richardson *et al.*,1990; Sivachelvan *et al.*,1996). This allowed bodyweight to be used to estimate the gestational age of the remaining 57 calves with reasonable accuracy ($r = 0.797$ $P < 0.01$).

It must however, be taken into consideration that at any one gestational age there is a range of sizes (weight, CRL) in any breed. This reduces the precision of using weight or CRL or any other physical parameter to determine gestational age. In the present study differences in gestational age of the calves account for 64% (0.797 x 0.797) of body weight variation. Differences in fetal genotype, maternal nutrition and placental size will contribute to the remaining 36% of weight variation at a given gestational age (Mellor,1983; Mellor,1987). The calves of known gestational age were of different breeds and crossbreeds. Using a single breed would have decreased this variation due to fetal genotype and increased the significance of the correlation between known gestational age and bodyweight. However, the calves in which bodyweight was used to estimate gestational age, also differed in breed and the equation used would have allowed for this. The correlations listed in Table 2.3 show that gestational age accounts for 64% of the variation in body weight, 49% of the variation in rectal temperature and 51% of the variation in breathing rate.

Strong correlations of the above parameters with estimated gestational age were retained when measurements of Hereford-Friesian and Friesian calves were analysed separately (Tables 2.5 and 2.6). In Hereford-Friesian calves' estimated gestational age accounted for 70% of the variation in CRL, 59% of the variation in tooth code, 47% of the variation rectal temperature and 44% of the variation in breathing rate. In Friesian calves estimated gestational age accounted for 75% of the variation in CRL and 48% of the variation in tooth code These figures indicates that these parameters are applicable to a range of calf breeds and breed crosses. The absence of such correlations in the Jersey-Friesian and Murray Grey-Friesian crossbred calves was likely to be due to a small sample size and a narrow range of gestational ages.

The calves were divided into subgroups that were 0-2 weeks premature, 2-3 weeks premature and 3-5 weeks premature to illustrate the change in mean CRL, body weight, rectal temperature, tooth code and breathing rate as their gestational ages approached full term (Table 2.7). Breed differences in these parameters are illustrated in Table 2.8, where Hereford-Friesian crossbred calves and Friesian calves are shown.

Table 2.7: The mean and standard deviation, 95% range, standard error and standard deviation of parameters measured in calves of all breeds estimated to have been born 0-2 weeks, 2-3 weeks and 3-5 weeks premature.

Parameter	Weeks premature	0-2	2-3	3-5
Crown Rump Length (cm)	Mean ± SD	85 ± 4	82 ± 4	72 ± 5
	95% Range	77-93	74-90	62-82
	Number (n)	15	18	12
	SEM	0.99	0.98	1.41
Rectal Temperature (°C)	Mean ± SD	39.2 ± 0.4	38.8 ± 0.5	38.5 ± 0.8
	95% Range	38.4-40	37.8-39.8	36.9-40.1
	Number (n)	16	21	15
	SEM	0.1	0.1	0.2
Number of Incisors	95% Range	4-8	2-8	0-8
	Number (n)	17	21	16
Breathing Rate (breaths/minute)	Mean ± SD	60 ± 15	53 ± 20	43 ± 17
	95% Range	30-90	13-93	9-77
	Number (n)	15	18	14
	SEM	4	4.75	4.5

95% Range = Mean ± 2 standard deviations

SEM = Standard error of the mean

2.4.2 Body Size

As expected premature calves born at an earlier gestational age have lighter body weights and shorter CRLs (Tables 2.7 and 2.8). The differences between the age groups (0-2 weeks, 2-3 weeks and 3-5 weeks premature) in Table 2.7 suggested a change in overall body structure in calves born 3-5 weeks premature compared with those born from 0-2 weeks premature.

Table 2.8: Means and ranges of parameters measured in Friesian and Hereford-Friesian calves estimated to have been born 0-2 weeks and 3-5 weeks premature.

Parameter		Friesians		Hereford - Friesians	
Weeks Premature		0-2	3-5	0-2	3-5
Crown Rump Length (cm)	Mean	85	73	85	71
	Range	81-92	68-80	79-89	69-75
	Number (n)	7	7	6	4
Temperature (°C)	Mean	39.1	38.6	39.3	37.7
	Range	38.5-39.5	37.6-39.7	38.9-39.9	36.9-38.4
	Number (n)	7	7	5	4
Number of Incisors	Range	6-8	0-8	4-8	0-2
	Number (n)	7	7	6	4
Breathing Rate (breaths/min)	Mean	54	44	65	43
	Range	36-84	18-72	54-84	30-66
	Number (n)	5	7	6	4

Between 245 (5 weeks pre-term) and 266 days of gestation (2 weeks pre-term), CRL and body weight continued to increase. At around 2-3 weeks pre-term, the growth patterns appear to change. From 266 days of gestation until full-term (280 days) the CRL grew at a slower rate while the body weight increased at a similar rate to that observed during the earlier period. This resulted in an increase in the body weight to CRL ratio from about 0.33 kg body weight /cm CRL to 0.39 kg bodyweight/cm CRL during the last 2-3 weeks before birth.

These patterns of CRL and body weight increase are supported in studies on both lambs and calves (Committee for Bovine Reproductive Nomenclature, 1972; Sivachelven *et*

al., 1996). A slowing of CRL growth as full-term is neared was shown by Richardson *et al.*, (1990) who studied fetal calves as well as by Mellor (1983) who studied individual fetal lambs. However slowing of the rate of CRL growth was not observed by Evans and Sack (1973). Richardson *et al.*(1990) also found a decrease in the rate of body weight increase between 260 and 280 day

A mean CRL of 82 ± 1 cm (Mean ± SD; Table 2.7) at 259-266 days gestation was within in the ranges listed by the Committee for Bovine Reproductive Nomenclature (1972), Evans and Sack, (1973) and Richardson *et al.*, (1990). The mean weight of 27.2 ± 0.2 kg was within the range reported by the Committee for Bovine Reproductive Nomenclature (1972) and higher than that reported by Richardson *et al.*,(1990). The discrepancy between these findings and those of Richardson *et al.*,(1990) is likely to be due to the fact that Richardson *et al.*,(1990) used Jersey fetuses and calves whereas the present study included a variety of breeds and crossbreeds many of which are on average heavier than Jerseys.

Although CRL and body weight were useful parameters for estimating the gestational age of premature calves, it is not advisable to use these parameters in isolation due the variability imposed by prenatal genetic and environmental factors (Sivachelvan *et al.*,1996). As previously mentioned gestational age accounts for only 64% of weight variation and estimated gestational age accounts for 54% of the variation in CRL when all calf breeds in the present study were considered together.

2.4.3 Rectal Temperature

Neonatal calves born up to 3 weeks premature were able to maintain a normal body temperature over the range of mean environmental temperatures of 7-13°C (Dirks,1995) experienced during spring in the Manawatu region. There was a wide range in the rectal temperatures that suggested variation in the thermogenic and thermoregulatory capacity of the calves. The normal rectal temperature of cattle is 38 - 39.5°C (Siegmund *et al.*,1973; Blood and Radostits,1989). Young animals have more labile temperatures

than older animals, and are subject to somewhat greater diurnal temperature fluctuations (Siegmund *et al.*,1973).

The majority of calves (55) had temperatures within the normal range. Three calves had temperatures above 39.5 °C and ten had temperatures below 38°C. Seven of the calves with low temperatures (<38°C) were more than 3 weeks premature (Appendix 2.1). Of the remaining three, two were listless and moribund at the time of collection.

Possible Causes of a Low Rectal Temperature

Below average rectal temperatures in some of the more premature calves may be a symptom of an impaired ability to produce or retain sufficient heat, excessive heat loss or the inability to effectively balance heat production with heat loss.

An impaired ability to produce heat may be due to immature thermogenic mechanisms or insufficient amounts of glycogen and BAT. Based on work by Alexander *et al.* (1973) and Mellor and Cockburn, (1986) it is likely that calves born more prematurely will have a lower summit metabolism and smaller quantities of glycogen and BAT than those born at full-term. The degree of impairment will become greater in calves born further from full-term. An increasing difficulty in maintaining body temperature in the smaller, light weight calves was illustrated by strong positive correlations of rectal temperature with body weight and CRL.

A greater rate of heat loss in lighter weight premature calves is supported by Randall (1978) and Carstens (1994). The insulative layer created by piloerection of the hair coat would be thinner in the more premature calves as they tended to have shorter hair. Decreased insulation allows a greater rate of heat loss.

Reduced thermoregulatory ability may be the result of functional immaturity or the inability to increase heat production to the level required to counter a high rate of heat loss. The CNS controlling the physiological systems involved in thermogenesis and

thermoregulation may be immature in calves with low rectal temperatures (Eales *et al.*,1982; Urban *et al.*,1997). An immature CNS would also be likely to contribute to the lack of co-ordination seen in some of the more premature calves. Based on the study of premature induced lambs by Alexander *et al.* (1973), it is likely that premature calves have a lower summit metabolic rate due to the interruption of BAT development and non-shivering thermogenesis capacity that occurs with premature birth, and a reduced ability to produce heat by shivering.

Some of the variation in rectal temperature may have been due to the fact that in some cases different thermometers were used, different people were doing the measurement and therefore the technique used, in terms of the depth to which the thermometers were inserted into the rectum, would have varied.

2.4.4 Eruption of Deciduous Incisor Teeth

Calves born at an earlier gestational age had fewer erupted incisors than calves born closer to full term. Three to five weeks before term a calf was likely to have had only 2 or 4 incisors erupt (Tables 2.7 and 2.8). In comparison, calves born within two weeks of full-term were likely to have 6 or 8 incisors erupt (Tables 2.7 and 2.8).

Sisson and Grossman (1953) stated that the eruption of deciduous teeth varies somewhat and that about 75% of calves have all incisors erupted at birth. With the exception of this commentary, the prenatal eruption of the deciduous incisors is not well documented in the literature. While there was variation in the tooth code of calves of a given gestational age, it was a parameter that was unlikely to vary with breed to the same degree as weight and CRL. Used in conjunction with parameters such as CRL, weight, rectal temperature and breathing rate it may add accuracy to the estimation of a premature calf's gestational age.

The postnatal age of the calves (days and hours after birth) in the present study varied possibly accounting for some of the variation in the number of teeth erupted at the time

of collection. As teeth may be cut in the process of feeding, those calves that had fed prior to their collection may have had more teeth through than those that had not. Even so, when tooth eruption was followed postnatally it did not occur rapidly and may therefore be predominantly a developmental event rather than being affected by behaviour or postnatal age.

2.4.5 Hoof Membranes

Hoof membranes were worn in 87% of the calves indicating that most viable induced calves born up to 5 weeks prematurely were able to stand and walk. However, it must be noted that only the live calves were collected therefore this figure does not accurately represent all induced calves. The two calves in which the hoof membranes were unworn at the time of collection were moribund and estimated to be 260 and 266 days gestational age. Six of the calves with half worn hoof membranes were under 1 day old at the time they were collected, and therefore may have had little opportunity to wear the membranes off their hooves. Presence of the hoof membrane is not a useful measure of gestational age in calves, however it does indicate whether the calf has been able to stand and walk.

2.4.6 Breathing Rate

Despite the fact that breathing rate may be influenced by many factors including panting to increase heat loss and the emotional state of the calf, the results of the present study indicate that 51% of the variation in breathing rate could be attributed to gestational age. Based on a positive correlation between breathing rate, gestational age and other parameters that vary with gestational age (CRL and weight; Tables 2.3, 2.5 and 2.6), it can be said that the calves born at an earlier gestational age tend to breath at a slower rate. Reference to Tables 2.7 and 2.8 suggests that a calf with a resting breathing rate of less than 30 breaths/minute is likely to be more than two weeks premature. Even so, 49% of the variation in breathings rate is due to factors other than gestational age so that it could not be used to provide a very precise estimate the age of a premature calf.

The high mortality rate of induced calves is likely to be the result of a number of factors, however the ability to undergo effective respiratory adaptation at birth is likely to be a

major limiting factor. Normally the pre-partum cortisol surge stimulates maturation of the fetal lungs shortly before full-term birth (Liggins,1969). Thus the lungs of a calf born prematurely as the result of corticosteroid-induced parturition will be more mature than those of a calf delivered by caesarian at the same gestational age.

The degree to which lung maturation is accelerated by the corticosteroids used to induce parturition may be a function of the gestational age of the calf as well as the drug and treatment regime used. A calf's lungs may be more likely to mature to a greater degree under induction treatments that more closely mimic the changes in corticosteroid concentration that occur during a natural full-term birth.

When long-acting corticosteroids are used to induce parturition the neonatal mortality rates of induced calves are higher than when either the short-acting drugs or a combination of drugs were used (Bunny,1992). Neonatal mortality is the same as would be expected in untreated cows when short-acting formulations are used in the last two weeks of gestation (Bunny,1992).

Although an excessively slow breathing rate may indicate that a calf is having difficulty breathing it is a parameter that is likely to vary with its emotional state and the required rate of heat loss. The emotional states of the calves, their awareness of the environment and reaction to being handled would have been likely to have varied. If the calves had been given time to stabilise in an environment with a similar environmental temperature before breathing rate was measured and if more than one measurement was made this parameter may have been of greater use for comparison between animals of groups of different gestational age. It is possible that a slow rate of breathing was associated with deeper breathes, however this was not investigated in the present study.

2.4.7 The Immune Status of Induced Calves

GGT activity is an accurate indicator as to whether a calf has consumed colostrum, given that the amount of time between a colostrum feed and blood sampling is sufficient

for absorption of GGT (Thompson and Pauli, 1981). Variation in GGT activity between calves of a similar gestational age was highly likely to have been influenced by whether the calves had been fed prior to their collection.

While there was no correlation between gestational age and GGT activity all calves with GGT activities greater than 1000 U/l were estimated to have been no greater than 3 weeks premature (Appendix 2.1). Calves induced closer to term are more likely to have been given short-acting corticosteroids. It has been found previously that the immune status of calves induced in this manner is not adversely affected (Muller *et al.*, 1975; Beardsley *et al.*, 1976; Naylor, 1986).

The reduced ability of some of the more premature calves to feed due to weakness and the inability to coordinate sucking and swallowing, would result in either no colostrum being swallowed or the swallowing of smaller volumes. Weakness and an impaired ability to feed have also been observed in premature calves by Adams (1969), Welch *et al.*,(1973) and Bailey *et al.*,(1973). If smaller volumes of colostrum or no colostrum is ingested the amounts of energy-substrates and immunoglobulins available for absorption (therefore the maximum attainable GGT level) are also reduced. Calves with lower GGT levels are more susceptible to disease, possibly contributing to the higher mortality rate in induced calves.

2.4.8 Heart Rate

Like breathing rate heart rate is strongly influenced by an animal's emotional state, therefore it is not surprising that heart rate showed no correlation with gestational age. Because of the great potential for variation due to emotional state it is of little use as an index of a comparatively fixed variable like gestational age unless measurement were taken when each calf was in a similar state such as sleep.

2.4.9 Gum Colour

Gum colour was of little use as an index of gestational age. This is not surprising given that gum colour is more likely to be affected by postnatal factors such as body

temperature than by prenatal factors that would vary with gestational age. Tissue oxygenation would have been better assessed through analysis of blood samples. The capillary refill time of the gums would have been a better measure of circulatory efficiency than gum colour.

2.4.10 Determining the Postnatal Age of Induced Calves

State of the Umbilical Cord Remnant

Under the conditions of this study (calves kept inside in close contact with others), the umbilical cord remnant of induced calves had usually become shriveled by four days of age (73% of calves). An induced calf with a shriveled umbilical cord remnant was likely to be a minimum of three days of age. The cord remnant was still wet by four days of age in 13% of calves. Thus, a calf with a wet cord remnant had a 73% chance of being less than two days of age.

Evidently it was possible for cord remnant drying to be slowed for some reason in 27% of calves. Persistence of a wet umbilical cord remnant in these calves may be due the calves being kept indoors in close contact with other calves. Close contact with other calves permitted cross sucking thereby preventing it from drying. The shed in which the calves were kept was relatively draft-free. Had the calves been exposed to the wind and sun the umbilical cord remnant may have dried more rapidly. Disease or persistence of a patent urachus may have also slowed drying. Urine escaping from a patent urachus would slow or prevent the umbilical cord remnant from drying.

Tooth Code

Although generally the number of incisors that had erupted increased between birth and four days of age, there is no clear pattern. The number of incisors that are present at four days of age is dependent on the number erupted at birth and therefore also the gestational age of the calf. For these reasons tooth code is not a useful parameter for determining the post-natal age of induced calves.

2.5 General Discussion

The main focus of this chapter was to study the physical and physiological state of induced calves with a view to determining factors that may increase the potential for welfare compromise compared to calves born at full-term. Three functions adversely affected by premature birth that are likely to contribute to the greater mortality rate of induced are the maintenance of body temperature, the ability to breathe and the ability to feed effectively by coordinating sucking and swallowing calves (MacDiarmid,1980; Welch and Kaltenbach,1977; Welch *et al.*,1979; Bunny,1992).

Compromise of these functions was evident only in calves that were greater than 3 weeks premature. This observation supports that made by Welch and Kaltenbach (1977) who found that the mortality rate of induced calves increased when they were more than 10 kg lighter than the average. According to the equation that related bodyweight and gestational age in the present study calves greater than 2 weeks premature would have been at least 10 kg lighter than those born at full-term.

Below average rectal temperatures indicated that some calves were unable to maintain their body temperature. Slower breathing rates in calves born at an earlier gestational age may indicate breathing difficulty. Low GGT levels and the observation of some calves having difficulty coordinating sucking and swallowing indicated that calves born greater than 3 weeks premature have difficulty feeding. These calves would be more likely to experience undernutrition and increased susceptibility to disease through an inadequate intake of colostrum and milk.

As evidence of compromise occurs mainly in calves born earlier than 3 weeks before full-term it is useful to be able to identify these calves. This study has shown that if a calf of any of the breeds examined here:

- weighs less than 25 kg,
- has a crown rump length of less than 74 cm,

- has a rectal temperature lower than 37.8°C,
- has less than 4 erupted incisors,
- takes less than 30 breaths per minute,

it is likely to be greater than 3 weeks premature (< 259 days gestational age).

The welfare of calves that are unable to maintain their temperature, breathe and feed effectively will be compromised to a greater degree than in full-term calves. These calves may suffer unnecessarily if they are kept for rearing or sent by truck for slaughter. The Animal Welfare Advisory Committee states that calves weighing less than 15 kg are usually too premature to survive the bobbying process and should be humanely destroyed rather than bobbied (AWAC,1994). It must be noted that a Jersey calf weighing 15 kg is likely to be more mature than a 15 kg Hereford-Friesian crossbred calf. Thus introducing other parameters such as the number of deciduous incisors erupted may be of greater use as a minimum standard than body weight alone. A 15 kg Jersey calf would be likely to have more teeth erupted than a 15 kg Hereford-Friesian calf.

Although calves weighing more than 15 kg may survive, from the results of the present study it could be suggested than some of these calves may be suffering unnecessarily. Raising the minimum recommended weight may be of advantage, as may introducing other criteria that exclude some of the more compromised premature calves from being sent for slaughter. Indeed, the potential for compromise of all induced calves, whether reared or bobbied, would be reduced if induction of parturition were only allowed in the last 3 weeks of pregnancy.

The usefulness and accuracy of the findings in the present study would have been increased if the gestational ages of all calves had been known and if their treatment from birth onward in terms of feeding had been similar.

2.5.1 Future Research

Future research could include the following.

- Inducing parturition in cows at specific times, such that the degree of prematurity of the calves is known and then assessing the physical and physiological states of these calves. This would increase knowledge of the maturational changes that occur in late gestation.

- Determine the amount of BAT and glycogen present in neonatal premature induced calves of varying gestational ages. This would allow estimation of the period for which premature calves could maintain their body temperature at different environmental temperatures when fasted (Mellor & Cockburn,1986).

- Measure the maximal heat production capacity through both shivering and non-shivering thermogenesis in neonatal premature calves born at differing gestational ages. This would allow estimation of a calf's ability to maintain its body temperature at varying environmental temperatures and gestational ages.

- Measure maximum heat production in individual calves as their postnatal ages increase to assess changes in their ability to maintain their body temperature in cold conditions (Alexander *et al.*,1973).

- Measure the hair length on calves of different gestational ages. This would allow the degree of insulation provided be the hair coat to be estimated.

- Study behaviour modification responses of premature calves in different air temperatures. This may differ from that of full-term calves and affect their ability to reduce heat loss under cold environmental temperatures.

- Measure the degree of blood oxygenation in calves of different gestational ages to study differences in lung function and maturity. Lung maturity is a major factor that influenced the viability of premature calves.

- Quantify the degree of coordination of calves at different gestational ages through a similar method to that used in human babies (Carter & Bowen,1994) in order to asses when premature calves develop coordination similar to that of a full-term calf. This may be important in determining appropriate feeding methods for premature calves.

Chapter Three: Feeding and Fasting of Induced Calves

Abstract

The present study has shown that viable induced calves born up to approximately five weeks prematurely tend to have slightly lower plasma glucose concentrations and slightly higher β-hydroxybutyrate concentrations than those born at full-term. This is likely to indicate a reduced availability of carbohydrates and therefore a greater dependence on lipids to provide their body's energy requirements. The degree to which premature calves depend on an increased rate of lipid catabolism to provide their energy requirements decreases as their gestational age at birth nears full-term. Premature calves were found to gain no apparent advantage (when in a fed state) in terms of energy availability from being fed a similar volume of milk over four feeds as opposed to twice within a ten hour period of each 24 hour day.

The ability of these calves to tolerate 30 hours fasting in terms of the availability of energy, increased as their gestational age at birth neared full-term. Fasting for this duration did not cause dehydration as indicated by the absence of significant increases in packed cell volume or total plasma protein concentration. The energy availability in fasted calves (as indicated by plasma glucose concentration) was greater in those calves fed 50 ml colostrum / kg body weight rather than 25 ml/kg at the onset of fasting. Four day old and ten day old calves showed no difference in their metabolic responses to fasting or different feeding frequencies (twice or four times daily).

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Chapter 3: Feeding and Fasting of Induced Calves

3.1 Introduction

Many premature induced calves die on farms, but others born closer to their due date may survive and be sent for slaughter along with full-term calves (Bunny,1992). The process in which surplus and unwanted calves are sent for slaughter is called bobbying. It involves transport and fasting both of which are potential stressors. The physiological responses of cattle of varying ages to fasting and transport have been examined (Crookshank *et al.*,1979; Cole *et al.*,1986; Warriss *et al.*, 1995), but the responses of premature calves have not been examined. Although bobbying involves both transport and fasting, the experiment described here was designed to assess only effects of fasting on premature induced calves without the added effects of transport.

3.1.1 Changes in Nutrient Supply During the Perinatal Period

In *utero*, the energy and other nutrient requirements of the bovine fetus are supplied continuously from the maternal circulation by way of the placenta and umbilical cord (Mayor and Cuezva, 1985; Ktorza *et al.*,1985). Glycogen, the form in which the body stores carbohydrates, accumulates in most fetal tissues, but predominantly in the liver and skeletal muscles (Mellor and Cockburn,1986). Liver glycogen is deposited at an accelerated rate just before birth, mediated by insulin and the preparturient increase in glucocorticoid secretion (Mayor and Cuezva,1985; Mellor 1988; Mellor,1993). Increases in muscle glycogen also occur before birth in the fetuses of many species, however this is not apparently linked to the pre-partum cortisol surge (Barnes, 1977; Mellor, 1993).

Lipid energy is stored as triglycerides within brown and white adipose tissue. Approximately 2% of the bodyweight of a full-term newborn Friesian calf is brown adipose tissue (BAT) (Alexander *et al.*,1975). The small amounts of white adipose tissue (WAT) present at the time of birth in full-term calves are restricted to thin (< 1 mm thick) subcutaneous depots (Alexander *et al.*,1975).

Hormonal changes occurring during the perinatal period stimulate the physiological adjustments necessary for the perinatal calf to adapt to the change in energy supply that occurs at birth (Ktorza *et al.*,1975; Mayor and Cuezva,1985). Once it is born energy substrates and nutrients are provided intermittently to the calf in colostrum or milk. The neonatal calf must therefore tolerate fluctuations in nutrient supply characterised by periods of fasting between bouts of sucking. The release of catecholamines associated with birth causes a decrease in the insulin/glucagon ratio, triggering the breakdown of liver glycogen and the production of energy substrates through gluconeogenesis (Mayor and Cuezva,1985). Similar hormonal changes occur during the variable periods of fasting that separate bouts of sucking. These allow the energy and nutrient requirements of the tissues to be met through mobilisation of body energy reserves during such periods of fasting.

3.1.2 Assessing Metabolic Responses to Feeding and Fasting

Metabolic responses to feeding and fasting can be evaluated by observing the changes in the plasma concentrations of carbohydrate, lipid and protein metabolites over time (Kinsbergen *et al.*,1994). During periods of fasting most of the body's energy requirements are initially provided through the mobilisation of glucose from glycogen reserves and lipids from adipose tissue. As these reserves become depleted the fasting body becomes increasingly dependent on protein catabolism to provide energy (Alexander, 1962).

3.1.3 Carbohydrate Metabolism

Glucose provides energy through its breakdown to carbon dioxide and water by way of the glycolytic pathway. The plasma glucose concentration reflects the balance between entry of glucose into the blood, from either the digestive tract or liver, and the uptake of glucose by the tissues. It provides an indication of the amount of glucose that is available to the tissues. An increase in plasma glucose indicates that the rate at which glucose is entering the blood is greater than its rate of uptake by the tissues. Raised plasma glucose concentrations stimulate the synthesis and secretion of insulin (Kaneko, 1989). Insulin facilitates the uptake of glucose by peripheral tissue cells (Kaneko, 1989).

As the blood glucose concentration decreases glycogen is broken down to provide glucose through the process of glycogenolysis. The breakdown of liver glycogen allows release of glucose into the blood stream, thereby making it available to all tissues.

Muscle glycogen is mobilised during contraction of the muscles as in exercise or shivering. Mobilisation of muscle glycogen releases glucose thereby making energy directly available to the working muscle, but glucose derived from the muscle glycogen cannot be released into the circulation. However, when muscular contraction occurs in the absence of sufficient oxygen, unmetabolised lactate is formed through the process of anaerobic glycolysis and is released into the blood (Mellor and Cockburn, 1986). Lactate may then be taken up from the plasma by the liver and further metabolised to provide energy.

Decreased plasma glucose concentrations (below approximately 4-5 mmol/l in the case of calves) would indicate a decrease in the availability of energy to the tissues, including heat producing tissues, and to the brain for which glucose is the sole energy source. Maintenance of adequate plasma glucose concentrations is necessary to prevent hypothermia at cooler air temperatures (0-10°C) and cerebral compromise at warmer air temperatures (Mellor and Cockburn,1986).

3.1.4 Lipid Metabolism

Lipids are stored in the form of triglycerides within BAT and WAT. Triglycerides themselves cannot be used directly to supply energy, however they may be broken down into one molecule of glycerol and three fatty acids from which energy can be derived. Glycerol can then enter the glycolytic pathway. Fatty acids can be used directly as a source of energy by some tissues. Lipids stored within WAT are liberated into the blood stream as free fatty acids (FFAs) to be made available to all tissues. Those stored within brown adipose tissue (BAT) are used within the tissue itself to generate heat (Schoonderwoerd *et al.*,1986).

When excess fatty acids have been mobilised they can be broken down by the liver to form the ketone bodies acetoacetate, β-hydroxybutyrate and acetone, which can be used by many tissues as a source of energy. Increases in plasma β-hydroxybutyrate

concentrations are associated with increases in the rate of fat mobilisation (Kaneko,1989).

3.1.5 Protein and Amino Acid Metabolism

When the readily available carbohydrate stores of glycogen and lipid become depleted the body then catabolises protein to supply its energy requirements. Proteins are made up of amino acids. Amino acids are deaminated during gluconeogenesis or they are catabolised completely to carbon dioxide, water and energy. Deamination causes the release of ammonia which then forms urea which may be excreted in the urine. Deaminated amino acids may be used directly in the tricarboxylic acid cycle to create glucose through the process of gluconeogenesis. Provided that the kidneys are functioning properly an increase in plasma urea concentration may therefore indicate an increased rate of protein breakdown (Blaxter and Wood, 1951; Kinsbergen *et al*, 1994; Kaneko,1989), and that an animal was having to breakdown amino acids to meet its body's energy requirements. Use of carbohydrates, lipid and protein results in weight reduction as the stored sources are metabolised.

3.1.6 Hydration State During Fasting

Milk and colostrum are the only external source of water available to a young calf. Therefore fasting could be associated with dehydration. Two parameters of dehydration are the packed cell volume and total plasma protein concentration (Kaneko,1989; Michell *et al.*,1989). The body is constantly losing fluid through insensible loss as well as the excretion of urine and faeces. When normal fluid loss is not replaced through eating or drinking the body becomes increasingly dehydrated. During this process the plasma water content is depleted so that the proportion of blood cells to plasma increases causing an increase in packed cell volume, and there is an associated increase in total plasma protein concentration. Decreases in plasma volume as indicated by packed cell volume may be masked if the change in osmotic balance of the plasma associated with dehydration causes withdrawal of water from within the red blood cells resulting in their shrinkage.

3.1.7 The Potential Effects of Prematurity on the Metabolic Responses to Feeding and Fasting.

In *utero* the hormonal environment is such that deposition of energy reserves is usually promoted (Mayor and Cuezva, 1985; Ktorza *et al.*,1985). Given that muscle glycogen

and lipids are all deposited at a linear rate during gestation and that this rate does not increase in response to the usual corticosteroid surge which causes labour (Mellor, 1983; Mellor and Cockburn, 1986), a calf born before full-term will have reduced quantities of these energy substrates. Birth induces hormonal changes that prevent the further development of BAT and promote the breakdown of glycogen rather than its continued accumulation (Alexander *et al.*, 1973; Mayor and Cuezva, 1985; Ktorza *et al.*,1985). Unlike these other energy substrates the rate of white fat deposition increases after birth with the onset of colostrum and milk intake and the conversion of BAT to WAT (Alexander *et al.*,1973; Alexander *et al.*,1975).

Liver glycogen deposition is stimulated by glucocorticoids (Mayor and Cuezva, 1985). Thus, induction of premature birth using synthetic glucocorticoids is likely to increase the amount of glycogen available compared to that in calves delivered by casearian at the same gestational age, provided that synthetic hormones cross the placenta in sufficient quantities for a sufficient period to mimic the effect of the usual endogenous cortisol surge. Even so, the extent of liver glycogen deposition in an induced calf may still be less than that in a full-term calf because of their decreased gestation period.

The duration of fasting that an animal can tolerate is dependent on the extent of its glycogen and lipid reserves as well as environmental conditions (Alexander, 1962; Mellor and Cockburn,1986; Schoonderwoerd *et al.*,1986). Smaller quantities of liver glycogen and WAT will lessen the period for which the body's energy requirements can be provided through catabolism of these substrates (Mellor and Cockburn,1986). Depletion of WAT and liver glycogen reserves would result in an increasing dependency on protein catabolism (Alexander, 1962).

Muscle glycogen and BAT are energy reserves that are specifically used in shivering and non-shivering thermogenesis (Mellor and Cockburn,1986; Schoonderwoerd,1986). A smaller quantity of these substrates is likely to reduce the period for which the body temperature could be maintained through a rate of thermogenesis above that of the basal metabolism. The thermogenic rate of a low weight induced calf is likely to be greater than that of a full-term calf due to an elevated rate of heat loss (see Chapter 2).

3.1.8 The Effect of Postnatal Age on the Responses to Feeding and Fasting

A premature calf's ability to tolerate fasting and adapt to extra-uterine conditions may increase with its postnatal age. In those that survive, physiological systems will mature over time. In full-term lambs for instance the stomach and intestines continue to develop after birth which presumably indicates a change in the capacities for digestion and absorption of food (Mellor,1993).

With time, the thermogenic capacity of a calf will increase and the rate at which it loses heat will decrease. The insulation from external temperatures provided by the cutaneous tissues of full-term neonatal calves is low at birth and increases by 37% during the first 14 days of life (Carstens,1994) with the intake of lipid rich colostrum and increasing thickness of subcutaneous WAT. Growth of the hair coat would also provide increased insulation, and the ability to reduce heat loss through vasoconstriction of cutaneous vessels also increases (Carstens,1994). With increasing gestational age calves and lambs become less dependent on non-shivering thermogenesis to produce heat and instead generate more heat through shivering (Alexander *et al.*,1973; Carstens,1994).

3.1.9 Recommendations of The Animal Welfare Advisory Committee

The Animal Welfare Advisory Committee (AWAC) produces codes of recommendations and minimum standards with a view to advising the Minister of Agriculture on issues relating to the treatment of animals in New Zealand. Four of these codes contain recommendations regarding the feeding, postnatal age, minimum weight and duration of transport of induced or otherwise premature calves. These are the codes relating to the welfare of dairy cattle, bobby calves and animals transported within New Zealand (AWAC, 1992; 1993; 1994; 1997). One aim of this research was to asses some of the recommendations and minimum standards listed in these AWAC codes regarding the treatment of premature calves.

Recommendations Regarding Body weight and Postnatal Age

The AWAC codes state that calves weighing less than 15 kg should be humanely destroyed rather than sent for slaughter as these calves are likely to be too premature to survive (AWAC,1992;1993). They also state that calves should be a minimum of 4 days of age when they are sent for slaughter. As postnatal age has the potential to

influence a calf's response to fasting or to feeding frequency, premature calves of both four and ten days of age were used in this study.

Recommendations Regarding the Feeding of Calves

If calves are to be accepted for slaughter at four days of age or older, they must be treated in such a way that an adequate level of welfare is maintained (see Chapter 1). This means providing colostrum and milk at regular intervals and in appropriate volumes. Intake of a sufficient volume of colostrum within the first 24 hours is necessary if a calf is to acquire the passive immunity it requires to resist neonatal disease (see Chapter 5). Colostrum and milk are also the young calf's only source of nutritients and fluid intake. Inadequate supply could result in undernutrition and/or dehydration.

The code recommends that all young calves receive a volume of milk or colostrum equivalent to 10-12% (100-120 ml/kg) of their bodyweight (AWAC,1993). This volume is intended to provide for the energetic and nutritional requirements of a young calf. It is recommended that full-term calves be fed either *ad libitum* or half the total volume twice daily and for premature calves correspondingly smaller volumes 4 to 6 times each day during the first week (AWAC,1993).

Recommendations Regarding the Maximum Duration of Fasting

During pre-slaughter penning, transport and lairage the maximum time calves are likely to go without food was calculated to be 30 hours. According to AWAC (1994) calves should be fed their usual volume of milk in the morning, before being placed in a roadside pen. During collection of such calves it must not take longer than 12 hours before the first calf collected reaches its final destination. Calves are then held in lairage and may not be slaughtered until the next day. By this calculation the maximum time between the morning feed before collection and the time of slaughter should be no longer than 30 hours.

While the code recommends a maximum transport duration of 12 hours for full-term calves, this is shortened to 4 hours for induced calves (AWAC,1994). Despite this recommendation, it is most likely that any premature calves that are sent for slaughter would be treated similarly to the full-term calves rather than be given special treatment. By using 30 hours we take into consideration the maximum likely duration of fasting

and from this the metabolic responses to any shorter duration of fasting can also be assessed.

3.1.10 Aims of the Present Experiment

The aims of the present experiment were to:

- study the metabolic responses of premature calves to two different feeding frequencies (50 ml/kg and 100 ml/kg).
- observe effects of 30 hours fasting on premature induced calves.
- determine whether postnatal age affects the responses of premature calves to feeding frequency or a 30 hours fast.
- study the metabolic state of viable premature calves of varying gestational ages, and
- assess some of the recommendations and minimum standards listed in the AWAC codes regarding the treatment of premature calves.

3.2 Materials and Methods

3.2.1 Animals

Sixty-two induced calves were collected from dairy farms around the Manawatu district, most within two days of their birth. The calves varied in breed and degree of prematurity. Breeds included Friesian, Jersey, Jersey x Friesian, Hereford x Jersey, Hereford x Friesian and Murray Grey x Friesian (see Chapter 2). While the date of premature birth for all calves was known, the expected full-term birth date was given for only eleven of them. Weight, crown rump length (CRL) and a tooth code were used to assess calf maturity where the full-term due date was not known (discussed in chapter 2). Comparison with calves of a known degree of prematurity suggested that all calves were between 1 day and 5 weeks premature. The calves ranged in weight from 16.5 kg to 38 kg with a mean weight of 24.4 ± 0.6 kg.

3.2.2 Housing and Husbandry

From the time of their arrival the calves were kept in indoor pens (1.7m x 2.43m, and 0.83 m high). Pen floors were covered by a rubber mat. Calf cereal (Top Calf Cereal Mix: Harvey Farms, New Zealand) and water were available *ad libitum* in each pen. The pens were hosed out twice daily after the morning and afternoon feeds. At this time the calf cereal and water were also replaced.

3.2.3 Feeding

From the time of collection one half of the calves were fed twice daily and one half were fed four times daily. Calf assignment to groups ensured that equal weight ranges were represented, but was otherwise random. All calves were kept on their designated feeding frequency for a minimum of two days before being entered in an experiment as either a control or fasted animal.

Each calf was fed pooled colostrum from a local dairy farm using an individual calf feeder (Skellerup 'Udder Mother'; Hamilton, New Zealand). Calf feeders were cleaned, disinfected (Chloride of lime - bleach; Ecolab) and rinsed between successive calves to minimise the spread of disease. The calves were collected and entered into the

experiment over a four week period, thus the source of colostrum and the concentrations of its constituents may have varied over time.

Calves fed twice daily

Calves fed twice daily were fed as recommended for full-term calves (AWAC,1993). They were fed 50 mg/kg of colostrum (or electrolyte solution) at 0800 and 1600 hours daily. On the days of the experiment control calves were fed at 0515 hours (t = - 0.75h), at 1600 hours (t = 10h) and at 0800 hours (t = 26h) the following day.

Calves fed four times daily

These calves were fed as recommended for premature calves (AWAC,1993). They were given 25 mg/kg of colostrum (or electrolytes) four times at 0800, 1200, 1600 and 2000 hours. On the days of the experiment control calves were fed their specified volume of colostrum at 0515 (t = -0.75h), 0800 (t = 2h), 1200 (t = 6h) 1600 hours (t = 10h) and again at 0800 (t = 26h) and 1200 (t = 30h) on the second day. Calves were fed four times over a 10-12 hour period (during normal waking hours) as this was thought to be more practical than feeding at 6 hourly intervals over a 24 hour period.

3.2.4 Feeding of Sick Calves

Calves with diarrhoea were fed 100-120 ml electrolytes /kg bodyweight per day split into either two or four feeds depending on their treatment group ('Lectade Plus'-SmithKline-Beecham). Electrolytes were given for 48 hours and then the calves were fed colostrum. Diarrhoetic calves were separated from healthy calves as soon as diarrhoea was noticed to minimise the spread of infection. They were returned to their groups once the diarrhoea was finished and returned to milk feeding.

Although diarrhoetic calves were fed electrolytes for up to four feeds prior to the experiment, all were returned to colostrum feeds for the 34 hours of the experiment over which blood samples were taken. Data from calves that were noted as having diarrhoea within 24 hours of the experiment were excluded from the analysis.

3.2.5 Postnatal Age

The effects of feeding frequency and fasting on calves at four or ten days after birth were studied. Some of the calves that were treated as controls at four days of age were

used again at ten days of age to minimise the total number of animals used in the experiment.

3.2.6 Fasting

On the day of the experiment the calves were divided into those to be treated as controls and those to be fasted and were distributed evenly by weight to create an equally wide variation in degree of prematurity in fasted groups and control groups.

All calves were fed according to their prior feeding regime at 0515 hours on the day of the experiment (t = -0.75). That is, calves that had previously been fed four times daily were fed 25 ml/kg body weight of colostrum at this time and calves previously fed twice daily were fed 50 ml/kg bodyweight. Fasted calves were not fed again until 30 hours later (t = 30).

During the period of fasting these calves had access to fresh water but not to the calf cereal mix, colostrum or electrolyte solution. At 30 hours, all fasted calves were fed 50 ml/kg bodyweight. Control calves were fed as designated throughout the experiment.

3.2.7 Treatment Groups

Eight treatment groups were created allowing for all combinations of feeding frequency, postnatal age and fasting (Table 3.1). Originally each group comprised of nine to eleven calves. Once the plasma samples were analysed it became apparent that some of the calves had plasma substrate concentrations that were considerably different (either lower or higher) from the means for the rest of the calves at the time of the pre-treatment sample. Subsequent clinical assessment of these parameters in conjunction with notes made during the experiment and pathology reports revealed that these calves had recently had diarrhoea and were metabolically abnormal as a result.

The plasma metabolite concentrations of many of these calves were outside two standard deviations of the mean and indicated dehydration (high PCV and TPPs) compromised energy status (high β-hydroxybutyrate and urea with low glucose concentrations), and low immune status (low GGT level). These combined factors were

considered adequate reason to withdraw the plasma metabolite concentrations of these calves from the final analysis of results. Withdrawal of these calves left each group with a total of seven to nine calves.

Table 3.1: Summary of the eight treatment groups and the codes used to descibe them.

Treatment Groups	Postnatal Age		Feeding Frequency (feeds daily)		Treatment During Experiment	
Code	4 days	10 days	2 times	4 times	Fed as Usual	Fasted
4D:4F-Control	√			√	√	
4D:4F-Fasted	√			√		√
10D:4F-Control		√		√	√	
10D:4F-Fasted		√		√		√
4D:2F-Control	√		√		√	
4D:2F-Fasted	√		√			√
10D:2F-Control		√	√		√	
10D:2F-Fasted		√	√			√

Ã = A feature of the groups treatment

Four day old calves, fed four times daily - Control (4D:4F-Control)

These calves were four to eight days old on the first day of the experiment. They were fed four times daily prior to and during the experiment.

Four day old calves, fed four times daily - Fasted (4D:4F-Fasted)

These calves were four to eight days old and were fed four times daily prior to the experiment. On the day of the experiment they were fed their usual volume of colostrum (25 ml/kg bodyweight) at 0515 hours (t = -0.75h) and were then fasted for 30 hours after which they were refed (50 ml/kg bodyweight).

Ten day old calves, fed four times daily - Control (10D:4F-Control)

All calves in this group were ten to fourteen days old at the start of the experiment. They were fed four times daily prior to and during the experiment.

Ten day old calves, fed four times daily - Fasted (10D:4F-Fasted)

Calves in this groups were ten to fourteen days old at the start of the experiment. They were all fed four times daily prior to the experiment. On the first day of the experiment the calves were fed 25 ml/kg bodyweight at 0515 hours (t = -0.75h) and were then fasted for 30 hours after which they were refed (50 ml/kg bodyweight).

Four day old calves, fed twice daily - Control (4D:2F-Control)

These calves were four to eight days old on the first day of the experiment. They were fed twice daily prior to and during the experiment.

Four day old calves, fed twice daily - Fasted (4D:2F-Fasted)

These calves were four to eight days old and were fed twice daily prior to the experiment. On the day of the experiment they were fed their usual volume of colostrum (50 ml/kg bodyweight) at 0515 hours (t = -0.75h) and were then fasted for 30 hours after which they were refed (50 ml/kg bodyweight).

Ten day old calves, fed twice daily - Control (10D:2F-Control)

All calves in this group were ten to fourteen days old at the start of the experiment. They were fed twice daily prior to and during the experiment.

Ten day old calves, fed twice daily - Fasted (10D:2F-Fasted)

Calves in this groups were ten to fourteen days old at the start of the experiment. They were all fed twice daily prior to the experiment. On the first day of the experiment the calves were fed 50 ml/kg bodyweight at 0515 hours (t = -0.75h) and were then fasted for 30 hours after which they were refed (50 ml/kg bodyweight).

3.2.8 Blood Sampling

Blood samples (up to 10 ml) were taken by venepucture into heparinised vacutainers from either jugular vein while the calves were gently restrained either by the bleeder or by another person.

A pre-treatment blood sample was taken at 0500 hours (t = -1 hour) on the first day of the experiment. Samples were then taken from all calves at t = 0, 3, 6, 9, 12, 15, 18, 24,

27, 30, 31, 32 and 33 hours. The packed cell volume (PCV) of each blood sample was measured using the capillary tube centrifugation method.

Blood samples were stored on ice until the PCV had been measured and recorded, they were then centrifuged ('Labofuge' 400e, Heraeus Instruments) at 3000 rcf for 20 minutes, and the plasma was separated and stored at -20°C to await analysis.

3.2.9 Analysis of Plasma Samples

The plasma samples were analysed for glucose (Boehringer-Mannheim, kit 14486698; Trinder, 1969), β-hydroxybutyrate (Sigma, procedure 310-UV; Li, 1985), urea (Boehringer- Mannheim, kit 1489364; Neumann and Ziegenhorn,1977) and total plasma proteins (Boehringer-Mannheim, kit 1553836; Weichselbaum, 1946) using a Hitachi 704 Multi-channel Analyser. Plasma triglyceride concentration were measured with a Hitachi 717 Autoanalyser (30°C) (Boehringer-Mannheim, catalogue no. 816370; Trinder, 1969). Appendix 1 lists the times at which plasma samples were analysed for the different substrates.

3.2.10 Rectal Temperature

During the experiment at 1h, 15h and 30h the rectal temperatures of all calves were measured at an approximate depth of 20-30 mm using a clinical mercury thermometer.

3.2.11 Statistical Analysis

Results are expressed as the mean ± the standard error of the mean (SEM). Significant differences between mean values were determined using Single Factor Analysis of Variance and Student's t test assuming unequal variance (Microsoft Excel V 5.0, Microsoft Corporation, USA).

Correlations between metabolic parameters and physical features were analysed by linear regression (Microsoft Excel V 5.0, Microsoft Corporation, USA).

3.2.12 Effects of Prematurity on Metabolic Parameters

Relationships between metabolic parameters and physical parameters that vary with age (as discussed in chapter 2) were determined using linear regression. The importance of

indices of gestational age on metabolic status was evaluated by reference to the significance of the regression coefficient (Clarke and Cooke,1978).

3.2.13 Effects of Calf Breed

Breed may affect the way in which parameters change with increasing gestational age. Thus in some cases during evaluation of the results the calves were separated into groups of the different breed or breed crosses.

3.3 Results

Energy Status

3.3.1 Glucose

The Effect of Feeding Frequency

At the start of the experiment the calves had a mean plasma glucose concentration of 5.0 ± 0.1 mmol/l (Table 3.2). During the first 10 hours all control calves were given the full recommended volume of milk (100-120 ml/kg bodyweight) split into either two or four feeds. Thereafter they were not fed for 16 hours overnight, and fed again at 26 hours after the start of the experiment (Appendix 3.1). Plasma glucose concentrations tended to increase in response to feeding with the magnitude of the increase in response to each feed usually being greater the larger the feed. Glucose concentrations usually started to decline between three and six hours after the most recent feed.

Control Groups Fed Four Times Daily (4D:4F-Control and 10D:4F-Control)

Control calves fed 25 ml colostrum/kg bodyweight four times during the first 10 hours exhibited a gradual rise in plasma glucose concentration during the period of feeding, with a peak concentration reached at 12 hours (Fig 3.1 and 3.2). Thereafter, the concentration decreased to reach its lowest value at 24 hours. Feeding at 26 and 30 hours resulted in subsequent increases in plasma glucose concentration to values which were similar to those seen at the beginning of the experiment.

Control Groups Fed Twice Daily (4D:2F-Control and 10D:2F-Control)

Control calves fed 50 ml colostrum/kg bodyweight exhibited transient rises in plasma glucose concentration after each feed, except after the first feed in 10D:2F-Controls (Figs 3.3 and 3.4).

Table 3.2: Means and standard errors (mean ±SEM) of all parameters measured

Parameter	Sample Time	4D:4F-Control	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
Glucose	-1	4.8±0.3	4.8±0.4	5.2±0.3	5.1±0.3	5.6±0.2	4.6±0.2	4.5±0.3	5.3±0.4
(mmol/l)	12	6.0±0.5	4.1±0.3	6.3±0.4	4.0±0.2	6.4±0.4	4.6±0.3	5.4±0.4	4.9±0.3
	24	4.4±0.4	3.0±0.2	4.1±0.4	2.9±0.3	4.9±0.2	2.9±0.3	3.4±0.3	3.2±0.3
	30	5.0±0.3	3.4±0.1	4.6±0.3	3.3±0.3	6.1±0.5	2.7±0.3	5.5±0.3	3.0±0.2
Triglycerides	-1	0.38±0.09	0.47±0.2	0.40±0.12	0.32±0.07	0.54±0.08	0.23±0.05	0.19±0.05	0.43±0.11
(mmol/l)	30	0.21±0.04	0.15±0.02	0.23±0.09	0.17±0.03	0.23±0.07	0.19±0.03	0.10±0.02	0.20±0.05
B-hydroxybutyrate	-1	0.09±0.01	0.07±0.01	0.13±0.01	0.12±0.03	0.10±0.02	0.15±0.03	0.22±0.05	0.11±0.04
(mmol/l)	30	0.1±0.02	0.39±0.07	0.16±0.03	0.38±0.08	0.07±0.01	0.65±0.08	0.23±0.05	0.52±0.06
Urea	-1	2.7±0.3	2.9±0.5	3.8±0.4	3.8±0.5	2.6±0.5	3.6±0.3	4.9±0.5	3.0±0.6
(mmol/l)	30	3.6±0.5	6.4±0.8	3.9±0.4	8.8±1.9	2.7±0.5	5.0±0.3	5.6±0.9	5.2±0.6
Packed Cell Volume	-1	38±2	37±2	36±2	41±2	37±1	40±2	39±3	39±2
(%)	30	36±2	37±1	35±2	42±2	37±2	41±2	39±2	40±2
Total Plasma Proteins	-1	46±1	46±3	54±2	55±2	65±7	65±2	69±4	59±4
(g/l)	30	52±4	48±3	51±2	61±6	62±6	65±5	67±5	67±5
Temperature	-1	38.5±0.2	39.0±0.2	38.6±0.1	38.8±0.1	38.3±0.3	38.4±0.2	38.8±0.1	38.4±0.2
(C)	30	38.6±0.2	38.1±0.3	38.7±0.2	38.2±0.3	38.9±0.1	38.7±0.3	38.6±0.3	38.3±0.3

Table 3.3: The changes (mean ±SEM) in parameters from the initial value.

Parameter		4D:4F-Control	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
Glucose	Change to 12 hours	1.1±0.4	-0.1±0.3	1.0±0.2	-1.1±0.2	0.8±0.2	0±0.2	0.9±0.6	0±0.2
(mmol/l)	Change to 24 hours	-.04±0.3	-1.8±0.4	-1.2±0.3	-2.2±0.3	-0.7±0.3	-1.7±0.3	-1.1±0.4	-2.1±0.4
	Change to 30 hours	0.2±0.2	-1.5±0.4	-0.6±0.4	-1.8±0.4	0.6±0.4	-1.9±0.3	1.0±0.4	-2.3±0.4
Triglycerides (mmol/l)	Change to 30 hours	-0.17±0.06	-0.32±0.18	-0.17±0.12	-0.2±0.07	-0.3±0.07	-0.04±0.03	-0.09±0.04	-0.23±0.11
β-hydroxybutyrate (mmol/l)	Change to 30 hours	0.01±0.02	0.33±0.06	0.02±0.03	0.26±0.09	-0.03±0.01	0.5±0.07	0±0.06	0.41±0.07
Urea (mmol/l)	Change to 30 hours	0.87±0.41	3.74±0.57	0.15±0.54	4.97±1.62	0.13±0.4	1.43±0.31	0.65±0.89	2.16±0.41
PCV (%)	Change to 30 hours	-1.1±0.5	0±0.6	-0.2±1.3	1.1±1	-0.7±0.5	1.8±0.6	-0.6±2.7	0.4±0.5
TPP (g/l)	Change to 30 hours	2.54±1.07	2.58±1.84	-3±1.35	-2.4±1.39	-3.58±1.33	0.56±1.54	-0.92±1.71	-2.07±1.54
Temperature (C)	Change to 30 hours	0.2±0.2	-1±0.4	0.1±0.3	-0.6±0.3	0.5±0.3	0.2±0.3	-0.2±0.3	-0.2±0.3

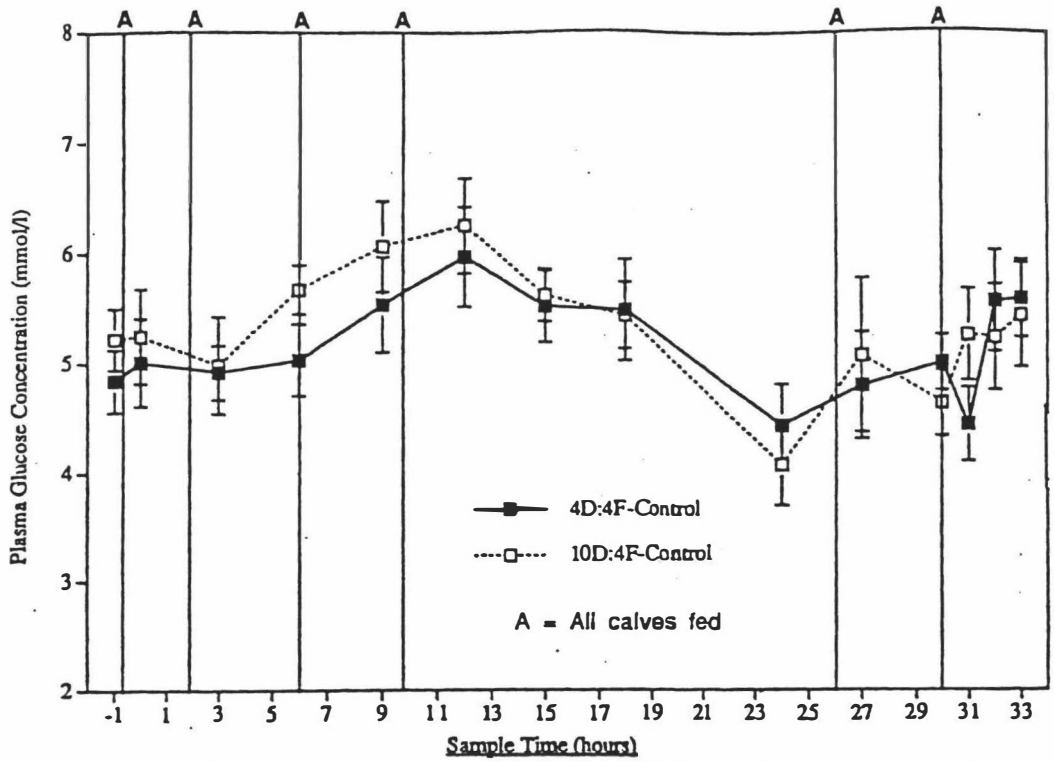

Fig 3.1: The plasma glucose concentrations (mean ± SEM) in calves that were either 4 or 10 days of age and were fed 25 ml colostrum/kg bodyweight four times daily.

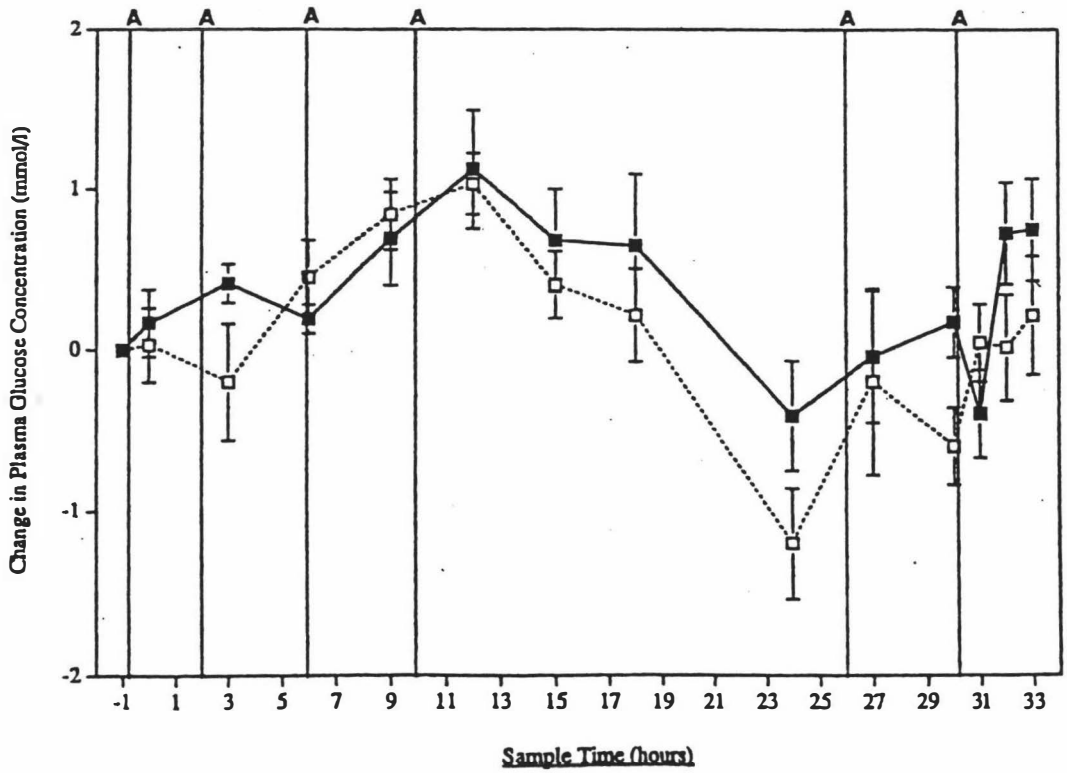

Fig 3.2: The change in plasma glucose concentration (mean ± SEM) over time from that of the intial sample, in calves that were either 4 or 10 days of age and were fed 25 ml colostrum /kg bodyweight four times daily

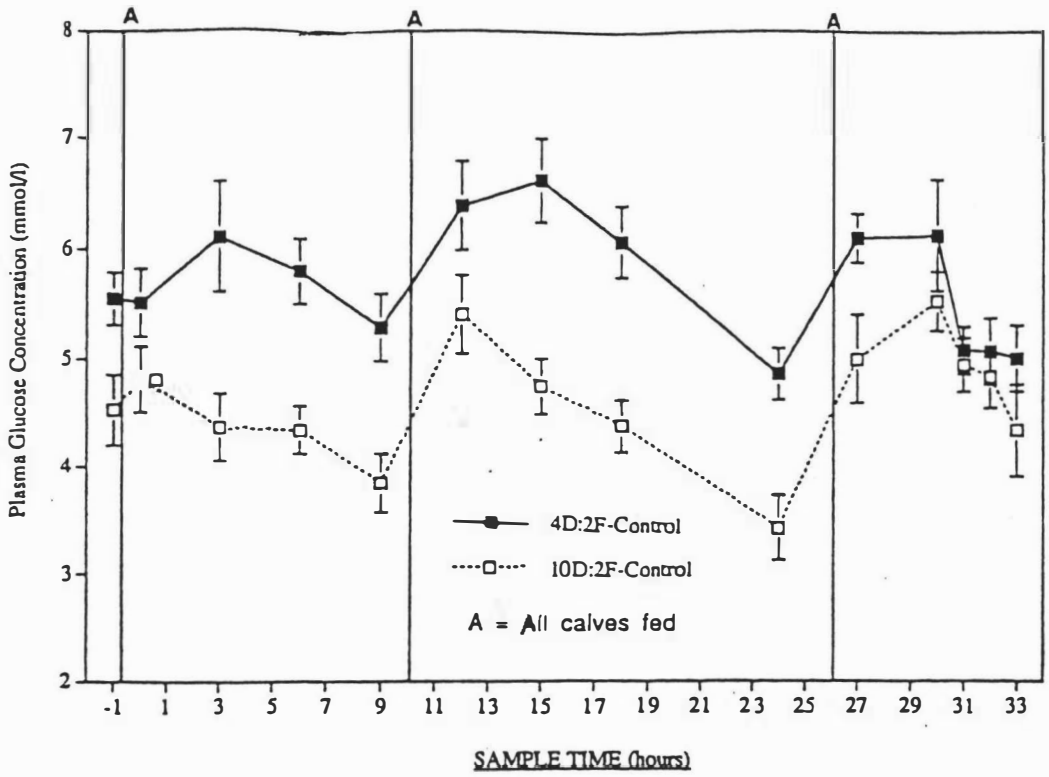

Fig 3.3: The plasma glucose concentrations (mean ± SEM) in calves that were either 4 or 10 days of age and were fed 50 ml colostrum/kg bodyweight twice daily.

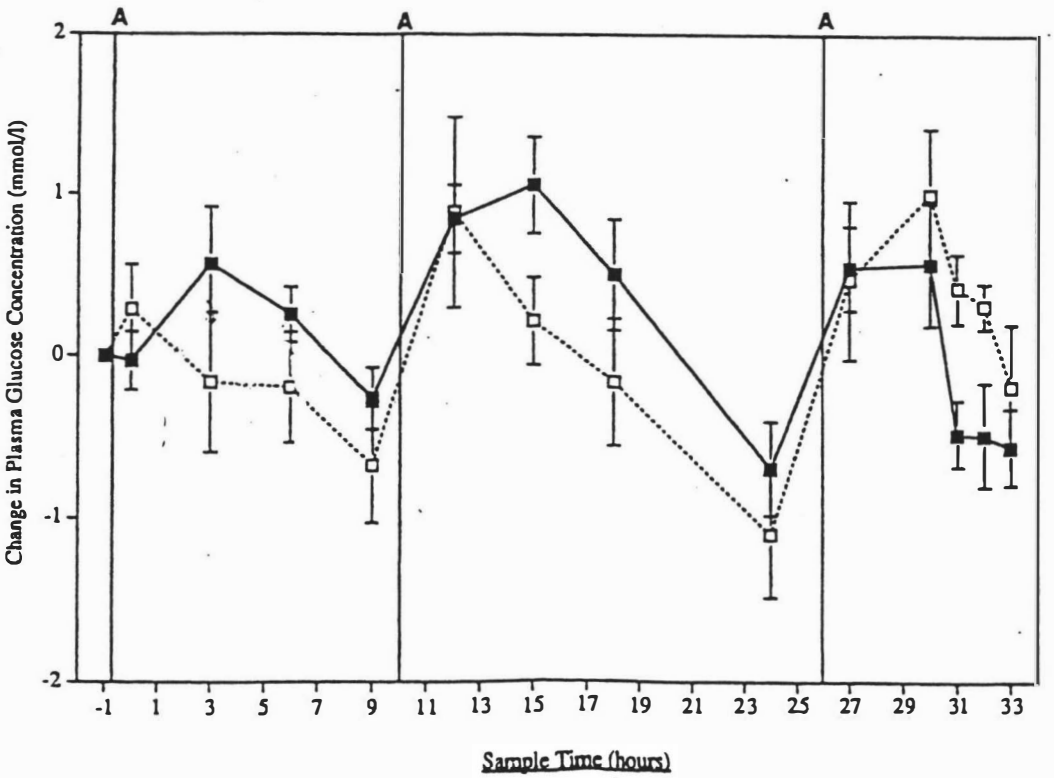

Fig 3.4: The change in plasma glucose concentration (mean ± SEM) over time from that of the intial sample, in calves that were either 4 or 10 days of age and were fed 50ml/kg twice daily.

Comparison of All Control Groups

At the start of the experiment there was no difference in the plasma glucose concentrations of the calves that had been on different feeding frequencies (Appendix 3.2). The major difference between the groups was seen 9 hours after the start of the experiment (Figs 3.5 and 3.6). By this time the concentrations in the 2F control groups had declined on average by 0.4 ± 0.2 mmol/l to reach a mean of 4.7 ± 0.3 mmol/l. In comparison, the concentrations in the 4F control groups had increased by 0.8 ± 0.2 mmol/l from their initial values to reach 5.8 ± 0.3 mmol/l.

The overall glucose concentration difference between groups created by the two feeding frequencies was no longer present by 12 hours, at which time the plasma concentrations of all control groups had reached a similar mean high of 6.0 ± 0.2 mmol/l. The magnitude of the decrease in concentration during the overnight fast was similar across all control groups, having a mean value of -1.78 ± 0.2 mmol/l. At 24 hours a low mean concentration of 4.3 ± 0.2 mmol/l was reached.

The Effect of Postnatal Age

4F-Control Groups:

The plasma glucose concentrations of the two 4F control groups started at 4.8 ± 0.3 mmol/l (4D:4F-Control) and 5.2 ± 0.3 mmol/l (10D:4F-Control). Both increased and decreased to the same degree in response to feeding and fasting thereby remaining similar throughout the 34 hours during which regular blood samples were taken (Figs 3.1 and 3.2).

2F-Control Groups:

4D:2F-Control calves had a significantly ($P<0.05$) higher plasma glucose concentration than did 10D:2F-Control calves in the initial sample (5.6 ± 0.2 mmol/l and 4.5 ± 0.3 mmol/l respectively, see Table 3.2 and Appendix 3.2). This difference was retained except when the plasma glucose concentrations of both groups increase to become similar in response to feeding at 12 hours and again from 30-33 hours (Figs 3.3 and 3.4). The peak in plasma glucose concentration reached by all control groups

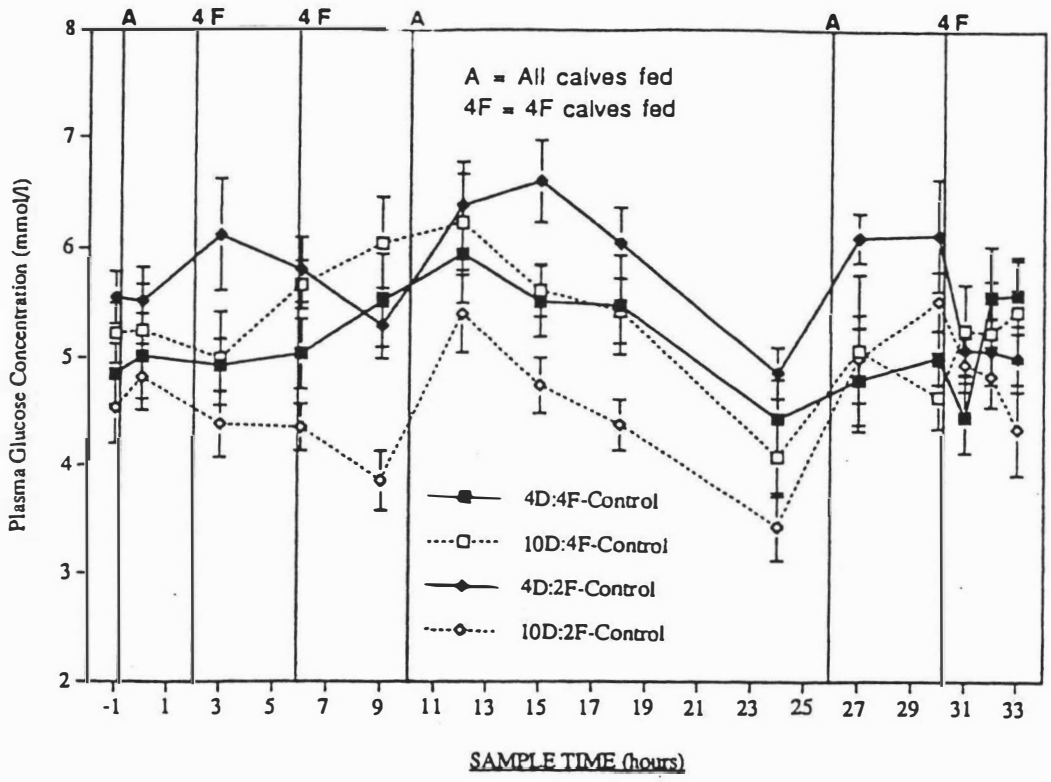

Fig 3.5: The plasma glucose concentrations (mean ± SEM) in calves that were fed two or four times daily.

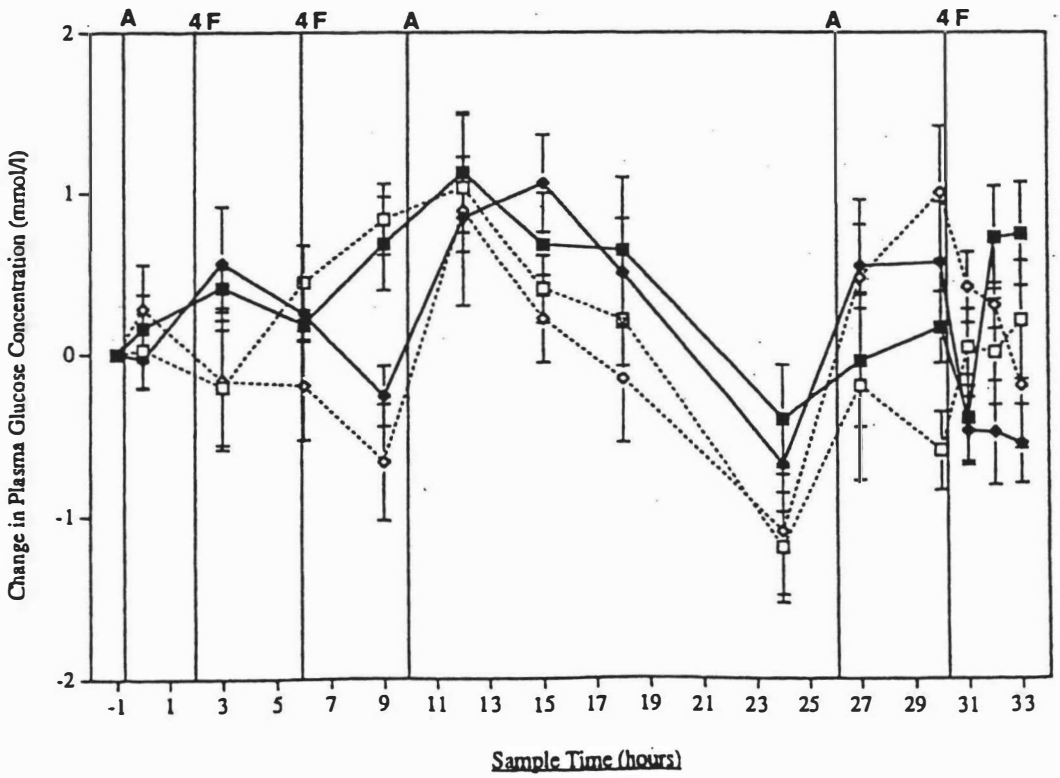

Fig 3.6: The change in plasma glucose concentration (mean ± SEM) over time from that of the intial sample, in calves that were fed either two or four times daily.

at 12 hours had increased further to 6.6 ± 0.4 mmol/l in 4D:2F-Control calves by 15 hours (t = 15). In comparison, the concentrations in 10D:2F-Control calves began to decline from 12 hours and had an mean value of 4.7 ± 0.3 mmol/l 15 hours after the start of the experiment.

Fasted Calves

Three hours after the pre-fasting feed, the plasma glucose concentrations of the 2F-Fasted groups had increased to a significantly greater degree ($P<0.01$) than those of the 4F-Fasted groups (0.9 ± 0.2 mmol/l and 0.5 ± 0.4 mmol/l, respectively, Figs 3.7 and 3.8). From this time (t = 3h) there was a gradual decrease in plasma glucose concentration until 24 hours, at which time the low mean of 3.0 ± 0.1 mmol/l was reached by all fasted groups. This low was maintained until 30 hours after the start of the experiment, at which time the fasted calves were refed.

The only significant difference ($P < 0.05$) between groups occurred between 10D:4F-Fasted and 10D:2F-Fasted calves between 9 and 18 hours after the start of the experiment, during which time the plasma glucose concentration of 10D:2F-Fasted calves was greater (Appendix 3.2). In terms of the change in plasma glucose concentration from the initial value the difference between these groups was only significant between 12 and 15 hours ($P< 0.05$) (Appendix 3.3). Otherwise, the patterns of changes in plasma glucose concentration over the 30 hour fast were similar across all four groups.

The Response to Refeeding

In all groups of previously fasted calves that were fed 50 ml colostrum/kg bodyweight at 30 hours, there was a marked increase in plasma glucose concentrations during the following 3 hours (Figs 3.7 and 3.8). The concentrations equaled or exceeded pre-treatment values by 3 hours after refeeding. Feeding frequency prior to fasting had no effect on the glucose response to refeeding, but the postnatal age did. The increase in glucose concentration within 3 hours of refeeding (between t = 30h and t = 33h) was significantly greater ($P<0.05$) in the 4-day-old calves (4D:4F-Fasted and 4D:2F-Fasted combined) than in 10-day-old calves

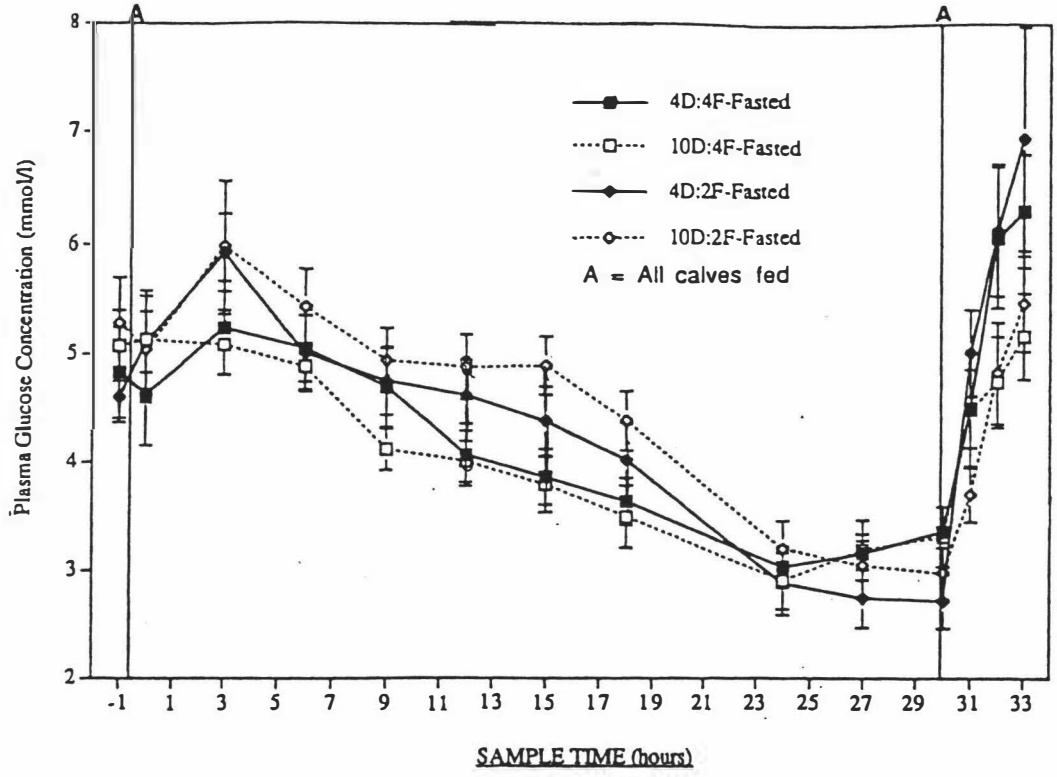

Fig 3.7: The plasma glucose concentrations (mean ± SEM) in calves that were fed either 25 ml or 50 ml colostrum/kg bodyweight, fasted for 30 hours, then fed 50 ml colostrum/kg bodyweight.

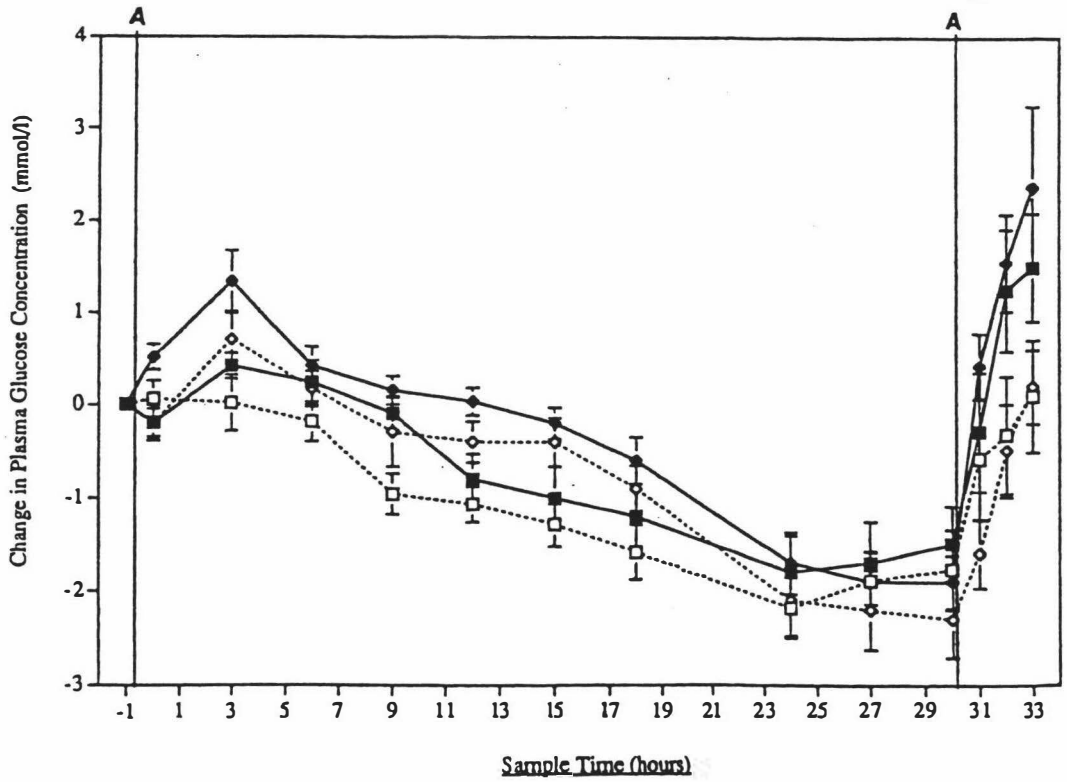

Fig 3.8: The change in plasma glucose concentration (mean ± SEM) over time from that of the initial sample, in calves that were fed either 25 ml or 50 ml colostrum/kg bodyweight, fasted for 30 hours, then fed 50 ml colostrum/kg bodyweight.

(10D:4F-Fasted and 10D:2F-Fasted combined), being 3.6 ± 0.6 mmol/l and 2.1 ± 0.3 mmol/l respectively.

Control vs. Fasted

The effects of the different treatments (fed or fasted) on blood glucose concentration became apparent in all groups, except 10D:2F-Control, between 6 and 12 hours after the start of the experiment. At this time the plasma glucose concentrations of the control and fasted groups began to diverge.

4D:4F

The plasma glucose concentrations of fed calves began to increase and those in fasted calves began to decrease about 6 hours after the start of the experiment (Figs 3.9 and 3.10). By 12 hours the mean plasma glucose concentration of control calves had increased by 1.1 ± 0.4 mmol/l and that of fasted calves had decreased by 0.1 ± 0.3 mmol/l making the concentrations in these two groups significantly different ($P < 0.01$). Thereafter, the plasma glucose concentrations of the fed calves remained greater than those of the fasted calves until after the fasted calves were refed (at t = 30h).

10D:4F

The plasma glucose concentrations of fed calves had increased by 0.5 ± 0.2 mmol/l, and those of the fasted calves had declined by 0.2 ± 0.2 mmol/l by 6 hours after the start of the experiment, such that the concentration difference between the two groups was significant ($P < 0.05$) (Figs 3.11 and 3.12). By 9 hours (t = 9h) this difference had become greater as the plasma glucose concentrations in fed calves continued to increase and those in fasted calves continued to decline. As with 4D:4F calves this difference was maintained until the fasted calves were refed.

4D:2F

Although the plasma glucose concentration of 4D:2F-Control calves was significantly higher ($P < 0.05$) than that of 4D:2F-Fasted calves in the pre-treatment sample (5.6 ± 0.2 mmol/l and 4.6 ± 0.2 mmol/l, respectively), the degree of change in the control and fasted groups remained similar until 12 hours after the start of the experiment (Figs 3.13 and 3.14). By this time the plasma glucose concentration of 4D:2F-Control had increased

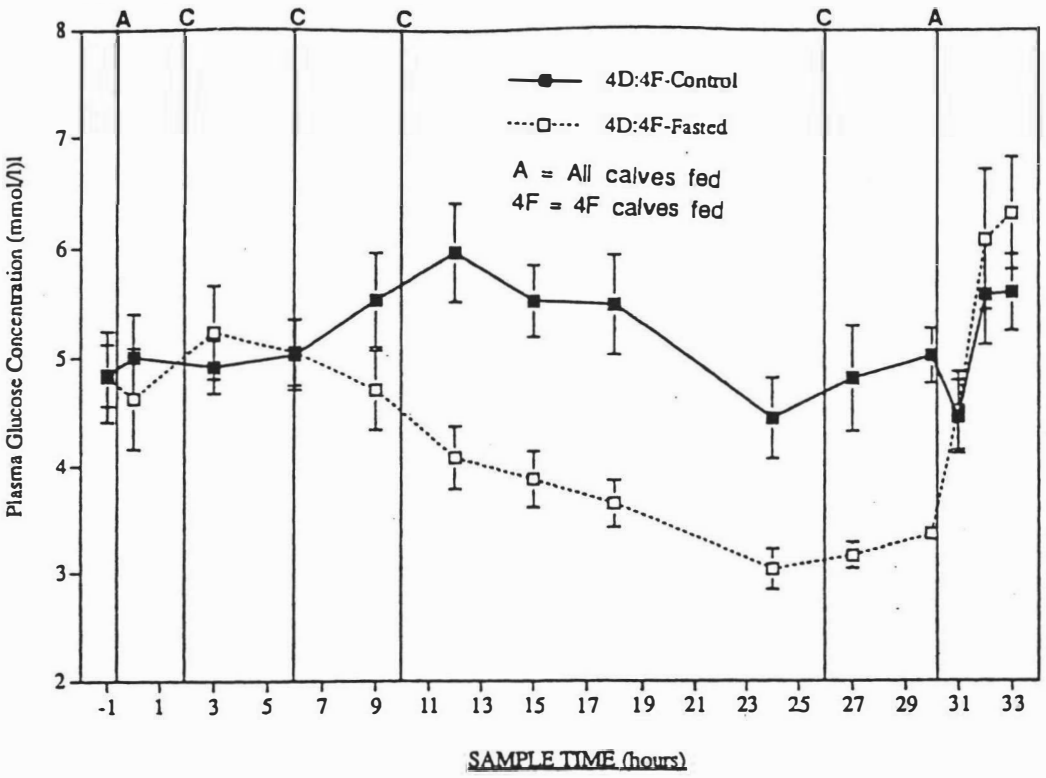

Fig 3.9: The plasma glucose concentrations (mean ± SEM) in four day old calves that were either fed four times daily or fasted for 30 hours.

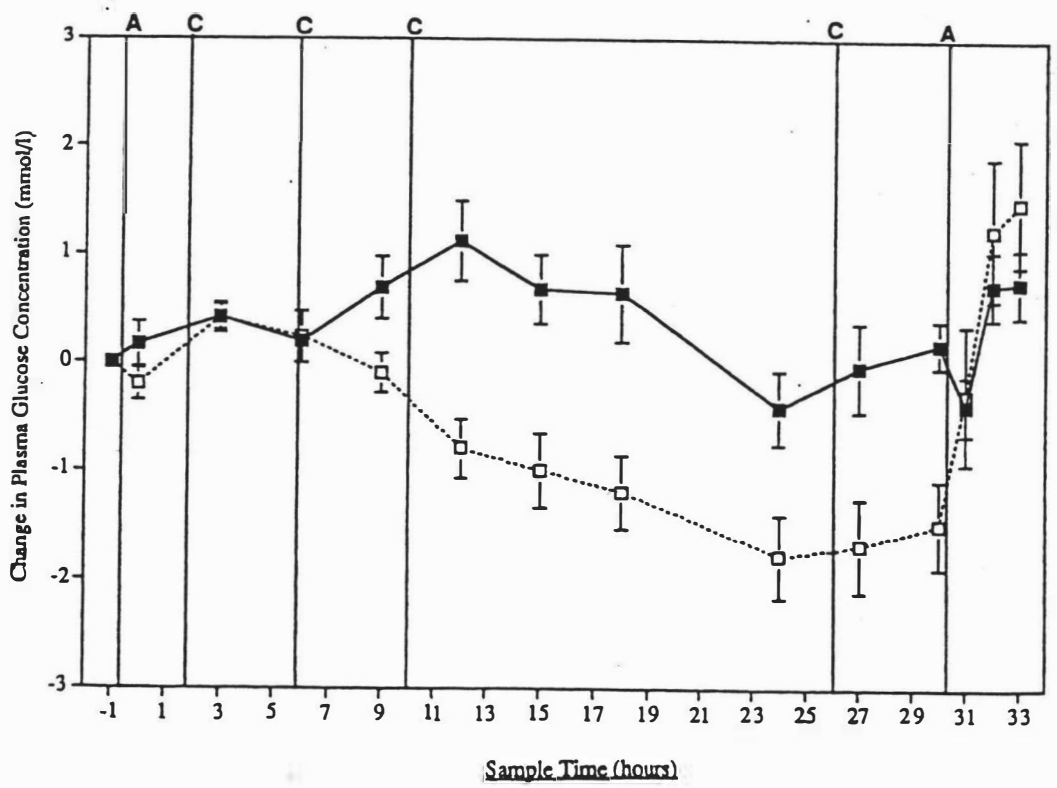

Fig 3.10: The change in plasma glucose concentration (mean ± SEM) over time from that of the intial sample, in four day old calves that were either fed four times daily or fasted for 30 hours.

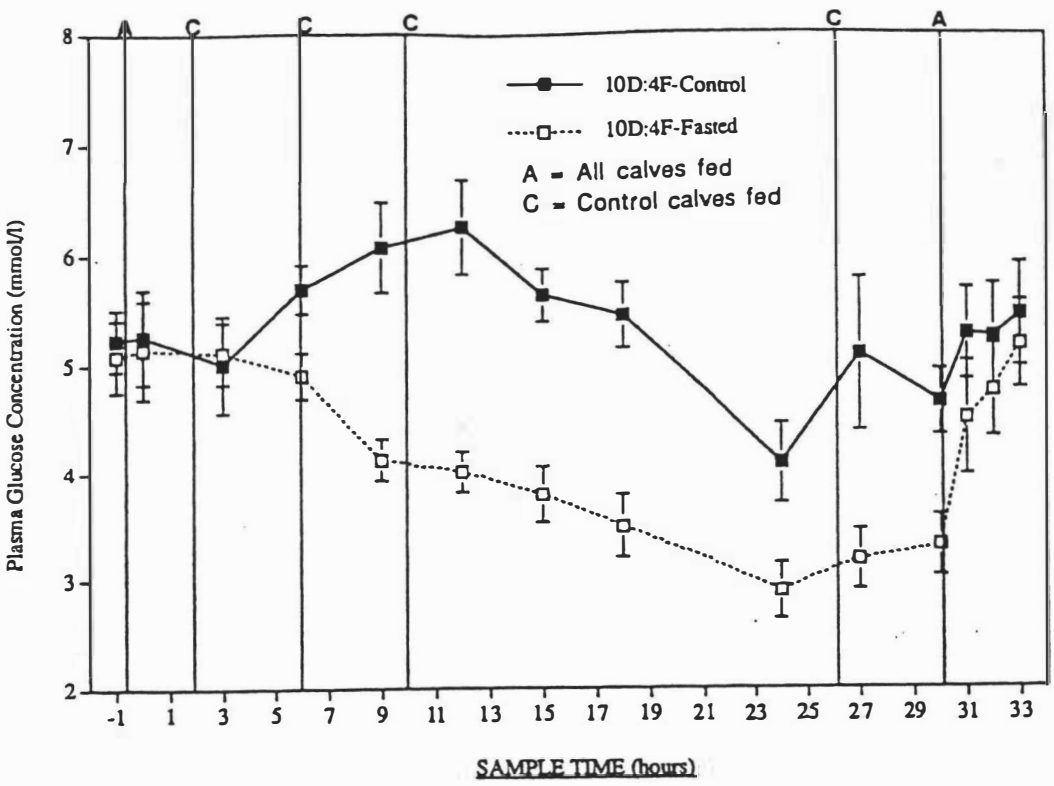

Fig 3.11: The plasma glucose concentrations (mean ± SEM) in ten day old calves that were either fed four times daily or fasted for 30 hours.

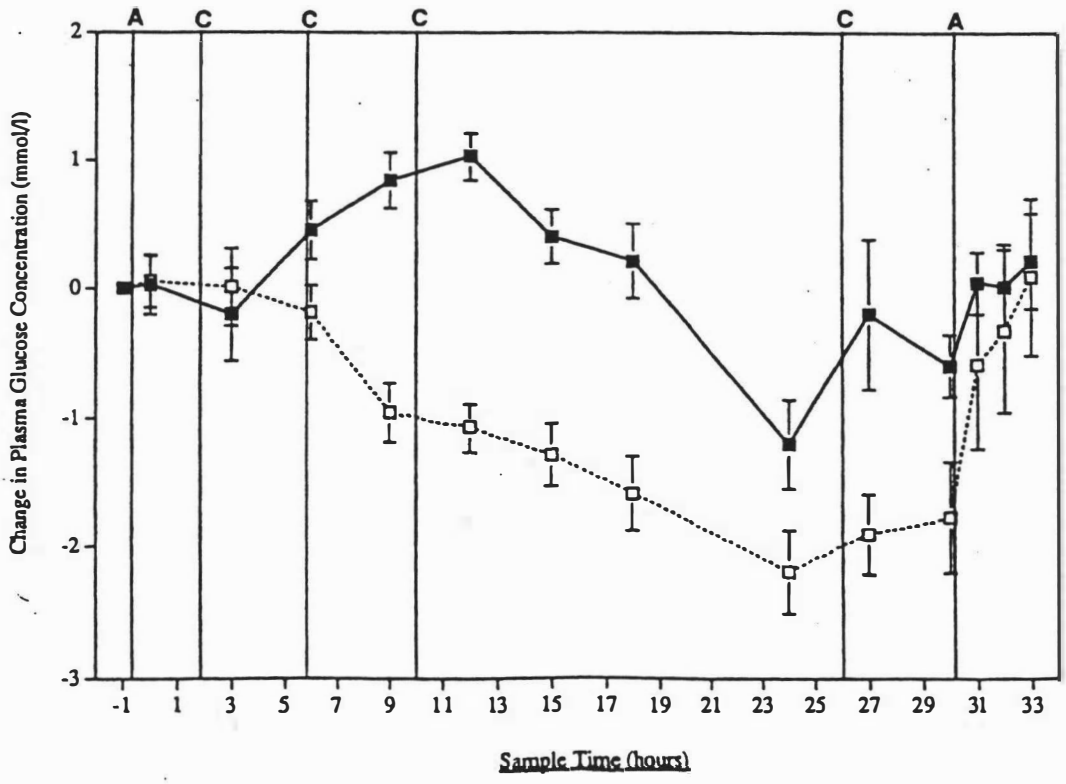

Fig 3.12: The change in plasma glucose concentration (mean ± SEM) over time from that of the intial sample, in ten day old calves that were either fed four times daily or fasted for 30 hours.

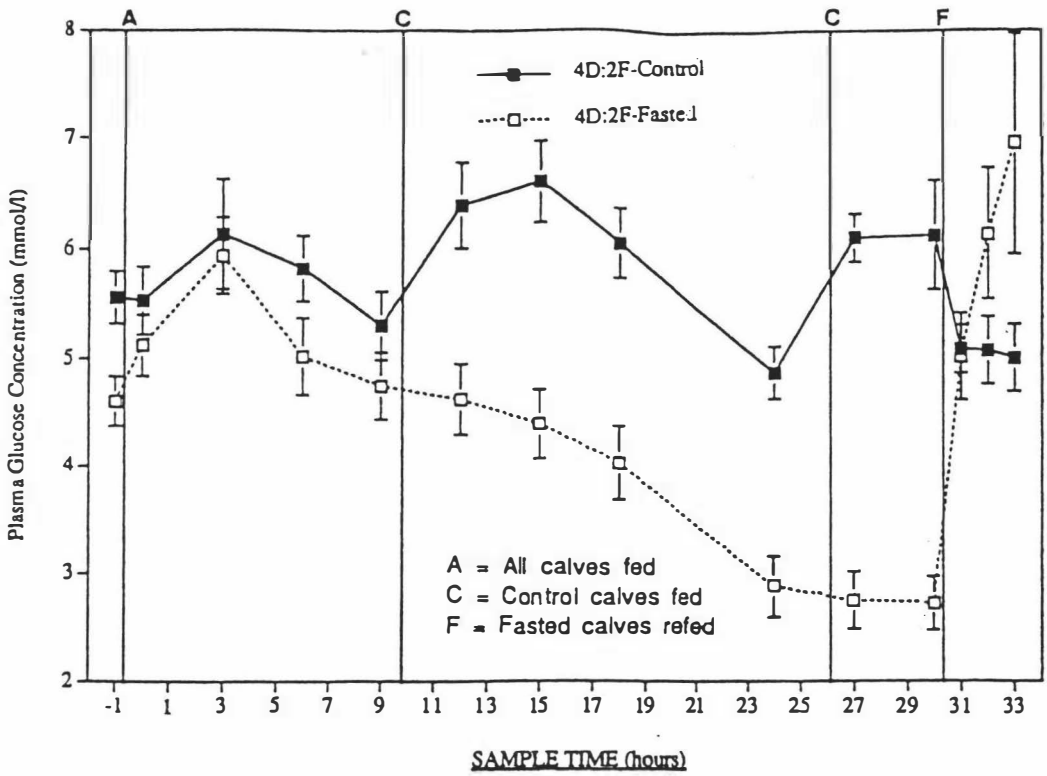

Fig 3.13: The plasma glucose concentrations (mean ± SEM) in four day old calves that were either fed twice daily or fasted for 30 hours.

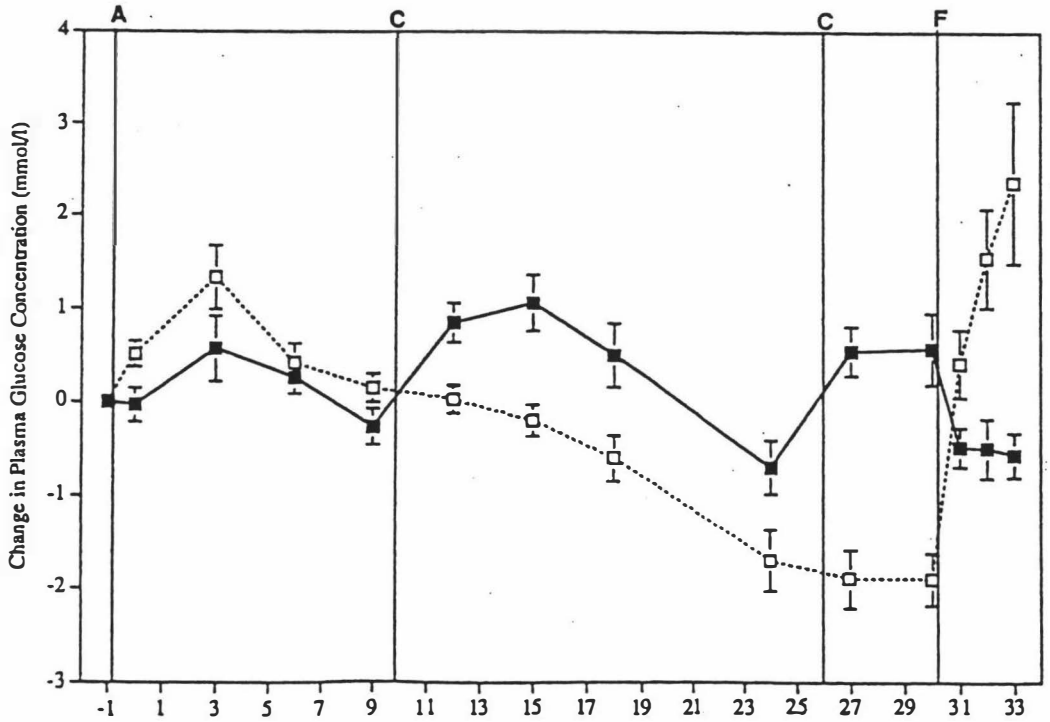

Fig 3.14: The change in plasma glucose concentration (mean ± SEM) over time from that of the intial sample, in four day old calves that were either fed twice daily or fasted for 30 hours.

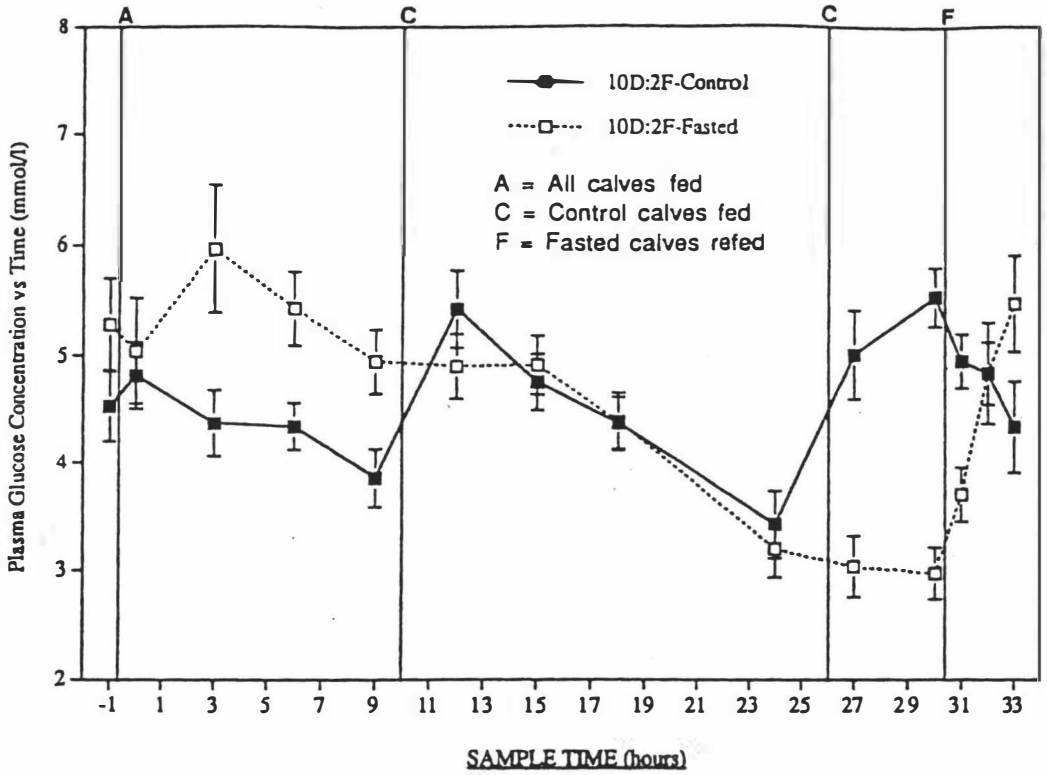

Fig 3.15: The plasma glucose concentrations (mean ± SEM) in ten day old calves that were either fed twice daily or fasted for 30 hours.

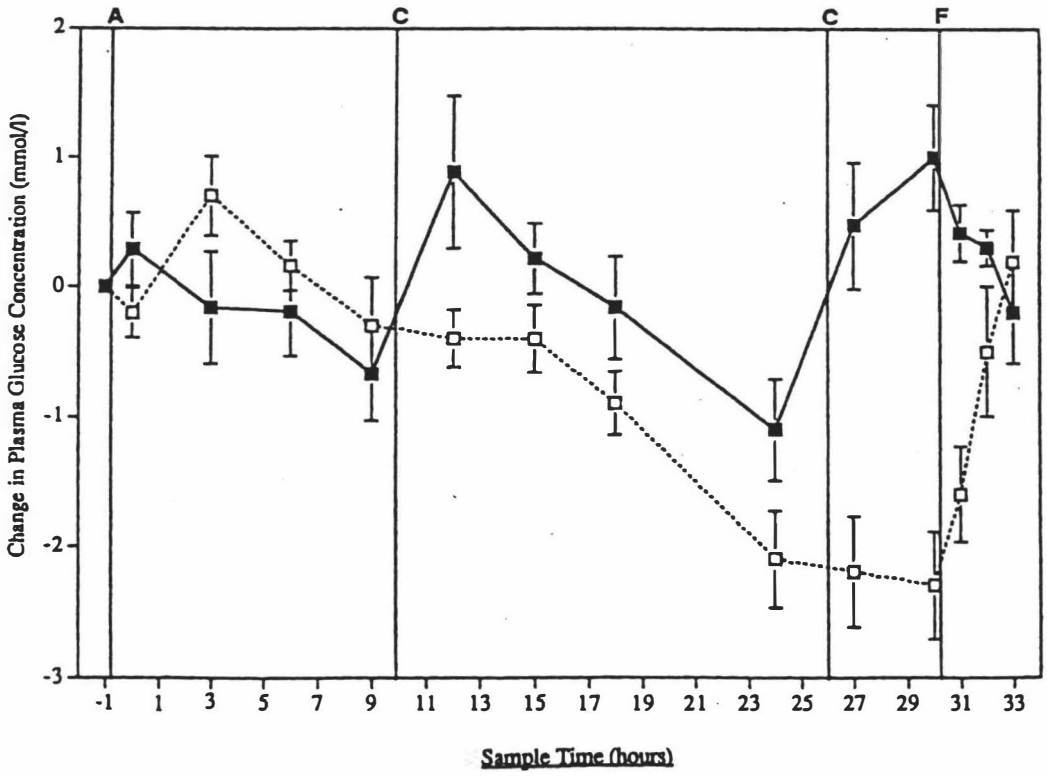

Fig 3.16: The change in plasma glucose concentration (mean ± SEM) over time from that of the intial sample, in ten day old calves that were either fed twice daily or fasted for 30 hours.

by 0.8 ± 0.2 mmol/l, while that of the fasted calves remained similar to the initial value, creating a significant difference between the two groups ($P < 0.01$). This difference remained until the 4D:2F-Fasted calves were fed 30 hours after the start of the experiment.

10D:2F

Unlike the other groups, the plasma glucose concentrations of 10D:2F-Control and 10D:2F-Fasted calves did not diverge significantly after either the first or second feed (at t = - 0.75h and t = 10h) (Figs 3.15 and 3.16). The concentrations in these groups did not become significantly different until after the feed given 26 hours after the start of the experiment (t = 26h). At this time 10D:2F-Control calves showed a large increase in plasma glucose concentration (1.6 ± 0.5 mmol/l) such that the two were significantly different from each other from 27 to 30 hours ($P < 0.001$).

Other Metabolites

3.3.2 Triglycerides

There were significant differences between the mean initial plasma triglyceride concentrations of the treatment groups (Appendix 3.6). They ranged from 0.19 ± 0.05 mmol/l (10D:2F-Control) to 0.54 ± 0.08 mmol/l (4D:2F-Control), with a overall mean value of 0.37 ± 0.04 mmol/l (Table 3.2). Triglyceride concentration decreased in all groups but one (4D:2F-Fasted) over the 30 hours studied, however the degree of triglyceride decrease was not consistent between groups of different postnatal age, feeding frequency or treatment (fed or fasted) (Fig 3.17).

Increases in plasma triglyceride concentration were seen in all groups but one (10D:2F-Control) within 3 hours of the feed given at 30 hours (Fig 3.18). Once again, the magnitude of this increase was not consistent between groups of similar feeding frequency, postnatal age or treatment.

3.3.3 β-hydroxybutyrate

There were significant differences ($P < 0.05$) between the groups in the plasma β-hydroxybutyrate concentration of the pre-treatment sample (Appendix 3.7). Mean initial concentrations ranged between 0.07 ± 0.01 mmol/l (4D:4F-Fasted) and 0.22 ± 0.05 mmol/l (10D:2F-Control) with an overall value of 0.12 ± 0.01 mmol/l (Table 3.2).

Because of this, the change in concentration over the 30 hours studied (between t = -0.75h and t = 30h) was used for analysis rather than the absolute concentrations.

Control Groups vs. Fasted Groups

All fasted groups showed an increase in plasma β-hydroxybutyrate concentration over 30 hours (Fig 3.19). Control groups showed little or no change. The increases in concentration in fasted groups ranged from 0.26 ± 0.09 mmol/l (10D:4F-Fasted) to 0.5 ± 0.7 mmol/l (4D:2F-Fasted), resulting in the development of significant differences ($P < 0.01$) between the mean concentrations in all the control and fasted groups (Appendix 3.8). There were no consistent differences in the degree of change between groups on different feeding frequencies or of different postnatal age. Even so, the absolute concentration of β-hydroxybutyrate of 4D:2F-Fasted calves after 30 hours was significantly greater than that of 4D:4F-Fasted calves ($P < 0.05$).

3.3.4 Urea

Initial samples had a range in plasma urea concentration of between 2.6 ± 0.5 mmol/l (4D:2F-Control) and 4.9 ± 0.5 (10D:2F-Control) with an overall concentration of 3.4 ± 0.2 mmol/l (Table 3.2). There were significant differences between the concentrations in these groups ($P < 0.01$), therefore the change in plasma urea concentration over 30 hours was used for comparisons rather than the absolute concentrations (Appendix 3.9).

Control Groups vs. Fasted Groups

Fasted calves showed a mean increase in plasma urea concentration of 3.1 ± 0.5 mmol/l over 30 hours, while there was no change in control groups (Fig 3.20). The increases in concentration seen in 4D:4F-Fasted and 10D:4F-Fasted groups were such that they became significantly different ($P < 0.01$ and $P < 0.05$ respectively) from their control groups. Although the plasma urea concentrations of 4D:2F-Fasted and 10D:2F-Fasted calves increased, 30 hours after the start of the experiment these increases were not significantly different from those of their controls (Appendix 3.9).

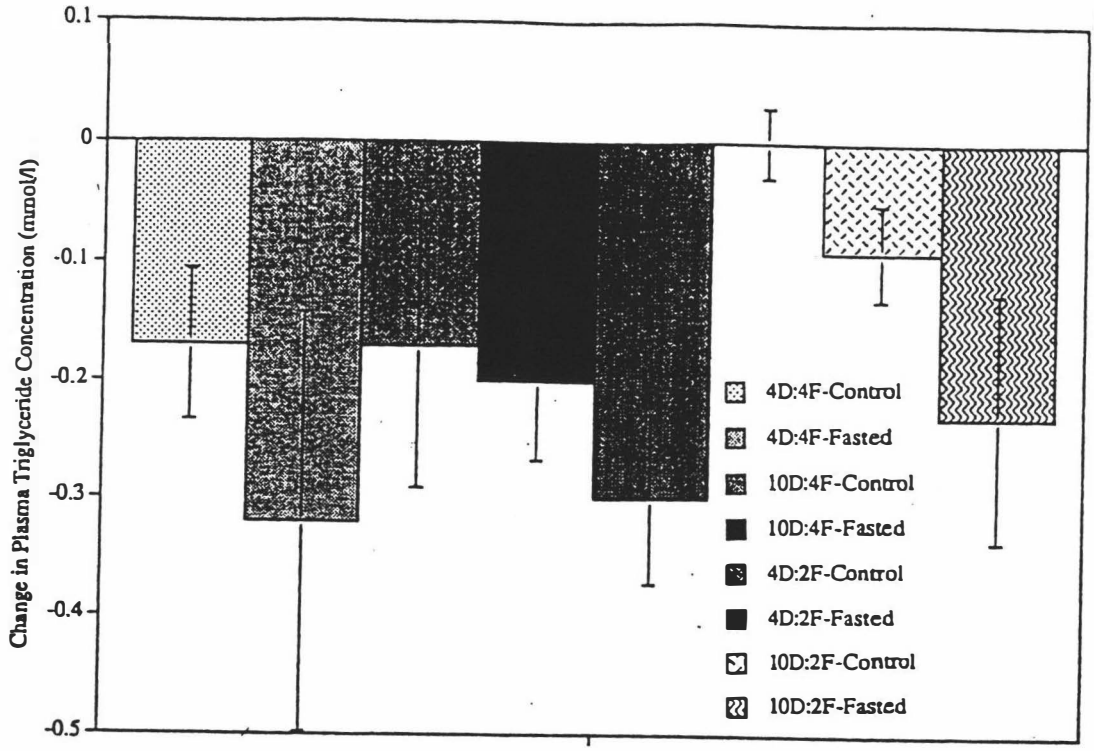

Fig 3.17: The changes in plasma triglyceride concentration (mean ± SEM) over 30 hours in all groups.

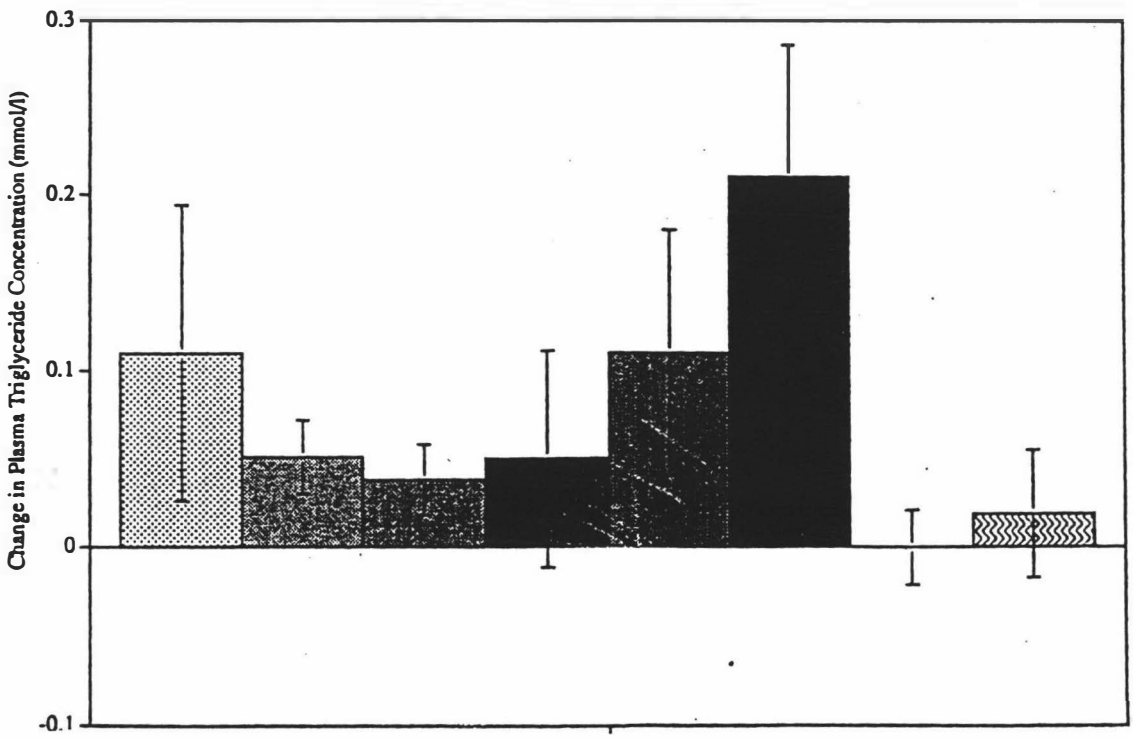

Fig 3.18: The change in plasma triglyceride concentration (mean ± SEM) in all groups over the first 3 hours after reffeding at 30 hours.

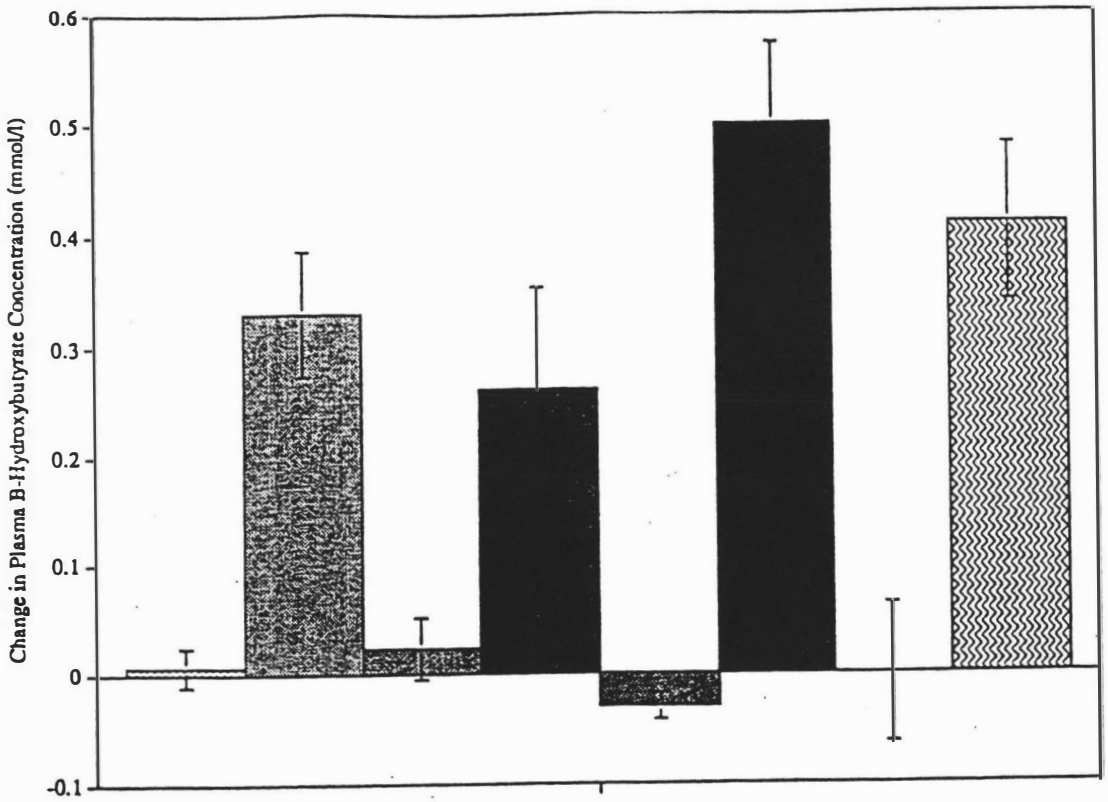

Fig 3.19: The changes in plasma β-hydroxybutyrate concentration (mean ± SEM) over 30 hours in all groups.

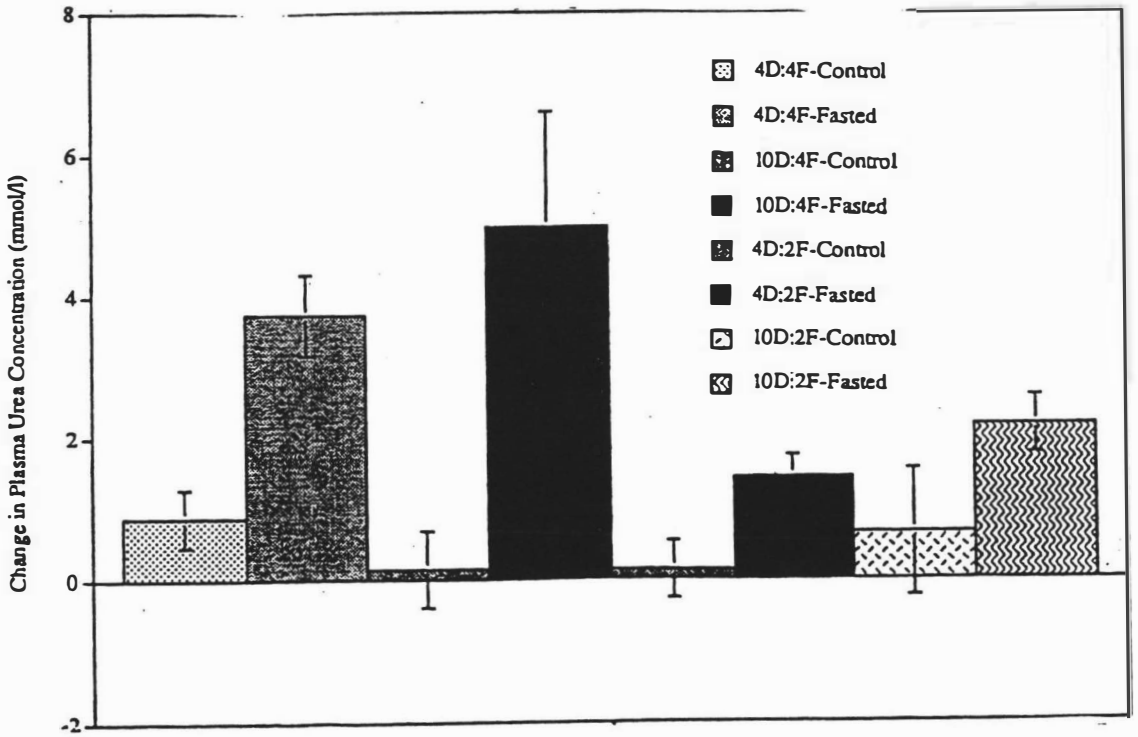

Fig 3.20: The change in plasma urea concentration (mean ± SEM) in all groups in over 30 hours.

3.3.5 Correlations Between Metabolic Parameters

Before the start of the experiment (at t=-1h) there were significant correlations between the plasma concentrations of glucose, triglycerides and urea (Table 3.4). Calves with a higher plasma glucose concentration tended to have a higher triglyceride concentration and a lower plasma urea concentration. Plasma urea concentration was also correlated with plasma triglyceride and β-hydroxybutyrate (Table 3.4). High plasma urea concentrations tended to be associated with low plasma triglyceride and high β-hydroxybutyrate concentrations.

Table 3.4: The correlations (r) between mean initial plasma glucose, triglyceride, β-hydroxybutyrate and urea concentrations.

Parameter	Triglycerides	β-hydroxybutyrate	Urea
Glucose	0.476***	-0.223	-0.304*
Triglycerides		-0.052	-0.358**
β-hydroxybutyrate			0.577 ***

* = P < 0.05; ** = P < 0.01; *** = P < 0.001

After 30 hours fasting a negative correlation between the plasma concentrations of glucose and β-hydroxybutyrate became apparent (Table 3.5).

Table 3.5: Correlations between plasma glucose, triglycerides, β-hydroxybutyrate and urea concentrations after 30 hours fasting.

Parameter	Triglycerides	β-hydroxybutyrate	Urea
Glucose	0.153	-0.580**	0.293
Triglycerides		0.183	-0.116
β-hydroxybutyrate			-0.293

** = P< 0.01

A corresponding correlation between the change in plasma β-hydroxybutyrate and change in plasma glucose concentration after 30 hours fasting was also present (Table 3.6). Lower plasma glucose concentrations were associated with higher β-

hydroxybutyrate concentrations. A larger decrease in plasma glucose levels during 30 hours fasting tended to be accompanied by a larger increase in β-hydroxybutyrate concentration over the same period.

Table 3.6: The correlations (r) between the changes in plasma glucose, triglycerides, β-hydroxybutyrate and urea concentrations over 30 hours fasting.

Parameters	Triglycerides	β-hydroxybutyrate	Urea
Glucose	0.220	-0.451*	0.241
Triglycerides		-0.030	0.169
β-hydroxybutyrate			-0.312

* = P< 0.05

3.3.6 Correlations Between Metabolic Parameters and Indices of Prematurity.

At the start of the experiment the plasma concentration of glucose showed strong positive correlations with both body weight and tooth code in Hereford-Friesian calves (Table 3.7). There were also negative correlations between initial plasma β-hydroxybutyrate and both body weight and tooth code in this breed (Table 3.7). The calves thought to be of an earlier gestational age tended to have lower plasma glucose and higher plasma β-hydroxybutyrate concentrations.

Table 3.7: The correlations (r) between indices of maturity and initial plasma glucose, triglyceride, β-hydroxybutyrate and urea concentrations in Hereford-Friesian calves.

Parameter	Glucose	Triglycerides	β-hydroxybutyrate	Urea
Body weight	0.738**	0.401	-0.628*	-0.142
Tooth code	0.854**	0.508	-0.701*	-0.277

* = P< 0.05; ** = P< 0.01; *** = P< 0.001

Table 3.8: The correlations (r) between indices of maturity and initial plasma glucose, triglyceride, β-hydroxybutyrate and urea concentrations in Friesian calves.

Parameters	Glucose	Triglycerides	β-hydroxybutyrate	Urea
Body weight	-0.035	-0.360	-0.028	-0.333
Tooth code	-0.326	-0.326	-0.348	-0.315

After 30 hours of fasting, tooth code was negatively correlated with plasma urea concentration in both Hereford-Friesian and Friesian calves (Tables 3.9 and 3.10). At

this time the body weight of Friesian calves was negatively correlated with urea and positively correlated with β-hydroxybutyrate (Table 3.10).

Table 3.9: Correlations (r) between indices of maturity and plasma glucose, triglyceride, β-hydroxybutyrate and urea concentrations in Hereford-Friesian calves after 30 hours fasting.

Parameters	Glucose	Triglycerides	β-hydroxybutyrate	Urea
Body weight	-0.295	0.534	0.471	-0.627
Tooth Code	-0.429	0.312	0.558	-0.814*

* = P< 0.05

Table 3.10: Correlations (r) between indices of maturity and plasma glucose, triglycerides, β-hydroxybutyrate and urea concentrations in Friesian calves after 30 hours fasting.

Parameters	Glucose	Triglycerides	β-hydroxybutyrate	Urea
Body weight	0.09	0.434	0.610*	-0.587*
Tooth Code	0.151	0.172	0.470	-0.662*

* = P< 0.05

After 30 hours fasting, calves of an apparently earlier gestational age tended to have lower β-hydroxybutyrate concentrations and higher urea concentrations. The change in urea over 30 hours fasting was correlated with crown rump length and the change in β-hydroxybutyrate was correlated with body weight (Table 3.11). Greater increases in urea concentration and decreases (or smaller increases) in β-hydroxybutyrate concentration were seen in calves of an apparently earlier gestational age when they had been fasted for 30 hours.

Table 3.11: Correlations (r) between the indicies of maturity and the change in plasma glucose, triglycerides, β-hydroxybutyrate and urea concentration over 30 hours fasting.

Parameter	Glucose change	Triglyceride change	β-hydroxybutyrate change	Urea change
Crown rump length	0.024	0.386	0.226	-0.408*
Body weight	0.093	0.295	0.389	-0.226
Tooth code	0.130	0.231	0.170	-0.216

* P< 0.05

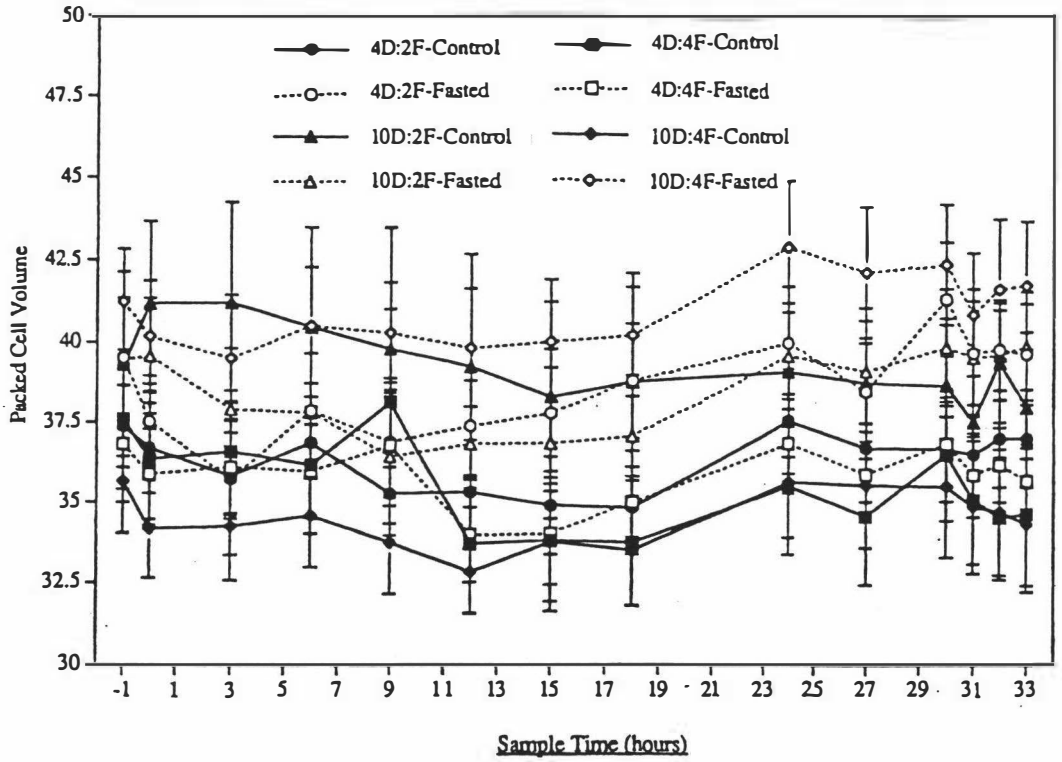

Fig 3.21: The packed cell volumes (mean ± SEM) in all groups over time.

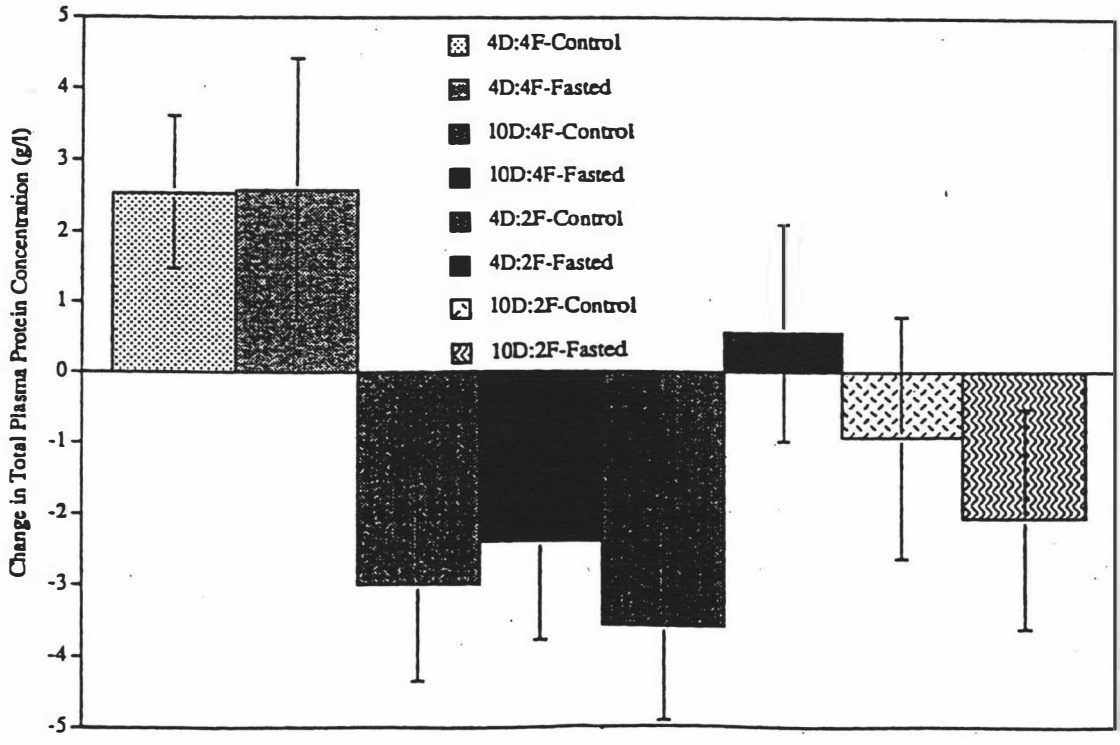

Fig 3.22: The change in total plasma protein concentration (mean ± SEM) in all groups over 30 hours.

Hydration State

3.3.7 Packed Cell Volume

The initial packed cell volume (PCV) varied widely among the calves and was not apparently associated with postnatal age or degree of prematurity. Of the calves used, the initial PCVs ranged between 28% and 51%, with a mean value of 39 ± 6%.
Changes in PCV over time were not apparently influenced by prematurity, feeding frequency, postnatal age or treatment (feeding or fasting) (Fig 3.21, Appendix 3.10).

3.3.8 Total Plasma Protein Concentration

Total plasma protein (TPP) concentrations were significantly different between groups in the initial sample, therefore the change in concentration over 30 hours was used for comparisons rather than the absolute concentration (Fig 3.22, Appendix 3.11). The mean TPP concentration in the initial sample was 57 ± 13 g/l. While there were changes in TPP concentrations over the 30 hours studied these could not be attributed to feeding frequency, postnatal age or fasting.

3.3.9 Effect of Prematurity on Total Plasma Protein Concentration

When the mean initial TPP concentration of the eight heaviest and eight lightest calves were compared, that of the heavier calves was significantly greater ($P < 0.01$) than that of the lighter calves.

As expected initial TPP concentration and GGT level were highly correlated ($r= 0.827$, $P< 0.001$). This suggests that differences in TPP in the initial sample were likely to have been due to the quantities of immunoglobulin absorbed via colostrum in the first 24 hours of life. Thus, initial TPP concentration may have been a function of access to colostrum and absorption of immunoglobulins rather than a property affected by the degree of prematurity.

3.3.10 Rectal Temperature

Initially there was no difference in rectal temperature between the groups which exhibited an overall mean value of 38.5 ± 0.7 °C (Table 3.2). Subsequently there was a tendency for the temperatures of fasted calves to decrease such that at 30 hours their mean rectal temperatures were significantly lower ($P<0.05$) than those of the control calves (38.3 ± 0.2 °C and 38.7 ± 0.1°C, respectively). The extent of temperature change

in fasted groups varied from -1.0 ± 0.4 °C in 4D:4F-Fasted to 0.2 ± 0.3°C in 4D:2F-Fasted.

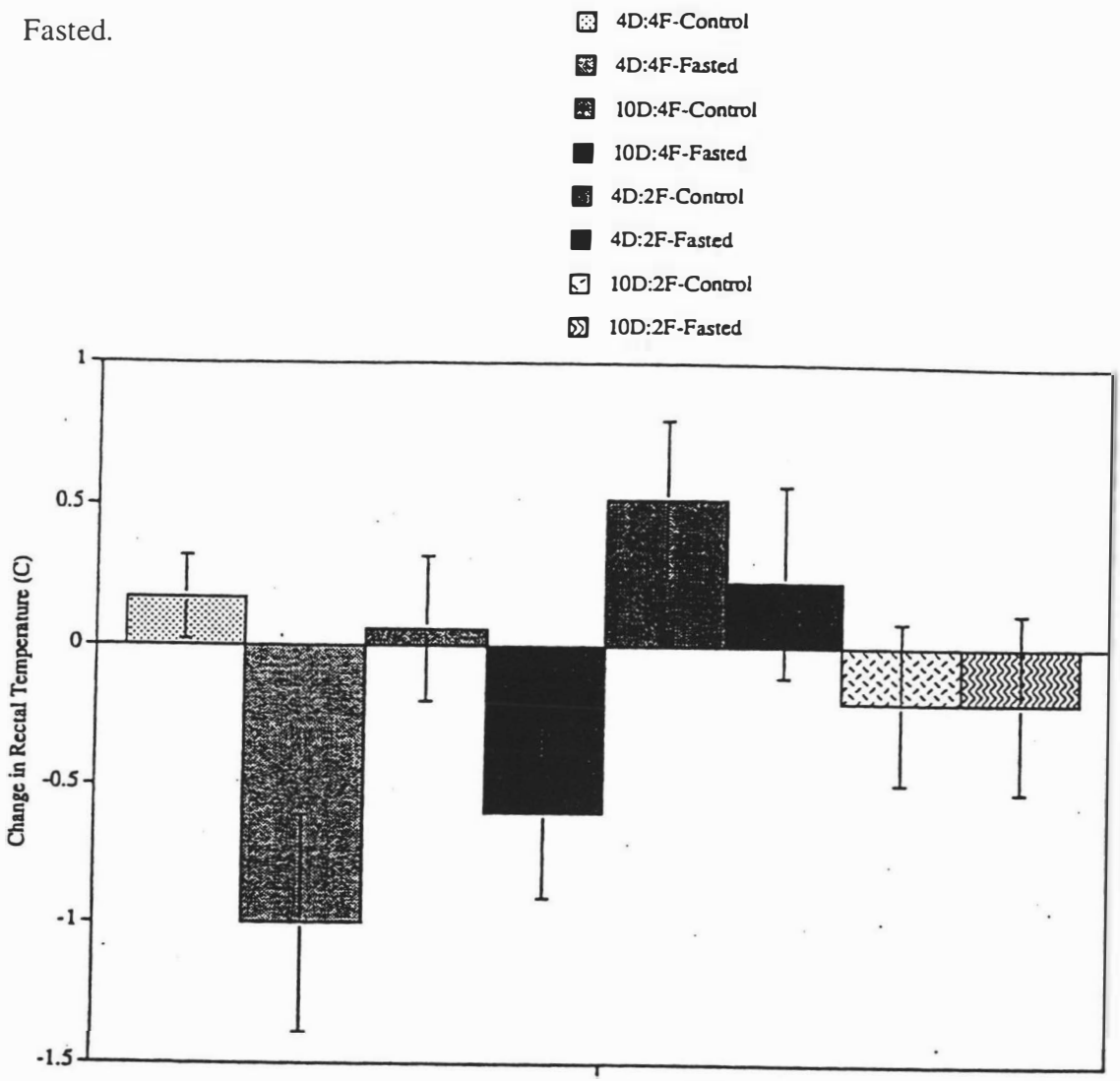

Fig 3.23: The change in rectal temperature (mean ± SEM) in all groups over 30 hours.

The trend for fasted calves to show a decrease in temperature and for this to be maintained in fed calves is reflected by the 4F treatment pairs. The mean rectal temperature of the calves in 4D:4F-Fasted decreased by 1°C over the 30 hours, whereas that of 4D:4F-Control did not change creating a significant difference ($P<0.05$) between these groups (Fig 3.23). Although the calves in 10D:4F-Fasted showed a mean decrease in rectal temperature it did not create a significant difference between this group and 10D:4F-Control. Fasting did not appear to have an effect on the rectal temperatures of calves in the 2F groups. These groups (2F) were fed twice the volume of colostrum of the 4F groups at the start of the experiment.

3.3.11 The Effects of Prematurity on Rectal Temperature

There was a positive correlation between the initial rectal temperature and known gestational age ($r = 0.674$, $P<0.05$). Calves born earlier tended to have lower rectal temperatures at the start of the experiment. Heavier calves (which tended to be of greater gestational age) had significantly higher rectal temperatures before the start of the experiment ($P <0.05$) and after 30 hours ($P <0.05$). When the mean rectal temperature of calves with teeth scores of 10-11 and 1-5 were compared, those with higher teeth scores also had significantly higher mean rectal temperature after fasting for 30 hours ($P < 0.01$).

3.4 Discussion

The conclusions from this research were as follows:

- Viable induced calves tend to have slightly lower plasma glucose and higher β-hydroxybutyrate concentrations than full-term calves (Kinsbergen *et al.*,1994; Todd, 1998, *unpublished data*), however there is a large variation of values. Variation in the metabolic state of an induced calf is primarily determined by its gestational age at the time of its birth. The degree to which it depends on a greater rate of lipid catabolism to provide its normal energy requirements decreases as its gestational age at birth nears full-term.

- Premature calves gained no apparent advantage (when in a fed state) in terms of energy availability from being fed a similar volume of milk over four feeds as opposed to twice within a period of ten hours.

- The ability of induced calves to tolerate 30 hours fasting showed great variation. Calves born at an earlier gestational age had a reduced ability to tolerate this period without food in terms of whole body and thermogenic energy requirements as a result of body energy stores that were probably smaller and partially depleted at the onset of fasting.

- The energy availability in fasted calves (as indicated by plasma glucose concentration) was greater in those fed 50 ml colostrum /kg bodyweight rather than 25 ml/kg at the onset of fasting.

- Plasma triglyceride concentration was not a meaningful index of lipid mobilisation as employed here.

- Fasting induced calves for up to 30 hours did not cause dehydration as indicated by the absence of significant increases in packed cell volume or total plasma protein concentration.

3.4.1 The State of the Calves at the Start of the Experiment

Metabolic state was assessed through combined analysis of indices of the energy and hydration states as well as the clinical condition as indicated by the rectal temperature. At the start of the experiment the calves had not been fed for 9-13 hours overnight. Consequently, the parameters measured at this time would be expected to reflect effects

of this previous treatment, and their state at this time is likely to influence their metabolic and other responses to fasting or to feeding frequency.

The State of Carbohydrate Metabolism at the Start of the Experiment

There is evidence that the mean initial plasma glucose concentration of these premature calves (Table 3.2) was slightly lower than that found in the 1-2 week old full-term calves studied by Todd (1998, *unpublished data*) and Kinsbergen *et al.*(1994).

As none of the calves had been fed for 9-13 hours prior to when the initial blood sample was taken, and control calves had been fasted for 14 hours overnight prior to when the 24 hour sample was taken, it would have been expected that the mean plasma glucose concentrations in the initial and 24 hour samples from the control groups would have been similar. Although the mean plasma glucose concentrations of the calves in 4D:4F-Control in the initial sample and at 24 hours were similar, those of the calves in 10D:4F-Control, 4D:2F-Control and 10D:2F-Control were not (see figs 3.9-3.16). The plasma glucose concentrations at 24 hours of the calves in 10D:4F-Control and 10D:2F-Control were significantly lower than they were at the start of the experiment ($P < 0.05$) and the mean concentration in the 4D:2F-Control calves was considerably lower at 24 hours although not significantly so ($P = 0.0586$).

It is possible that events surrounding the start of the experiment, such as lights being turned on, noise of people entering the shed and the novelty of being restrained for the pre-treatment blood sample, had stimulated the sympathetic nervous system causing an increase in the secretion of both cortisol and adrenaline. This is supported by observations made by Kinsbergen *et al.*,(1994) who measured cortisol concentrations and metabolic parameters first over 24 hours of normal feeding and then during 24 hours without food. On the first day of their study, plasma cortisol concentrations were highest at the onset of sampling than at any other point during the two days over which blood samples were taken.

Dineen *et al.* (1993) have claimed that an increase in cortisol concentration causes partial inhibition of insulin secretion and stimulation of glucagon secretion. This

increased the rate of gluconeogenesis in the liver and caused a decrease in hepatic glycolysis and glucose uptake by the cells (Dineen *et al.*,1993). The anxiety associated with a new or frightening situation stimulates the sympathetic nervous system causing the secretion of adrenaline. Adrenaline also strongly stimulates the breakdown of glycogen in the liver by inhibiting insulin release and activating the enzymes involved in glycogen breakdown. These combined effects of cortisol and adrenaline would be likely to have caused an increase in plasma glucose concentration.

When neonates are handled and blood sampled from the time of birth onward, such stimuli would no longer be novel for the young animals. Lambs that were fed and blood sampled at regular intervals from birth onward by Mellor (1987) did not exhibit elevated plasma glucose concentrations at the onset of sampling.

The State of Lipid Metabolism at the Start of the Experiment

The mean initial plasma triglyceride and β-hydroxybutyrate concentrations were greater than those found by Todd (1998, *unpublished data*,). A greater triglyceride concentration can be difficult to interpret due to the fact that higher values may indicate increased triglyceride synthesis by the liver, or increased lipid uptake from the gut. For this reason plasma triglyceride concentration is not considered to be a good indicator of lipid mobilisation from adipose tissue. This is supported by the absence of meaningful changes in this parameter during the present experiment.

A raised mean initial plasma β-hydroxybutyrate concentration in the present study showed that these induced calves needed to catabolise lipid reserves at a greater rate than full-term calves to meet their energy needs. Had the calves had sufficient carbohydrate reserves, the elevation of β-hydroxybutyrate concentrations would have been unlikely.

It is possible that elevated cortisol and adrenaline concentrations (as discussed above) may have stimulated an increase in the rate of lipolysis and ketogenesis resulting in a higher mean initial plasma β-hydroxybutyrate concentration than that found by Todd

(1998, *unpublished data*) (Dineen *et al.*,1993). However, given that the full-term calves studied by Todd (1998, *unpublished data*) were subjected to similar treatment at the start of the experiment to the calves in the present study, this is unlikely to be the cause of the observed difference in the initial mean concentration.

The State of Protein Metabolism at the Start of the Experiment

The mean initial plasma urea concentration was slightly greater than that recorded by both Todd (1998, *unpublished data*) and Kinsbergen *et al.* (1994), however the difference was not so great as to suggest that the majority of these calves required protein to supply their energy requirements.

The Hydration State of Calves at the Start of the Experiment

The mean initial PCV was within the range of values for full-term calves reported in other studies (Mollerberg, 1975; Katunguka-Rwakishaya *et al.*,1985; Katunguka-Rwakishaya *et al.*,1987; Kinsbergen *et al.*,1994; Todd,1998, *unpublished data*). However, mean TPP concentration was lower than those values reported by Kinsbergen *et al.*(1994); Katunguka-Rwakishaya *et al.*(1985) and Todd, (1998, *unpublished data*). There was wide variation in both PCV and TPP at the start of the experiment.

The relationship between TPP and GGT level suggests that variation in TPP concentration is likely to be a reflection of the amount of immunoglobulins a calf has. However, variation in the initial TPP concentration is not likely to interfere with the assessment of hydration status using this parameter as it is the *change* in TPP over the 30 hour fast that is used rather than the absolute values. Although there was no correlation between TPP concentration and PCV of indivdual calves the start of the experiment the absence of increase in these parameters over the following 30 hours indicates that the calves were not dehydrated at this time.

When the mean and range in GGT levels of the calves in the present study and those studied by Todd (1998, *unpublished data*) were compared, the mean value in the present study was greater though not significantly so (610 ± 161 U/l and 568 ± 65 U/l

respectively) and had a greater range in GGT levels (7-6727 U/l and 9-2507 U/l, respectively) than those found by Todd (1998, *unpublished data*).

Rectal Temperatures at the Start of the Experiment

The mean rectal temperature of the calves at the start of the experiment (38.5 ± 0.7°C) was within the lower end of the normal range of temperatures (38.0 - 39.5°C) expected in cattle (Siegmund *et al.*,1973; Blood and Radostits, 1989). As with the other parameters there was large variation between individual calves (see Chapter 2).

Rectal temperature shows diurnal variation, rising during the day and falling during the night (Siegmund *et al.*,1973). This variation is greater in younger animals than in older ones (Siegmund *et al.*,1973). The fact that the initial temperature was taken early in the morning (0500 hours) when the calves had not been fed for 9-12 hours and the air temperature was cooler may have contributed to the lower rectal temperatures at this time. The large variation in temperature is likely to be affected by the degree of prematurity of the calves as discussed in Chapter 2.

3.4.2 The Effect of Gestational Age at Birth on the Metabolic State of Induced Calves

The primary source of the wide variation of each blood or plasma parameter was gestational age rather than prior feeding frequency or postnatal age. This was indicated by the correlations between the metabolic parameters and the body weight or teeth code (the indices of gestational age), as well as by the absence of consistent significant differences between treatment groups at the start of the experiment.

Induced calves born closer to full-term (those with higher bodyweights and teeth codes) tended to have a greater availability of carbohydrate energy as indicated by higher plasma glucose and lower plasma β-hydroxybutyrate concentrations. The presence of glucose has been shown to stimulate the synthesis of triglycerides in the liver and adipose tissue and have to a sparing effect on the oxidation of fatty acids (Kaneko, 1989), hence the association of high plasma glucose concentrations with high triglycerides, low β-hydroxybutyrate and low urea concentrations in the more mature

calves. This indicated that the more mature calves had sufficient carbohydrates available. Compared to less mature calves, they have a reduced need to increase their rates of lipid and protein catabolism to provide energy during the 9-13 hour overnight fast. Conversely, the induced calves born at an earlier gestational age apparently required an increased rate of lipid and protein catabolism to meet their normal overnight energy needs before the morning feed, as indicated by raised plasma β-hydroxybutyrate and urea concentrations and lower glucose concentrations in some of the more premature calves.

Calves in a State of Negative Energy Balance at the Start of the Experiment

A state of negative energy balance is entered when the intake of energy substrates is not sufficient to meet the body's requirements. This is indicated by low plasma glucose concentrations in the presence of raised plasma β-hydroxybutyrate and urea concentrations (Blaxter and Wood, 1951; Alexander, 1962; Kinsbergen *et al.*,1994).

Negative energy balance after the overnight fast in some calves, particularly those of an apparently earlier gestational age, may have been caused by:

- these calves consuming less milk during the preceding feeds (see Chapter 2),
- differences in the duration of fasting,
- greater energy requirements in these calves (see Chapter 2).

Although all calves were offered 100 ml colostrum/kg bodyweight daily, it is possible that this full volume was not consumed by the calves in which negative energy balance was apparent from the plasma metabolite concentrations of the initial sample. Some of the more premature calves were observed to have trouble coordinating sucking and swallowing when feeding (See Chapter 2). As a result they may have spilt significantly more colostrum than the calves that showed no difficulty sucking. If so, the volume of milk consumed by these calves would have been proportionately less, reducing the availability of energy substrates and other nutrients.

All calves were fasted for 9-13 hours overnight. The variation in fasting time depended on whether they were fed at 1600 hours (2F calves) or 2000 hours (4F-calves) on the

previous day. The concentrations of glucose, triglycerides, β-hydroxybutyrate and urea from 4F and 2F treatment in the initial sample were not significantly different, thus the duration of the over night fast is unlikely to be the cause of negative energy balance.

A body's energy requirements depend on the metabolic rate of the animal concerned. The basal metabolic rate is determined by the background hormonal environment. Added to this are metabolic adjustments made by the body according to changes in the supply and demand for nutrients by the tissues. At this level the metabolic rate may be increased by exercise, thermogenic responses to external environmental temperature below the animal's critical temperature and the internal body temperature (Gonzalez-Jimenez and Blaxter, 1962; Schrama *et al.*,1993; Mellor and Cockburn, 1986).

Given that the environmental conditions and amount of exercise (not including shivering) experienced by the calves in the present study were similar, the factor most likely to have caused differences in metabolic rate was variation in the rate of thermogenesis required to maintain the internal body temperature. States of negative energy balance were more prevalent among the light-weight calves of an apparently earlier gestational age. As mentioned in the previous chapter, induced calves weigh less than full-term calves and have a higher surface area to body weight ratio. Thus the surface area over which heat is lost is greater in proportion to the amount of tissue that produces heat (Randall,1978; Alexander, 1979; Eales *et al.*,1982; Carstens, 1994). The fact that all calves were at least four days old at the start of the experiment demonstrates that they were able to maintain an adequate body temperature until this point in time. Even so, calves of lighter body weight would have been loosing heat at a greater rate per unit body weight and would have therefore required a greater rate of thermogenesis in order to maintain their body temperature. Maintaining a higher metabolic rate would have resulted in more rapid depletion of carbohydrate reserves and therefore a greater tendency to catabolise lipid and later protein to provide energy requirements (Alexander,1962; Eales *et al.*, 1982; Mellor and Cockburn,1986). This would explain the higher β-hydroxybutyrate and lower urea concentrations in lighter weight (more premature) calves.

3.4.3 The Response to Feeding Two or Four Times Daily

Absorption of nutrients containing carbohydrates after a fast of short duration (such as that overnight) is usually associated with an increase in plasma glucose concentration (Mellor, 1987; Kinsbergen *et al.*, 1994), so that a glucose rise was expected after feeding in all groups. The expected response was only seen in three of the groups at the start of the experiment groups (4D:4F-Fasted, 4D:2F-Fasted and 10D:2F-Fasted), but in all groups subsequently. The likely reason for no apparent increase in five of the groups is the artificial elevation of initial concentrations in response to novel stimuli as discussed earlier masking any post-prandial increase in plasma glucose that may have otherwise been seen.

By this reasoning, it is likely that in the absence of novel stimuli, the increases in plasma glucose concentration by all groups in response to the first feed would be similar to those seen between 24 and 27 hours in response to the feed given at 26 hours. The increases in response to this feed were 0.66 ± 0.29 mmol/l in calves fed four times daily and 1.38 ± 0.28 mmol/l in calves fed twice daily . Calves fed twice daily (2F) recieved twice the volume of colostrum given to the calves fed four times daily (4F) (50 ml/kg vs 25 ml/kg). Because of this the increase in plasma glucose in response to each feed tended to be greater in the 2F control groups.

The fluctuations in plasma glucose of calves fed twice daily and the progressive increase seen in calves fed four times daily are similar to those found by Mellor (1987) in lambs. Although the pattern of changes in plasma glucose concentration varied between the two feeding frequencies, this did not appear to affect the overall energy availability and capacity to tolerate the overnight fast as indicated by similar high concentrations being reached at 12 hours and similar low concentrations at 24 hours. Thus, feeding a similar volume of milk (100 ml/kg bodyweight) spread over four feeds within 10 hours, compared to two feeds, provided no apparent energetic advantage to premature induced calves.

In practical terms feeding four times daily was sometimes made more difficult by the fact that calves were reluctant to suck, unlike those fed twice, so that time and labour inputs were greater. The glucostatic theory of hunger and feeding regulation suggests that a decrease in plasma glucose concentration stimulates the sensation of hunger and the desire to feed (Storlein, 1985). It is possible that the decline in plasma glucose concentration seen in 2F-Control calves preceding the second feed may have stimulated the sensation of hunger and the consequent desire to feed, whereas the steady increase, and absence of a decline, in plasma glucose concentration in the 4F-control calves may have resulted in a lower drive to feed in these animals.

Had the four feeds been given at 6 hour intervals over the 24 hour period rather than during the 10 or 12 hour period, it is probable that a moderate plasma glucose concentration of 4 -5.5 mmol/l would have been maintained, rather than the steady increase then decrease seen in the 4F-Control calves. In terms of energy availability this may have been preferable to two large feeds. A greater interval between feeds may also have increased the calves' desire to feed.

3.4.4 The Effect of 30 Hours Fasting on Premature Calves

Fasting premature calves for 30 hours probably reduced their glycogen stores and increased catabolism of lipids and amino acids to provide energy. Calves born closer to full-term were apparently able to rely on an increased rate of lipid catabolism to a greater degree than those of an apparently earlier gestational age, and they therefore required less amino acid catabolism. Calves that were already exhibiting greater rates of lipid and amino acid catabolism (those with raised β-hydroxybutyrate and urea concentrations) at the start of the experiment would have had proportionately less lipid and glycogen stored at the onset of fasting and therefore would have relied to a greater degree of amino acid catabolism to supply their energy requirements when fasted.

The decrease in temperature in 17 of the 31 fasted calves (55%) indicates that their ability to produce heat was reduced by fasting. This may be due to depletion of muscle glycogen and BAT both of which are required for heat production above that of the basal metabolism.

The Effect of 30 Hours Fasting on Carbohydrate Metabolism

The decline in plasma glucose concentration three hours after the start of the experiment is likely to be due to reduced uptake of carbohydrates from the gut as the body cells continue to absorb glucose from the blood. Although the α and β cells of the pancreas (that secrete glucagon and insulin respectively) of the fetus at full-term are immature compared to those of adults, they are still able to respond to changes in plasma glucose concentration (Ktorza *et al.*,1985). Kinsbergen *et al.*(1994) showed that an increase in insulin concentration occurs in association with the post-prandial increase in plasma glucose concentration followed by a decline in both substances as fasting continues.

The decrease in plasma glucose would reduce the stimulus for insulin secretion and stimulate secretion of glucagon (Kaneko,1989). Glucagon promotes the maintenance of normoglycaemia by promoting the break down of liver glycogen and increasing gluconeogenesis, lipolysis and amino acid catabolism. (Ktorza *et al.*,1985). The significant difference between the glucose concentrations of 10D:4F-Fasted and 10D:2F-Fasted calves between 9 and 18 hours is likely to be due to the fact that 10D:2F-Fasted calves were given twice the volume of milk at the pre-treatment feed. These calves showed a greater increase in plasma glucose in response to feeding and would have been able to replenish their glycogen and lipid stores to a greater extent thereby allowing a slight delay in the subsequent concentration decrease.

Plasma glucose concentrations continued to decline until 24 hours at which time a minimum concentration was reached by all groups and then maintained until the calves were refed. Plasma glucose concentrations in calves fasted for 30 hours by Kinsbergen *et al.*,(1994) and Todd (1998, *unpublished data*) had reached minimum mean values of approximately 4.0 mmol/l and 3.4 mmol/l respectively. These minimum plasma glucose concentrations were greater than those found in the present study. This difference could indicate that the homeostatic mechanisms involved in regulating blood glucose may be functionally immature. The 'set-point' above which minimum plasma glucose is maintained may be lower than that in full-term calves or their ability to balance the availability and requirement for glucose may be impaired to some degree.

The Effect of 30 Hours Fasting on Lipid Metabolism

As already stated, changes in triglyceride concentration are difficult to interpret. However, the significant increase in the β-hydroxybutyrate concentrations of all fasted groups indicates that the calves were obtaining energy through the mobilisation and oxidation of fatty acids.

The decline in plasma glucose concentration removes inhibition of fatty acid use by lowering the α-glycerophosphate content of adipose cells that had previously promoted lipogenesis (Steinberg, 1963). Fatty acids released through the breakdown of triglycerides may be used directly by some tissues or oxidised to form ketone bodies. The increase in glucagon secretion associated with decreasing plasma glucose concentrations is thought to be responsible for the activation of ketone production by the liver (Kaneko,1989).

These observations are supported by those of Kinsbergen *et al.*(1994), who followed the changes in plasma concentration of both triglycerides and fatty acids during periods of fasting. The fasting plasma triglyceride concentration was shown to first increase as plasma glucose concentration declined, then to decrease gradually from the time at which the calves would normally have been fed. In comparison, the concentration of fatty acids increased as the plasma glucose concentration decreased then continued to increase to a concentration above that of the initial sample.

High plasma glucose concentrations and low β-hydroxybutyrate concentrations at the start of the experiment, changing to low plasma glucose and high β-hydroxybutyrate concentrations were features of the metabolic response to 30 hours of fasting in calves born closer to full-term. Over 30 hours the requirement for lipids to provide energy would have increased as glycogen deposits became progressively more depleted. These calves had sufficient glycogen and lipid reserves at the start of the experiment to allow these substrates to be used as the main source of energy during fasting.

Calves born at an earlier gestational age tended to have higher plasma β-hydroxybutyrate concentrations at the start of the experiment and had lower concentrations after 30 hours fasting. A greater rate of lipolysis at the start of the experiment would result in more rapid depletion of the already reduced amount of lipid available in the more premature calves. Maintenance of plasma glucose concentrations by calves with already depleted glycogen and lipid reserves under these conditions of fasting supports the notion that their body's energy requirements were being maintained mainly through gluconeogenesis rather than glycolysis.

The Effect of 30 Hours Fasting on Amino Acid Metabolism

Assuming that kidney function was normal, the increase in plasma urea concentration in all fasted groups shows that the rate of amino acid catabolism had become greater in these calves. The degree to which urea concentration increased varied considerably between the fasted calves. Differences in the degree of change in urea concentration appeared to be a function of the average gestational age of each group at birth (as indicated by teeth code and bodyweight) rather than their prior feeding frequency.

Greater increases in urea concentration were associated with either smaller increases (or in one case a decrease) in β-hydroxybutyrate with both of these changes occurring in calves of a lower gestational age (Tables 3.6, 3.7 and 3.8). These combined correlations suggest that calves born earlier deplete their lipid reserves at a greater rate and must therefore rely on amino acid oxidation to provide energy or as substrates for gluconeogenesis.

The lack of comparable increases in urea in the fasted full-term calves studied by both Kinsbergen *et al.*(1994) and Todd (1998, *unpublished data*) shows that the extent of glycogen and lipid reserves and therefore the duration of fasting that can be tolerated by induced calves is less than that of full-term calves. Increases in urea concentration associated with fasting became less as the calves gestational age at birth neared full-term.

The Effect of 30 Hours Fasting on Maintenance of Body Temperature

The development of a significantly lower mean rectal temperature in fasted calves compared to those that were fed suggests that in general, fasting impairs the ability of induced calves to maintain their body temperature. Once again, the degree to which fasting affected their ability to do so varied with gestational age. Given that body temperature represents the balance between heat production and heat loss and that there is no reason to think heat loss would have increased during the period of fasting, it is likely that the drop in temperature seen in many of the fasted calves was due to a decreased ability to produce sufficient heat to maintain their body temperature.

At the environmental temperatures experienced during spring in New Zealand, calves are likely to require a thermogenic rate above that of the basal metabolism. Increased thermogenesis depends on the utilisation of BAT and muscle glycogen for non-shivering and shivering thermogenesis respectively (Alexander *et al.*, 1973; Mellor and Cockburn, 1986; Carstens, 1994). Maintenance of an increased rate of thermogenesis without the required energy reserves being replaced through feeding will result in their gradual depletion and therefore a reduced ability to produce heat. Links between starvation and hypothermia are well established in lambs and piglets (Eales *et al.*,1982; Mellor and Cockburn,1986).

The absence of a temperature decrease in the full-term fasted calves studied by Todd (1998, *unpublished data*) suggests that the reduced thermogenic ability as a result of fasting observed here is a phenomenon associated with prematurity. The decline seen in premature calves suggests the exhaustion of the already reduced BAT and muscle glycogen reserves. It is probable that induced calves have smaller quantities of BAT and muscle glycogen at birth due to their shorter gestation and therefore shorter period for accumulation of body energy reserves. It has also been shown by Alexander *et al.*(1973) that the ability of premature lambs to utilise these reserves is less than that of lambs born at full-term.

3.4.5 The Metabolic Response to Refeeding

Refeeding resulted in increases in plasma glucose and triglyceride concentration. The increases in plasma glucose concentration to equal or exceed the initial value (-1 hours) indicate that after 30 hours fasting induced calves are capable of recovery in terms of glucose availability once fed.

The large increase in response to refeeding could be the result of physiological insulin resistance. This is the situation where negative energy balance stimulates the secretion of cortisol and adrenaline. Cortisol inhibits insulin secretion and stimulates glucagon secretion (Dineen *et al.*,1993). Adrenaline also inhibits insulin secretion. This causes a state of physiological insulin resistance that results in decreased rates of glucose uptake (Dineen *et al.*,1993). Reduced glucose uptake by the cells in the presence of an increased rate of glucose absorption from the gut causes a large increase in plasma glucose concentration than occurs if cellular glucose uptake uptake is not impaired.

The significantly greater increase in plasma glucose concentration of the 4 day old fasted calves may be due to a reduced sensitivity to plasma glucose concentration by the α and β cells of the pancreas. These pancreatic cells mature with age (Ktorza *et al.*,1985), thus their ability to respond to fluctuations in plasma glucose concentration is likely to increase. This may also explain the greater increase in plasma glucose concentration by 4D:2F-Control calves compared to 10D:2F-Control calves seen in response to the feed given at 10 hours.

3.4.6 Hydration State

Despite the wide variation in PCV and TPP at the start and end of the experiment the absence of significant changes in either suggest that the calves were not becoming dehydrated as the result of fasting for 30 hours. Fasting alone does not cause dehydration in premature calves. However, it is possible that at least some of the calves were drinking as they had access to a trough containing water throughout the experiment and the troughs required refilling at regular intervals.

Packed cell volume is sensitive to osmotic changes in the blood as well as operator error. Red blood cells may shrink in response to increasing plasma osmolarity or swell in response to decreasing plasma osmolarity causing apparent changes. PCV was measured manually and by many different individuals during the course of the experiment, leaving potential for operator error.

3.4.7 Group 10D:2F-Control

Fortuitously, the calves allocated to the group 10D:2F-Control started the experiment with the highest urea and β-hydroxybutyrate concentrations and the lowest plasma glucose and triglyceride concentrations. Assessment of the metabolic state as indicated by these parameters suggests that the calves were already experiencing a state of negative energy balance at this time. The calves in this group were used on different experimental days and were fed according to the code during the previous 10 days. Their compromised metabolic state at the start of the experiment led to their plasma glucose concentration remaining lower than all other control groups for most of the experiment. After 30 hours the absolute concentrations of plasma triglycerides and urea were more similar to those of fasted calves than of calves that had been fed.

This group did not have a greater proportion of calves of lower bodyweight or teeth code, therefore it is unlikely that these differences were due to prematurity. It is possible that a greater proportion of these calves had lingering effects of prior diarrhoea that were not evident from their clinical state 24 hours before the experiment. If so, the calves would have been fed electrolytes prior to but not during the experiment. The electrolyte solution contained glucose and electrolytes but no fats. After being fed electrolytes for 48 hours the lipid and liver glycogen reserves would be likely to have been considerably reduced, resulting in the lower glucose and triglyceride and higher urea and β-hydroxybutyrate concentrations in the initial sample.

3.5 General Discussion

Generally, premature induced calves are in a metabolically more precarious state than full-term calves as indicated by lower plasma glucose concentrations and higher β-hydroxybutyrate and urea concentrations at the start of the experiment and after 30 hours fasting. This may be due to the calves having smaller quantities of glycogen and lipid, more rapid utilisation of these reserves if they required a greater rate of thermogenesis and the functional immaturity of some physiological systems. Premature calves are likely to experience more rapid depletion of body energy reserves than full-term calves, therefore there is a greater potential for welfare compromise through undernutrition and hypothermia. Calves thought to have been born at an earlier gestational age (as indicated by bodyweight and teeth code) show greater increases in urea and larger decreases in rectal temperature indicating that the potential for welfare compromise increases with decreasing gestational age.

The findings of the present study can be used to assess the current recommendations and minimum standards of the Animal Welfare Advisory Committee.

3.5.1 Minimum Acceptable Weight of Calves to be Bobbied

The results of this experiment suggest that the recommended minimum weight for bobby calves of 15 kg may be too low if the calves are expected to be able to tolerate up to 30 hours fasting. Calves of different breeds weighing 15 kg vary in their degree of maturity. A Jersey or Jersey crossbred calf of this weight is likely to be better able to tolerate the transport and fasting associated with bobbying in terms of the extent of depletion of energetic body reserves than a Hereford crossbred calf of the same weight as it would be likely to be more mature and have proportionately more glycogen and lipid reserves.

Reference to chapter two shows that calves born up to two weeks premature did not show signs of immaturity such as low teeth score, rectal temperature or respiration rate. If the minimum weight could be raised such that most bobby calves were no less than 3 weeks premature when sent for slaughter the calves ability to tolerate the associated fasting is likely to be enhanced.

3.5.2 Minimum Postnatal Age of Bobby Calves.

Little apparent advantage in terms of fasting tolerance is gained by increasing the minimum age from 4 days to 10 days. The ability of a 4 day old induced calf to tolerate 30 hours fasting appears to be similar to that of a 10 day old calf. Keeping the calves for a further 6 days would involve extra time, effort and expense for no apparent gain making this option impractical.

3.5.3 The Feeding of Induced Calves

There was no observable energetic or thermogenic advantage to be gained by feeding induced calves smaller volumes four times over a 10 hour period as opposed to larger volumes twice over the same period. If the four feeds were given over a 24 hour period there may be an energetic advantage gained in terms of less depletion of glycogen and lipid reserves during the overnight fast in the more premature calves.

The recommended volume of milk (100 ml/kg body weight) appears adequate for maintaining plasma glucose concentrations. The higher plasma glucose concentrations of calves fed 50 ml/kg body weight in the pre-fasting feed may indicate that a larger feed gives these calves an advantage over those fed 25 ml/kg when fasting for up to 18 hours.

Based on the results from this study it could be recommended that in the morning feed before transport or fasting calves be fed at least 50 ml/kg bodyweight no matter what their prior feeding regime was. This would provide sufficient energy for the calves to tolerate up to 30 hours without being fed.

It must be taken into consideration that some of the more premature calves appeared to be less able to feed effectively by co-ordinating sucking and swallowing, thus although they may be offered proportionately similar volumes of milk as full-term calves the amount that reaches the stomach is likely to be less. Delivering an adequate volume of milk to such calves may be better accomplished by way of a stomach-tube until their coordination/ability to feed effectively improves. In this case delivery of smaller

volumes at more regular intervals may be advantageous as it would allow plasma glucose levels to be maintain at a moderate level (4-5 mmol/l) rather than fluctuation. It would also prevent over-loading of the calf's abomasum.

3.5.4 The Maximum Duration of Fasting for Induced Calves

If mobilisation of muscle protein to provide energy or a decrease in rectal temperature are seen as undesirable, a reduced duration of fasting or exclusion of calves greater than 3 weeks premature would be recommended. However if this is not a concern, the rapid recovery of all calves in response to refeeding as well as their ability to maintain a minimum plasma glucose concentrations from 24 to 30 hours of fasting, would suggest that most premature calves which have survived to four days of age are able to tolerate 30 hours fasting acceptably.

3.5.5 Improvements to the Design of the Present Experiment

Limitations Imposed by Obtaining Calves From Many Sources

The neonatal calves used in this experiment were obtained from eleven farms around the Manawatu district as they became available. This meant that we were unable to have the number of calves at the age we required them available at the same time, making a single experiment impossible. Instead, five similar experiments were conducted over a period of four weeks. Efforts were made to have several calves from each treatment group present on each experimental day to minimise any effect of climatic and any other conditions on any one group, but this was not always possible. For example, different calves from 4D:4F-Control were included in experiment one, experiment two and experiment four, but not experiment three.

Ideally the supply of calves would have been more reliable such that one or two larger experiments could have been carried out rather than five smaller ones. This would have allowed the conditions under which the experiment was held to be standardised. Knowing the actual ages of the calves would have allowed the effects of this variable to be known rather than approximate and would have helped with grouping the calves. Having large numbers of one or two breeds or crossbreeds would have been preferrable to using any calves that were available. Even so an advantage of this experiment is that

it deals with calves in a real life situation. That is, induced calves that were to be bobbied would be of a variety of breeds and gestational ages.

Parameters Used

The metabolic state of the calves would have been more accurately assessed if the concentrations of key hormones such as insulin and glucagon were measured regularly during the experiment. Measurement of the free fatty acid concentration in plasma would have been a more accurate indicator of the state of lipid metabolism than plasma triglyceride concentration. Interpretation of the state of protein metabolism would have been improved if urine output and urinary urea concentration had been measured.

The hydration state of the calves may have been monitored more closely if we had noted whether or not the fasted calves had been drinking water during the experiment. Urine analysis may have also provided meaningful data in addition to measuring blood haemoglobin and serum sodium concentration. Differences in the concentration of antidiuretic hormone in fed and fasted animals as well as at different times during the experiment would have also been useful.

Sick Calves

The results would have been more easily interpreted if a more accurate record of the calves that contracted diarrhoea and their treatment had been kept during the time the experiment was being conducted.

3.5.6 Future Research

Future research regarding the responses of premature induced calves to feeding and fasting could include the following:

- The use of premature induced calves of known gestational age and of a given breed or crossbreed. This would allow the specific effects of prematurity to be known and would reduce variation due to genetic factors.
- Compare the effect of 30 hours fasting on 4 day old calves born when 4 weeks premature, 2 weeks premature and at full-term, to assess the effects on metabolic responses and hydration status.

- Determine the effects of fasting and variable transport durations on premature induced calves of known gestational age at birth. This would allow the effects of the transport that occurred during the bobbying process to be assessed in addition to the affects of fasting.

- Assess the metabolic response of full-term and premature induced calves that are two and four weeks premature, to feeding 25 ml colostrum/kg bodyweight every six hours. Feeding 25 ml/kg colostrum every six hours is another way the AWAC recommendations could be interpretted. Thus it would be of use to asses the response of induced calves to this feeding regime.

- Compare the metabolic responses of full-term and premature calves known to be two and four weeks premature after they are fed required volumes of milk either by sucking or via a stomach tube. This may allow the extent of spillage at varying gestational ages to be assessed and compared to that in full-term calves.

Chapter Four:

Vascular Anastomoses of Twin Bovine Placentas

Abstract

In the present study the gross anatomy of the placentas from 18 twin-bearing cows was examined and the packed cell volumes of their calves at birth and at 24 hours of age were measured. The placentas were able to be divided into six classes on the bases of the degree of vascular anastomosis between the placentas of co-twins. The degree of anastomosis varied from none, to connection via common placentome, to extensive joining of large diameter blood vessels.

The PCV decrease over the 24 hours following birth differed to greater extent between twins from placentas exhibiting a high degree of anastomosis. It was concluded that these differences were likely to have been due to blood transfer within the joined placental vessels of the calves during birth.

The mean birth weight of twin calves was significantly greater than that of single calves.

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Chapter 4: Vascular Anastomoses of Twin Bovine Placentas

4.1 Introduction

In cattle, twinning normally occurs at a rate of 1 per 96 single births in dairy breeds and at 1 per 227 single births in beef breeds (Roberts,1986). Unlike fetal sheep, goats, pigs, deer and horses the placental blood vessels of adjacent bovine fetuses often join allowing the sharing of blood between the feto-placental circulations of each calf (Lillie,1922; Williams *et al.*,1963; Mellor,1969a).

4.1.1 Development of the Fetal Membranes and Placentas in Twin Bovine Pregnancies

The fetal membranes of cattle form two liquid-filled sacs (Sloss and Dufty,1980). Each sac is composed of two membranes formed by the junction of the chorion, allantois or amnion (Sloss and Dufty,1980; Mellor and Slater,1974). One envelops the fetus, it contains amniotic fluid and is made up of the amnio-chorion (the junction of the amnion and chorion) and the allanto-amnion (Mellor and Slater,1974). The other contains allantoic fluid and is made up of the allanto-chorion and allanto-amnion (Mellor and Slater,1974). The two sacs are enclosed within the chorion, the outer fetal membrane (Sloss and Dufty,1980; Mellor,1969b).

During development the allantois carries with it vessels from the fetal aorta and posterior vena cava (Sloss and Dufty,1980). Fusion of the chorion and allantois during early development of the fetal membranes causes vascularisation of the chorion (Sloss and Dufty,1980). When this vascularisation does not extend to the tips of the chorion, these become necrotic (Sloss and Dufty,1980). In bovine multigravid pregnancies the tips of adjacent chorionic membranes may either fuse or become necrotic and remain separate (Sloss and Dufty,1980). Hammond (1927) noted that necrosis of the tips of the allanto-chorion is less marked in the cow than in the sheep or pig. Thus, it was suggested by Williams *et al.*,(1963) that this may facilitate the increased incidence of fusion of adjacent chorionic sacs of bovine twins.

4.1.2 The Degree of Vascular Anastomosis Between Adjacent Fetuses

The relationship between adjacent fetal membranes and degree of anastomoses of placental blood vessels depends on the position of the embryos within the uterus (Williams *et al.*,1963; Hafez and Rajakoski,1964; Mellor;1969b). The positions of the embryos may result in a complete lack of attachment of adjacent chorions, partial attachment of adjacent chorions with no contact between the allantoic membranes, or complete attachment of the chorions and direct contact between allantoic membranes with subsequent anastomosis of the adjacent vascular supplies (Lillie,1922; Mellor,1969b; Hafez and Rajakoski,1964). The degree of anastomosis is most likely to depend on the extent to which adjacent allantoic membranes are apposed to each other (Hafez and Rajakoski,1964). Anastomosis of the vascular supply of allantochorions of adjacent bovine embryos was found by Hafez and Rajakoski, (1964) to have occurred as early as 30 days after conception.

Joining of the chorionic vessels was observed by Williams *et al.*(1963) to be more likely to occur in unicornuate twins (when both fetuses occupy one uterine horn) than in bicornuate twins (when the fetuses occupy separate uterine horns). These authors suggested that the relative crowding effect which occurs in unicornuate twin pregnancies may be a factor that leads to more frequent anastomosis of chorionic blood vessels (Williams *et al.*,1963). In unicornuate pregnancies the surface area over which the allantoic membranes abut is likely to be greater thereby increasing the degree of vascular anastomosis. Such pregnancies are also associated with lower embryo survival rates and higher incidences of abortion and still birth (Gordon,1996). Twins from bicornuate pregnancies are usually heavier and have longer gestation periods than those from unicornuate pregnancies (Sinclair *et al.*,1995abc; Penny *et al.*,1995).

4.1.3 The Effect of Twinning on Feto-placental Haemodynamics

Vascular anastomosis of twin placentas is likely to alter feto-placental haemodynamics. The extent to which blood flow is altered may depend on the magnitude of vessel sharing, the diameter of the vessels involved and the balance of blood pressure within the feto-placental unit of each calf. Lillie (1922) stated that:

> *"...whenever the arterial pressure is higher on one side than the other blood must be distributed from the side of the higher pressure to that of the lower pressure; it will thus reach the veins and the fetus of the opposite side; variations in pressure on the two sides must constantly occur, if there is any difference in the time of occurrence of systole and diastole of the twin hearts."*

Single calves normally have two umbilical arteries and one or sometimes two umbilical veins that divide repeatedly into a placental circulatory network of smaller diameter vessels that terminate at placentomes (Sloss and Dufty,1980). The twin bovine placentas described and illustrated by Lillie (1922) show one of the main umbilical arteries of one fetus dividing into three branches, one of which could be followed directly through to its connection with the placental arterial system of its twin. This arterial anastomosis was described as being a single strong vessel (Lillie,1922). Venous anastomoses were described as being much less viable (reducing the passage of blood) than arterial; macroscopically consisting exclusively of a connection between the two veins of one cotyledon (Lillie,1922).

Sharing of placentomes between adjacent fetuses has also been reported in cattle by Swett *et al.*(1940) and Lillie (1922), and in sheep by Mellor (1969b). Although intermixing of the blood of adjacent fetuses that share one or more cotyledon does occur in bovine twins (Lillie,1922), this is not the case in ovine twins (Mellor,1969b).

Sharing of Fetal Blood

Sharing of fetal blood results in the production of freemartins, erythrocyte mosaicism and chromosomal variations (Lillie,1917; Swett *et al.*,1940; Williams *et al.*,1963; Mellor,1969a; Roberts,1986). These conditions occur much more commonly in bovine twins than in other species (Williams *et al.*,1963; Mellor,1969a). In fetal sheep and goats a clearly delineated suture line separates the placental vessels and blood of each fetus (Mellor,1969a). Of the bovine twins studied by Williams *et al.* (1963), 81% showed erythrocyte mosaicism, which represents good evidence of fusion of their fetal circulations during pregnancy. Lillie (1922) reported that freemartins occurred in 80-90% of heterozygous bovine twins.

4.1.4 The Potential for Blood Volume Shifts or Loss at Birth Arising From Placental Vascular Anastomosis

The effects of placental vascular anastomosis on the feto-placental haemodynamics of twin calves during and soon after birth have not previously been studied. The large diameter of the anastomosed arteries described and illustrated by Lillie (1922) present the potential for movement of blood between the feto-placental circulations of the first and second calves during the perinatal period.

During parturition the blood vessels of the umbilical cord are stretched, constrict and subsequently rupture as the fetal calf engages and is then expelled through the maternal pelvis (Sisson,1978; Sloss and Dufty,1980). Stretching and constriction of the umbilical cord propels blood within the umbilical vessels into either the fetus or the placental circulation (Sisson,1978). When large diameter placental blood vessels of the first-born calf run directly to the umbilical blood vessels and placental circulation of a co-twin rather than solely terminating in placentomes (as illustrated by Lillie,1917) blood that would normally have moved into the placenta or fetus may be diverted into the feto-placental circulation of the second calf. Thus, the feto-placental circulation of the first-born calf may effectively provide a blood transfusion into that of the second.

Alternatively, the birth of the first calf may alter the balance of blood pressure within the shared placental vasculature. Rupture of the umbilical cord of the first-born calf may cause a decrease in blood pressure in its placental vasculature. This may allow blood from the feto-placental circulation of the second calf to move into these low pressure vessels. Thus, before its birth the second-born calf may lose blood into the placental vasculature of the first-born calf.

It is also possible that one or more of the major vessels of the shared placenta would be severed during the birth of the first calf. If these vessels did not constrict the blood from the feto-placental circulation of the second-born calf could be lost via haemorrhage into the uterine cavity.

Birth Order of Twin Calves

As illustrated above, birth order has the potential to affect the changes in blood volume that occur in the first 24 hours. Thus factors that affect birth order in bovine twins must be examined. Whether a twin pregnancy is bicornuate or unicornuate is a major factor effecting birth order. In unicornuate pregnancies the fetus nearest the cervix would invariably be born first (Mellor *et al.*,1977). However in bicornuate pregnancies both fetuses will usually have equal access to the cervix at the onset of labour. Mellor *et al.*,(1977) found that of five bicornuate twin sheep pregnancies, the cortisol increase that initiates labour (see Chapter 2) consistently began at least two days earlier in the first-born fetus than in its co-twin. The different corticosteroid patterns were considered to be the likely cause of development of greater myometrial contractions in the uterine horn occupied by the first-born twin (Hindson *et al.*,1968; Mellor *et al.*,1977). Given that the hormonal changes initiating parturition are similar in the sheep and cow, it is likely that similar mechanisms govern birth order in bovine bicornuate twin pregnancies.

However, it is likely that in bovine bicornuate twin pregnancies where there is a high degree of vascular anastomosis the intermixing of blood would reduce any differences in cortisol concentrations between the twins. It may be speculated that an increase in cortisol concentration of one twin would act on the placenta and myometrium of its own uterine horn and probably also that of its co-twin. If this were the case the uterine horns may contract with similar strength pushing both calves toward the cervix. If both calves were pushed toward the pelvis simultaneously the potential for calving difficulty would be likely to increase. In twins calves with a high degree of vascular anastomosis compared to those with less vascular anastomosis or separate placentas, both fetuses would be expected to become jammed in the maternal pelvis more often. Mellor *et al.*(1977) observed one pair of twin lambs, which exhibited simultaneous cortisol rises before birth, during which the head of one lamb and the legs of the other were delivered simultaneously.

4.1.5 The Present Study

Normally multiple births are relatively uncommon in cattle making the study of twinning and twin placental anatomy difficult (Roberts,1986). However access to a herd of beef crossbred cows in which twin embryos had been implanted made a large

number of twin calves and their placentas available for study. The aims of the present investigation were to:

- describe the gross anatomy of twin calf placentas, especially their vasculature,
- examine potential relationships between placental anatomy and birth weight,
- study the effects of birth order and twinning on PCV at birth and at 24 hours,
- study the effects of birth order on changes in PCV between birth and 24 hours, and
- examine potential relationships between placental anatomy and PCV changes within twin pairs, or PCV differences between co-twins at birth and 24 hours.

4.2 Materials and Methods

4.2.1 Animals:

The calves and placentas used in this study were sourced from 55 Hereford-Friesian cows originally impregnated with two embryos each through embryo transfer (McMillan and McMillan,1989; Pugh *et al.*,1989; Lambert *et al.*,1996) As often occurs, some embryos were lost during pregnancy (Sakakibara *et al.*,1996) giving rise to the birth of single calves rather than twins. In the spring of 1995 data were collected from 19 single born calves and 27 sets of twins. The remaining data were obtained from 9 sets of twins born in 1996.

4.2.2 Birth Observations and Packed Cell Volume

Calves were born outdoors on a research farm in the Manawatu region (AgResearch, Ballentrae). In 1995 the following details were recorded:

- The time and date of birth and birth order of twin calves.
- PCV at birth and 24 hours (duplicate)(see Chapter 3 for method).
- Birth weight.

Only the birth order and PCV at 24 hours were recorded from the twin calves born in 1996.

4.2.3 Placental Dissection

Placentas obtained from 18 of the cows bearing twins in 1995 were frozen for later dissection. The gross anatomy of the placentas was studied and the degree of vessel anastomosis described. The following details were recorded from each placenta.

- Placental Weight. Total weight of the fused placenta and fetal membranes from each cow.

  Weights of each half of a joined placenta, where the halves could be separated.

  Weights of separate placentas where they were not fused.

- Placentomes. Total number.

  Number of placentomes on each separated half of fused placentas.

- Umbilical Blood Vessels.

  Number of arteries and veins.

  Diameter of veins and arteries.

  Notes regarding anastomosis of umbilical arteries and/or veins.

- General notes describing placental anatomy and the degree of vascular anastomosis.

4.2.4 Derived Data

The observation of birth order and weight, and measurement of PCV allowed the following data to be derived:

- The time between birth of the first and second twin.
- Differences in the birth weight of twin pairs.
- Difference in PCV between the twins at birth and at 24 hours.
- The magnitude of PCV change between birth and 24 hours.
- An estimation of the volume of blood moved from the feto-placental circulation of either the first or second born calf of twins during birth (methods in appendices 4.2 and 4.3).

4.2.5 Estimation of the Volume of Blood Moved Between the Feto-placental Circulations of Twin Calves During Birth

Comparison of the packed cell volumes (PCV) of co-twins after birth may be used to estimate the shift in blood volume during parturition. Differences in PCV between the twins at birth and at 24 hours and changes in PCV that occur within this time, may indicate whether there has been a net movement of blood into or out of the feto-placental unit of each calf. Examination of placental anatomy and the degree of vessel sharing may then allow speculation about the effects of vascular anastomosis on net blood movement between the feto-placental circulations of both calves. The volume of blood moved between the feto-placental vasculature of twin calves can be calculated if the following assumptions are made.

Assumptions

1) No net blood loss to or gain from the second-born twin occurs before rupture of the umbilical vessels of the first-born twin.

2) Rupture of the umbilical blood vessels of the first-born calf causes changes in blood pressure that allow movement of blood between the anastomosed placental vasculature of the first- born twin and the feto-placental vasculature of the second-born twin.

3) Before birth the PCV of both calves is similar when significant vascular anastomosis is present.

4) Therefore, the PCV of the first-born calf immediately after birth is equivalent to that of the second-born calf before any net blood loss to or gain from the placenta of the first-born calf occurs after its umbilical cord ruptures.

5) Haemodilution, indicated by a decrease in PCV, occurs in response to drinking colostrum during the first 24 hours after birth (Adams *et al.*,1993).

6) Assuming similar intakes of colostrum per unit bodyweight, the decrease in PCV during the first 24 hours after birth in the first-born calf would be similar to that expected in the second-born calf if no net blood loss or gain occurred after the birth of its litter mate.

7) A smaller decrease in PCV during the first 24 hours in the second-born than in the first-born calf would therefore indicate a net gain of blood from, and a greater decrease in PCV a net loss of blood to the placenta of the first-born calf after the birth of the first calf.

8) The magnitudes of any such net blood losses from or gain by the second-born twin after the birth of its co-twin may be estimated from the PCV using the following additional information and the methods outlined in Appendices 4.2 and 4. 3.

Premise for Calculation of Transfused Blood Volumes (Appendix 4.2):

The 24 hour PCV of the first-born calf, and its PCV *decrease* between birth and 24 hours were used to estimate what the 24 hour PCV of the second-born calf would have been, had no transfusion occurred (Appendix 4.2).

The volume of blood transferred from the feto-placental vasculature of the first-born calf into that of the second was calculated using the difference between the actual PCV of the second-born calf at 24 hours and the estimated PCV, which would have been greater if transfusion had occurred (see assumption 7).

The blood volume of the second-born calf was estimated using its PCV at 24 hours, and based on the assumption that the plasma volume of newborn calves is 7.3% of their body weight (Dalton, 1964, Michell *et al.*,1989).

The feto-placental blood volumes were estimated using the PCV of the first-born calf at birth, and relied on the assumption that the feto-placental plasma volume of the first-born twin was 10% of its bodyweight as calculated by Caton *et al.*(1975) for full-term fetal lambs.

Premise for Calculation of Blood Volumes Lost by Twin 2 (Appendix 4.3):
The method for calculating the amount of blood lost by the second-born calf was similar to that for calculating the amount of blood transfused. However, rather than subtracting the difference in PCV decrease between the twins from the 24 hour PCV of the second-born calves, it was added (see assumption 7 above). Another difference is that the feto-placental blood volume was calculated for the second-born calf rather than the first (Appendix 4.3).

4.2.6 Statistical Analysis

Results are expressed in terms of the mean and standard deviation unless otherwise stated. Correlations between the parameters measured were analysed by linear regression (Microsoft Excel V 5.0, Microsoft Corporation, USA). Significant differences between mean values were determined using a Student's t-test assuming unequal variation (Microsoft Excel V 5.0, Microsoft Corporation, USA).

4.3 Results

4.3.1 Birth Observations

The Interval Between the Births of Co-twins

The time of birth was recorded for both members of a set of twins in 22 cases. The average period of time between the birth of the first and second twin was 31 ± 31 minutes with a range of 1 - 110 minutes.

Birth Weight

Single calves had a mean birth weight of 37 ± 6 kg with a range of 26 - 49 kg. In comparison the first-born twins had a mean birth weight of 33 ± 5 kg with a range of 27 - 43 kg. Twins born second weighed 31 ± 5 kg at birth, with a range of 20 - 39 kg. There was no significant difference between the birth weights of the first and second born calves, but singletons were significantly heavier than both first-born ($P < 0.05$) and second-born ($P < 0.001$) twin calves.

4.3.2 Placental Anatomy

Placental Types

Placentas and fetal membranes were obtained from 18 of the cows bearing twins. They were divided into six subgroups on the basis of the degree of joining of the membranes and amount of vascular anastomosis between the placental vessels of the two fetuses. The main feature allowing non-fused and fused twin placentas to be distinguished after they were delivered was the number of umbilical cords. That is, on non-fused placentas only one umbilical cord could be found and on fused placentas there was evidence of two cords.

Type A: Non-fused placentas from twin births.

Five of the twin sets had non-fused placentas (Figs 4.1 and 4.2). Both placentas were collected from one cow and one placenta was collected from the remaining four. In all a total of six non-fused placentas were derived from cows known to have given birth to twins. These placentas had one set of umbilical vessels each and there was no joining of membranes or of the placental vasculature. They could not be differentiated from those of single-born calves on the basis of their weight, number of placentomes or any other feature of their gross anatomy.

Five of the placentas had two umbilical veins and two umbilical arteries. The remaining placenta had two arteries and one vein. The width of the umbilical arteries ranged from 7 to 10 mm and that of veins ranged from 8 to 20 mm. In four of the six placentas the two umbilical arteries were connected by a short vessel of approximately 2 mm diameter near the point where they had been torn by fetal separation. This vessel ran perpendicular to their length and was termed an H-junction because of its shape.

Type B: Joined placentas with no obvious sharing of vessels or placentomes

Two placentas were collected which were effectively separate apart from joining of the chorionic membranes (Fig 4.3). They shared no placental vessels or common placentomes and had one set of umbilical vessels each.

The placentas from cow #274 had two veins and two arteries in each umbilical cord. The placental vessels from cow # 83 could only be described for one of the umbilical cords which had two arteries joined with an H-junction, and two veins. The umbilical arteries of these placentas had diameters of 7 to 8 mm and veins of 8 to 13 mm wide.

Type C: Joined placentas sharing placentomes only

The placentas from two cows had separate sets of umbilical vessels but had common placentomes without any additional macroscopic anastomosis of blood vessels (Fig 4.4). The placentas of both calves from cow #253 were joined by their membranes and had one common placentome. Blood vessels from each placenta of approximately 2 mm in diameter supplied the common placentome. The membrane joining the two placentas had few obvious vessels within it. A discernable white suture line that is often present in the fetal membranes of abutting twin sheep fetuses (Mellor, 1969b) was not obvious. When the two placentas were separated through this apparently avascular area they weighed 2931g and 1788g and had 37 and 18 placentomes, respectively. Each cord had two umbilical arteries and veins. The diameter of these arteries ranged from 5 to 8 mm and that of veins was 8 to 11 mm. Both sets of umbilical arteries were joined, those of the heavier placenta with an H-junction. Those of the lighter side were joined to create a stretched X-shape.

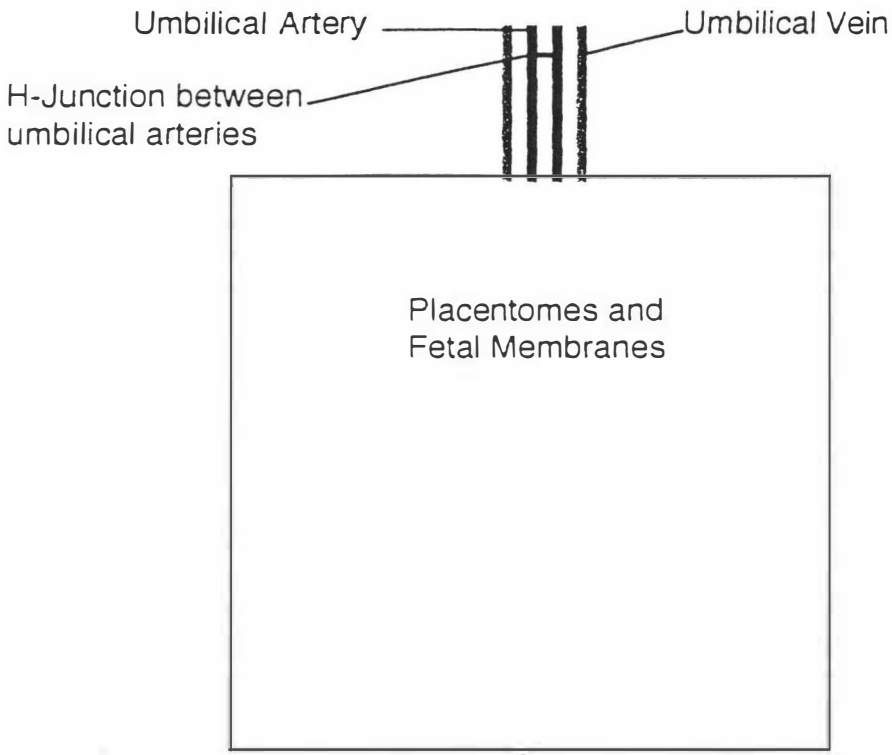

Fig 4.1: Type A: Non-fused placenta

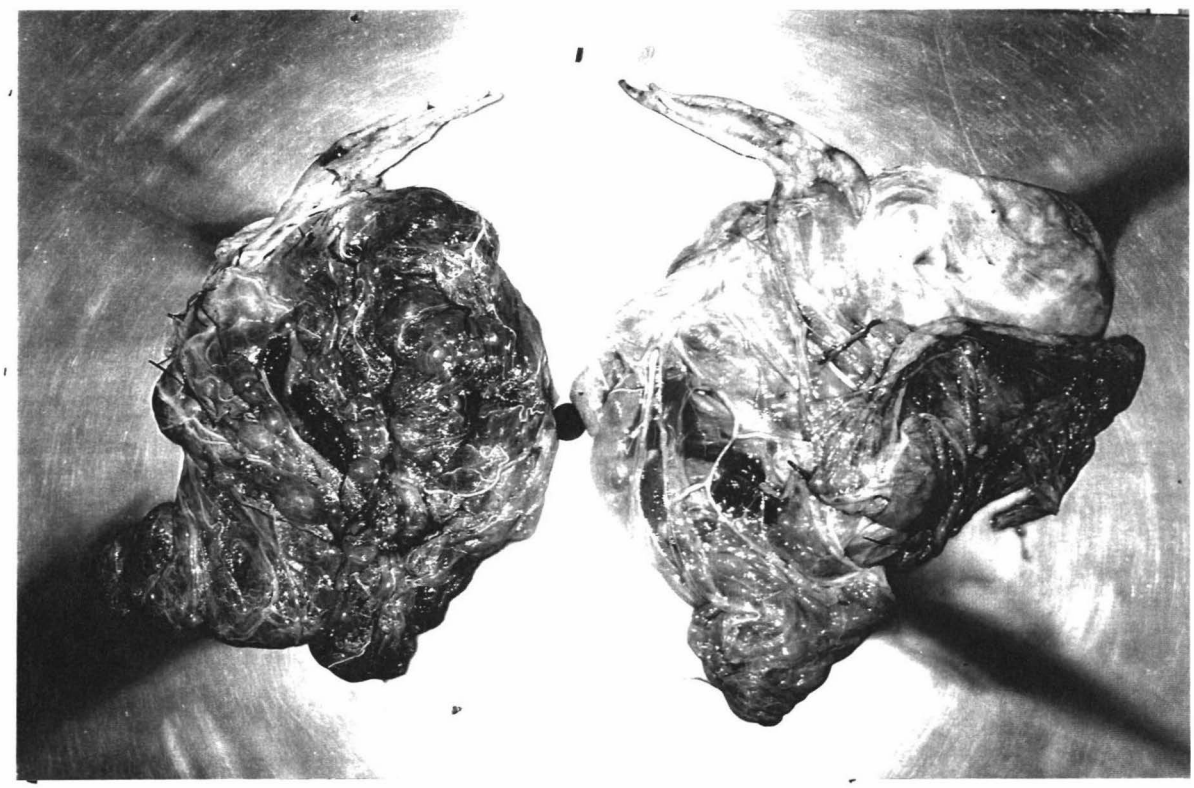

Fig 4.2 A pair of Type A placentas

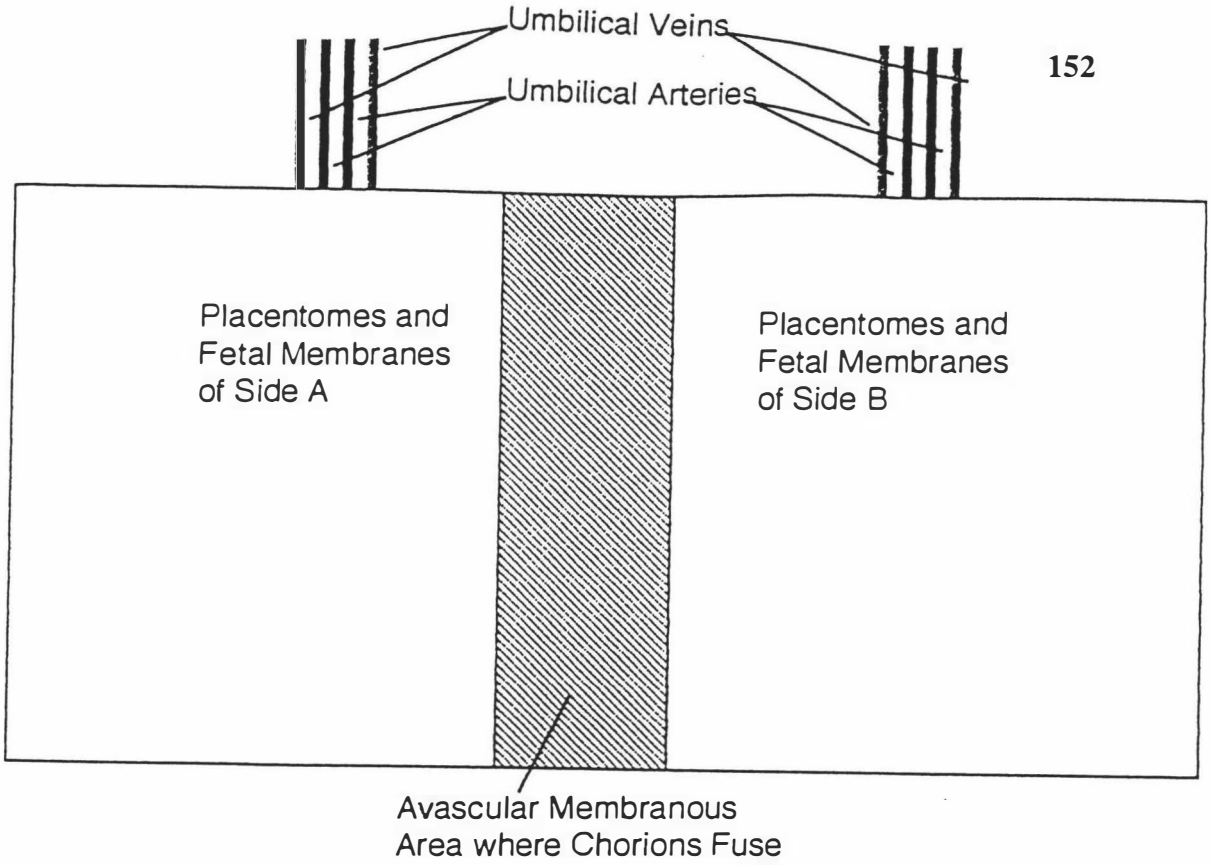

Fig 4.3: Type B: Joined placenta with chorionic fusion and no shared placentomes

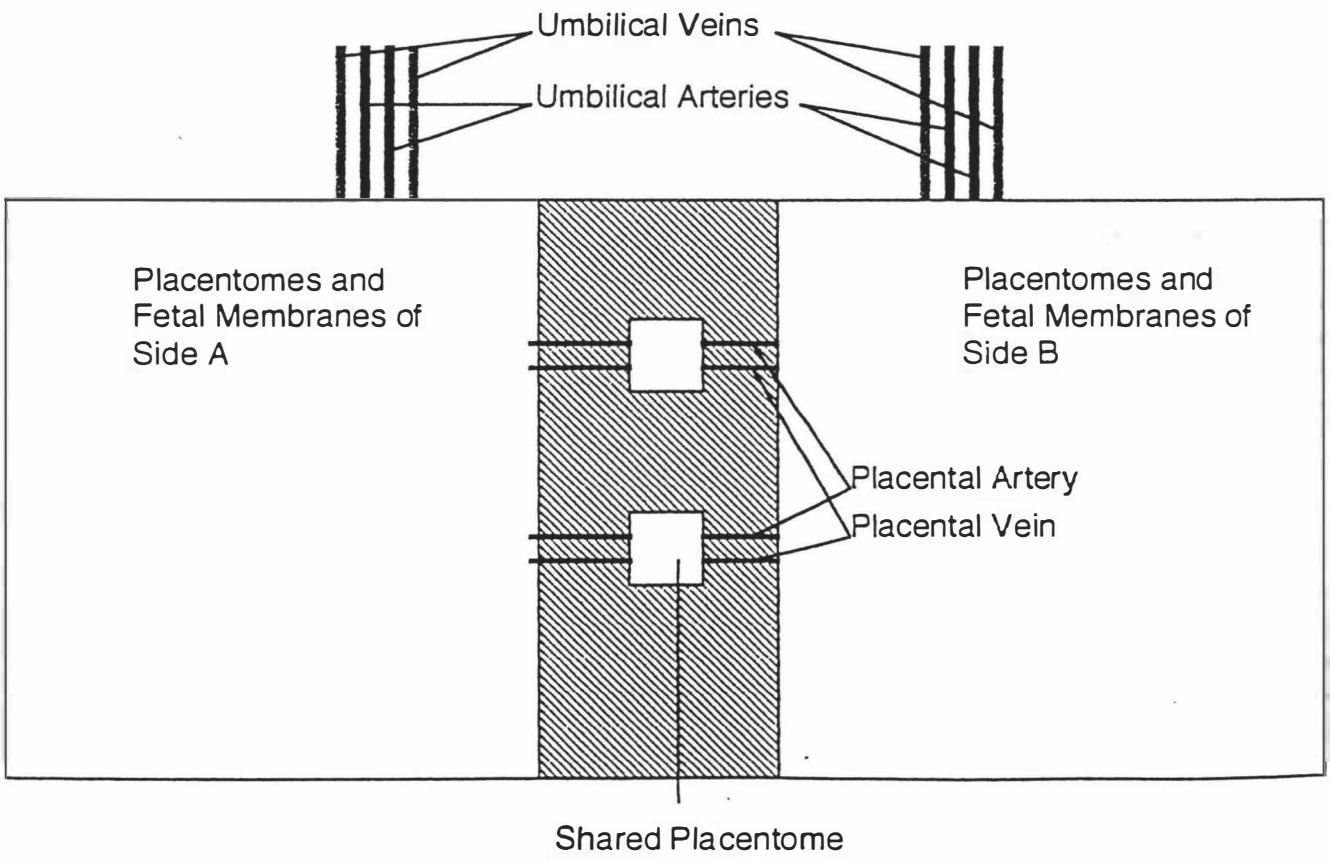

Fig 4.4: Type C: Joined placenta with chorionic fusion and shared placentomes

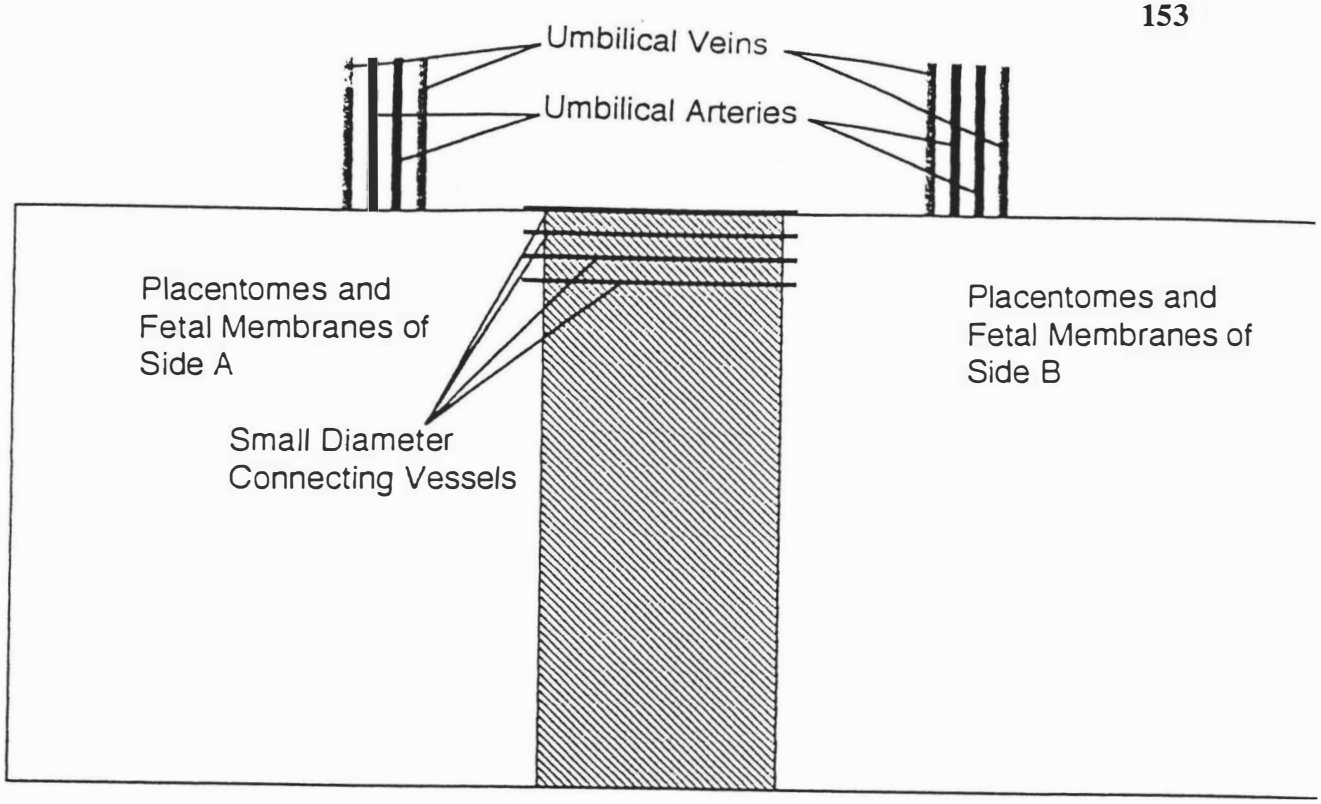

Fig 4.5: Type D: Joined placenta with chorionic fusion and small diameter connecting vessels

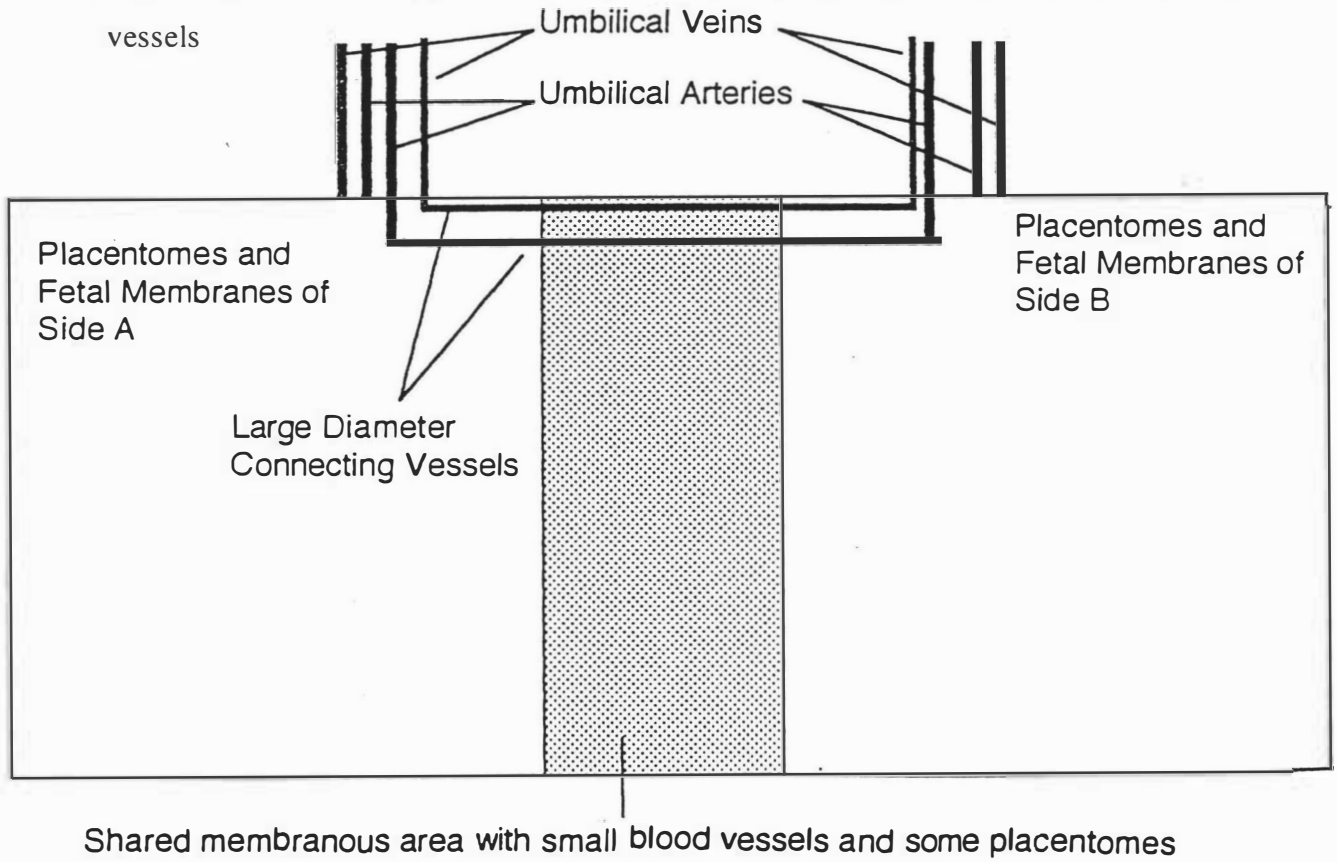

Fig 4.6: Type E: Joined placenta with chorionic fusion and large diameter connecting vessels

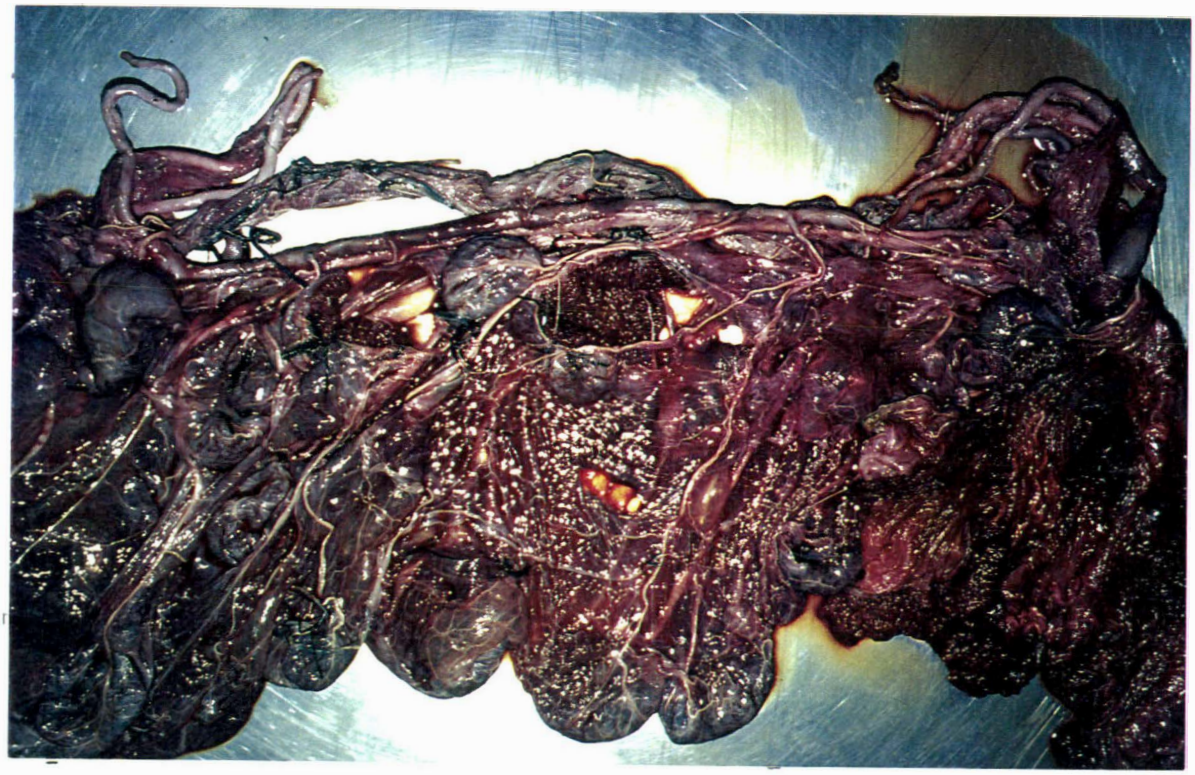

Fig 4.7: Type E placenta

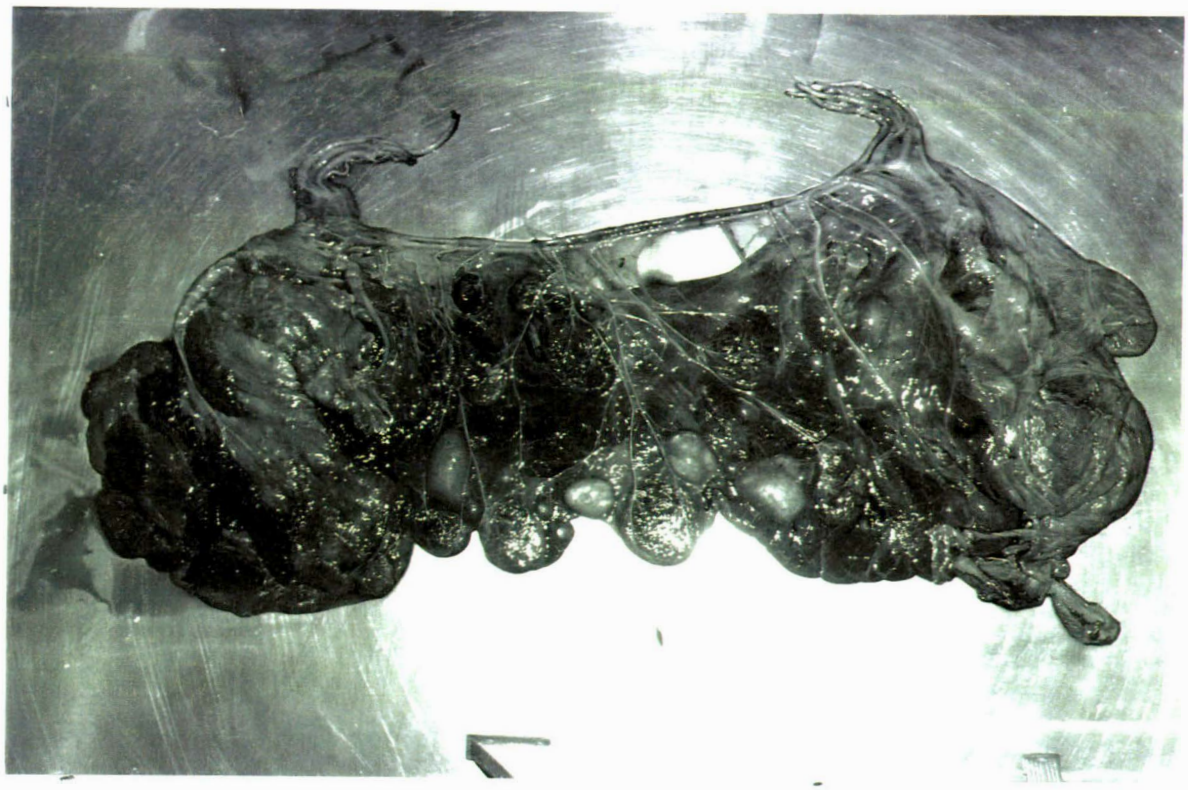

Fig 4.8: Type E placenta

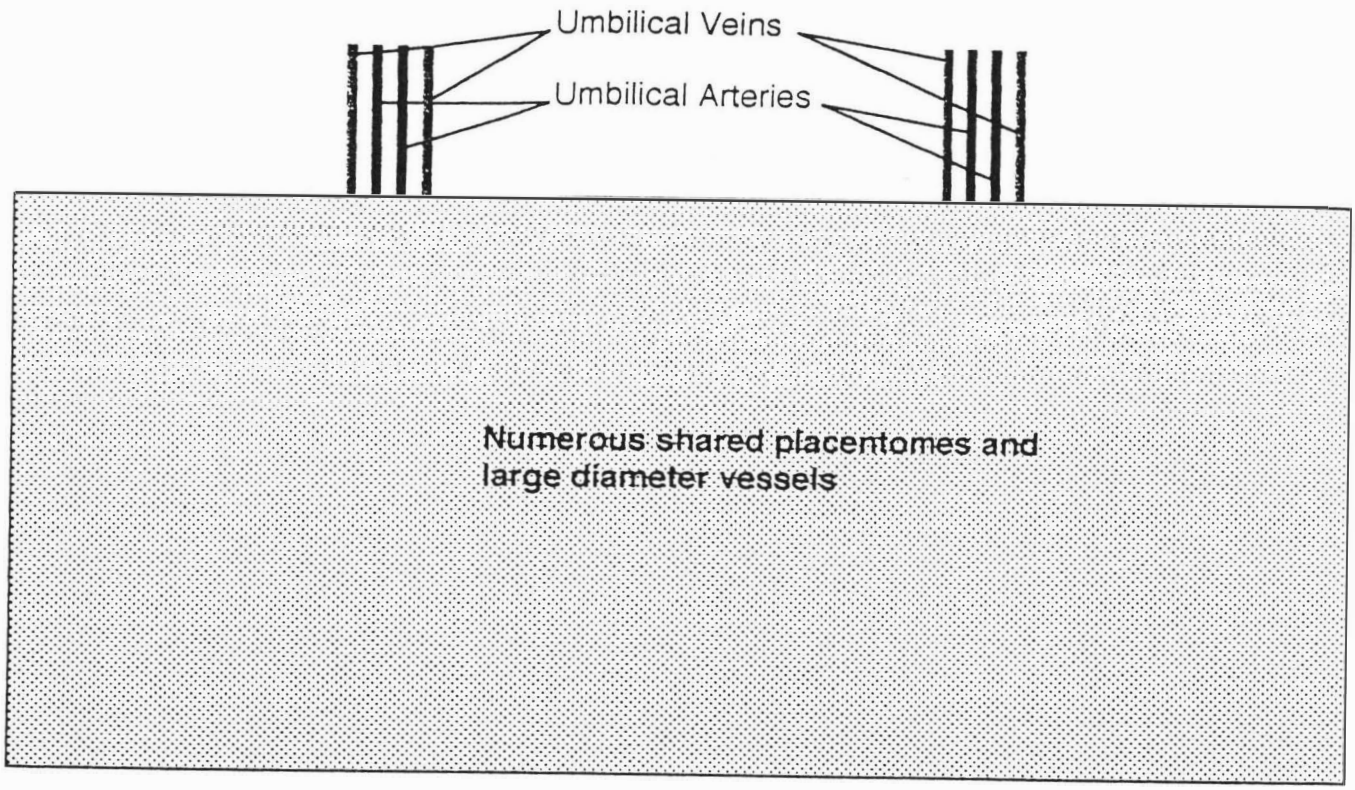

Fig 4.9: Type F: Joined placenta with a high degree of vascular anastomosis.

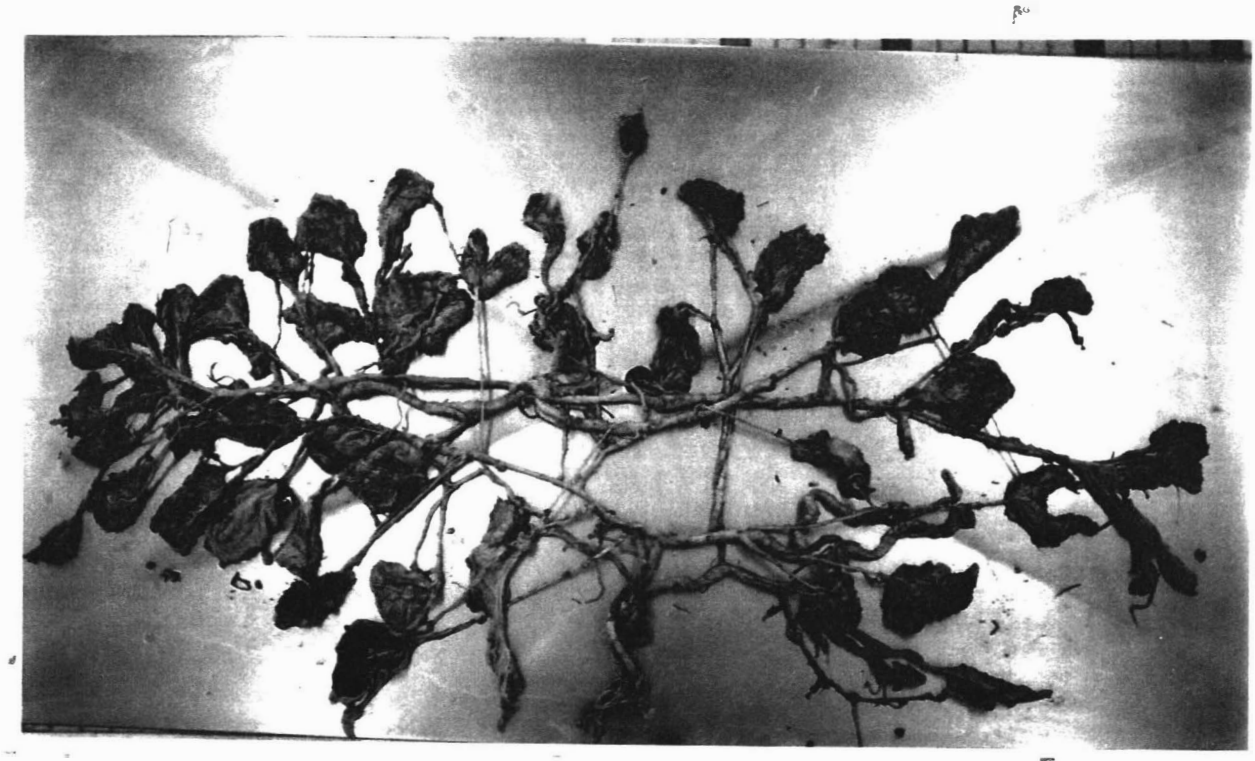

Fig 4.10: Type F placenta that has been preserved in formalin and the fetal membranes dissected from the placentomes and blood vessels.

The placentas from cow # 226 weighed 1153g and 1148g, and had 15 and 12 placentomes respectively. The umbilical cord of the heavier placenta had two arteries joined by an H-junction and two veins. One umbilical artery supplied the placental mass and the other divided with branches going to the shared placentomes. The umbilical cord of the lighter half had one artery and one vein. There were two common placentomes but no direct large vessel anastomoses. Blood vessels running to the shared placentomes were approximately 2 to 3 mm in diameter.

Type D: Joined placentas with small diameter connecting vessels

Two cows (# 53 and # 264) had placentas that were connected through the anastomoses of many small diameter vessels (Fig 4.5). The placentas from cow # 53 were joined by four small diameter arteries. They had a total weight of 6376g. When divided along an obvious dark suture line between the placentas, they weighed 3829g and 2653g and had 35 and 25 placentomes, respectively. The heavier placenta had two joined arteries with diameters of 7 and 10 mm and two veins with diameters of 7 and 9 mm. The two joined arteries of the lighter placenta measured 8 and 9 mm and the single umbilical vein had a diameter of 15 mm.

The umbilical arteries of each calf from cow #264 were joined by anastomoses of several small arteries (1-2 mm), one main connecting artery (3 mm vessel that ran from the large diameter vessels of one placenta to those of the other) and three common placentomes. The main connecting artery had several small branches coming off it. The placenta had a total weight of 6446g. When divided as before, the two placentas weighed 2553g and 3893g and had approximately 26 and 35 placentomes, respectively. The two umbilical arteries of the lighter placenta were 9 mm in diameter and joined by an H-junction. The veins had diameters of 7 and 9 mm. The umbilical vessels of the heavier placenta were not present.

Type E: Joined placentas with large diameter connecting vessels

These placentas had two sets of umbilical vessels joined directly via anastomoses of large diameter connecting vessels (Figs 4.6, 4.7 and 4.8). Five of the 18 placentas dissected were of this type. These double placentas weighed between 2256g and 3298g and had a total of 33-38 placentomes. Division was not appropriate as the separate placentas within each placental mass were not clearly defined.

In four cases one set of umbilical vessels was present while the other set were absent. The second set appeared to have been torn off at the level of the connecting arteries (the large diameter blood vessels running between the placentas) (the placentas from cows # 123, 259, 294, 297). The placentas with both umbilical cords intact had two arteries and two veins per cord (# 268). The diameters of umbilical arteries and veins were only recorded for one set of placentas (# 123) . These umbilical arteries had diameters of 10 mm and 3 mm. The diameters of the veins were 10 mm and 5 mm. The diameter of connecting vessels tended to be equal to if not greater than that of the umbilical vessels.

Type F: Joined placentas with a high degree of vascular anastomosis.

The placentas from two cows were highly fused, sharing numerous placentomes and large diameter connecting vessels (# 23 and # 144) (Figs, 4.9 and 4.10). These placentas were 'web-like' preventing their separation into obvious sides. The placentas from cow #23 weighed 6376g. Each umbilical cord had 2 arteries and 2 veins. The diameters of the arteries were 8 to 10 mm and those of the veins were 8 to 15 mm. The placentas from cow # 144 were not weighed but each umbilical cord was noted as having 2 arteries and 2 veins.

General Features

Placental Weights

Weights were recorded for seven of the non-fused (type A) placentas and ten fused placentas (types B, C, D, E and F). Non-fused placentas ranged in weight from 2197g to 3096g with a mean weight of 2532 ± 307g. In the case where both non-fused placentas were collected the combined wet placental weight was about 5570g. The mean combined weight of the ten joined placentas was 4311 ± 1857 g with a range of 2256 to 6446g.

Placentome Numbers

The mean number of placentomes was recorded for six type A placentas and nine joined placentas. Non-fused placentas had between 33 and 46 placentomes with a mean number of 37 ± 5. In the case where both non-fused placentas from a set of twins were collected they had a total of 79 placentomes. The mean total number of placentomes on nine joined placentas was 46 ± 17 with a range of 27 to 73.

Relationships between the features of placental anatomy

There was a significant positive correlation between the weight of the fetal membranes and placenta and the total number of placentomes in the 12 placentas in which both were recorded ($r = 0.782$, $P < 0.01$). When only the joined placentas (types B, C, D, E and F) were considered this correlation remained ($r = 0.812$, $P < 0.05$, $n= 7$). The total weight (membranes plus placentomes) and number of placentomes on each type A placenta also showed a significant positive correlation ($r = 0.584$, $P< 0.05$, $n= 16$).

Relationships between features of placental anatomy and calf birth weight

The total birth weight of a twin pair was not correlated with total placental weight or with the total placentome number. However, within twin sets the birth-weight difference and difference in placentome number, irrespective of placental type, did show a significant correlation ($r = 0.694$, $P <0.05$, $n = 9$).

4.3.3 Packed Cell Volume

The mean and range in PCVs of calves with different placental types are shown in Table 4.1. At birth the PCVs of twin calves tended to be lower than those of singles, however this difference was not significant probably due to the wide variation of values as shown by high standard deviations.

All calves showed a decrease in PCV between birth and 24 hours. There was a tendency for the second-born calf of twins to have a greater PCV at birth and at 24 hours than did the first-born calf. However this difference was only significant ($P< 0.05$) between twins of unknown placental type at 24 hours. The period between the birth of the first and second calf did not affect the PCV difference between twins at birth or 24 hours or the change in PCV to 24 hours. For each twin pair it was not possible to determine which calf and placenta or umbilical vessels had been connected to each other. Thus, potential relationships between PCV and placentome number could not be examined.

Table 4.1: Mean, Standard Deviations and Ranges in Packed Cell Volumes (%) of Single and Twin Calves

Calf and Placenta Type	0 hours (%)	n	24 hours (%)	n	Change 0 to 24 hours (%)	n
Single Calf	44 ± 6 (31-52)a	19	33 ± 6 (22-43)b	21	11 ± 4	17
Non-fused Placenta - Twin 1	40 ± 4 (34 -43)a	6	32 ± 4 (26-35)	5	9 ± 2	5
Non-fused Placenta - Twin 2	42 ± 7 (30 -49)	6	32 ± 7 (23-41)	5	9 ± 2	5
Joined Placenta - Twin 1	40 ± 8 (27 - 52)	10	30 ± 12 (13-48)	10	10 ± 6	10
Joined Placenta - Twin 2	42 ± 7 (29 - 54)	11	31 ± 8 (14 - 41)	11	11 ± 4	11
Unknown Placental Type - Twin 1 (1996)			30 ± 6 (18 - 40)c	11		
Unknown Placental Type - Twin 2 (1996)			38 ± 5 (29 - 43) b,c	11		

a = P = 0.055; b = P<0.05; c = P< 0.01

Twin 1 = First-born twin

Twin 2 = Second-born twin

Table 4.2: The PCV and Change in PCV (%) of Twins with Different Placental Types

Placental Type	No Vascular Connections (Types A and B)	Vascular Connections (Types C,D,E and F)
PCV at birth	41 ± 5 (30 - 49)	41 ± 8 (27 - 54)
PCV at 24 hours	32 ± 5 (23 - 41)	30 ± 10 (13 - 48)
Change in PCV	9 ± 2 (6 - 11.5)	11 ± 5 (1 - 17.5)

Although there were no significant differences in PCV or change in PCV in twins with different placental types (Table 4.2), trends were seen when individual data sets were studied (Appendix 4.1). The data in Table 4.2 show that twins with placentas having vascular connections (Types C, D, E and F) showed a greater variation in PCV decrease than did twins with placentas having no vascular connections (Types A and B).

Table 4.3: The Difference in PCV(%) Decrease Between Co-twins

Cow Number	Placental Type	Twin 1 PCV Decrease	Twin 2 PCV Decrease	Difference in PCV Decrease
66	A	6	6.5	0.5
41	A	8	8	0
20	A	10.5	8.5	2
83	B	8	9.5	1.5
274	B	7.5	8.5	1
253	C	16	14	2
226	C	17.5	12.5	5
264	D	6.5	8.5	2
268	E	12	5.5	6.5
123	E	15	13	2
259	E	4	13	9
297	E	2.5	12	9.5
294	E	1	9	8
23	F	14	5	9
144	F	13.5	8	5.5

The effect of placental type on the change in PCV over 24 hours

Effects of joined placentas were evident when the differences in PCV between co-twins at birth and at 24 hours were compared. Table 4.3 allows comparison of the difference in PCV decreases between co-twins with different placental types. The mean difference in PCV decrease between co-twins from type A and B placentas was 1 ± 1%, compared to the mean difference of 6 ± 3% for co-twins from placental types C, D, E and F. The degree of PCV change between these sub-groups was significantly different ($P < 0.001$) which indicates that anastomosis of vessels between placentas of twin calves apparently altered the proportionate changes in blood cell and plasma volumes during the first 24 hours after birth.

4.3.4 Estimations of the volume of blood moved from the feto-placental circulation of calves during birth

The first-born twins had a greater decrease in PCV than their second-born litter-mates in six of the eleven twin pairs having joined placentas (types C, D, E, F, Table 4.4). This suggested that there was a net gain of blood by the second-born calf from the placental circulation of the first-born calf during birth.

When the method described in Appendix 4.3 was followed the values in Table 4.4 were calculated. Estimated volumes ranging from 99 to 1053 ml of blood would need to have been transferred from the placental vasculature of the first-born twins into the feto-placental circulations of their co-twins to account for the observed changes in PCV. These volumes represented between 5% and 79% of the estimated placental blood volumes of the first-born calves concerned. With the exception of the calves from cow #226, the estimated volume of blood transferred increased as the degree of vascular anastomosis increased (Table 4.4).

Table 4.4: Volumes of Blood Transfused From Twin 1 to Twin 2

Cow Number	Placental Type	PCV Difference (%)	Estimated Blood Volume Transferred (ml)	Estimated Placental Blood Volume (ml)	Estimated Proportion of Placental Blood Volume of Twin 1 Transferred to Twin 2 (%)
253	C	2	99	2030	5
226	C	5	588	1130	52
260	E	6.5	409	1610	25
123	E	2	177	1850	10
23	F	9	1053	1330	79
144	F	5.5	562	1050	54

In four of the eleven twin sets the postnatal PCV decrease was greater in the second-born calf than in its co-twin. This suggests that there was a net loss of blood from the feto-placental circulation of the second-born calf after the birth of the first-born calf.

Table 4.5: Blood Lost From the Feto-placental Circulation of Twin 2

Cow Number	Placental Type	PCV Difference (%)	Estimated Blood Volume Lost (ml)	Estimated Placental Blood Volume of Twin 2 (ml)	Estimated Proportion of Placental Blood Volume Lost by Twin 2 (%)
294	E	8	666	1980	34
297	E	9.5	467	1480	32
259	E	9	537	2040	26
264	D	2	182	1380	13

The estimated blood volumes lost by the second-born twins were calculated using the method described in Appendix 4.4 and ranged from 182 to 666 ml (Table 4.5). This represented 13 to 34% of the estimated placental blood volumes of these calves. The estimated proportion of blood lost was greater in calves from type E placentas than from the calf with a type D placenta.

4.4 Discussion

The main conclusions of the present study are as follows:

- The mean birth weight of twin calves is significantly lighter than that of single calves.
- Six classes of placental vascular anastomosis were identified varying from none to extensive.
- Vascular anastomosis of twin calf placentas may cause differences in postnatal haemodilution between co-twins. These differences are likely to have been influenced by blood transfer between the joined placental vessels of the calves during birth.

4.4.1 Birth Weight

As expected, the mean birth weight of the twin calves was less than that of singletons. The birth weights of twins as a percentage of the birth weights of singletons were reported by Gordon (1996) to range between 61% and 83%. Lighter birth weights in twin calves were also reported by Adams *et al.*,(1993) in Angus, Hereford and crossbred cattle and by Sakakibara *et al.*,(1996) in Holstein and Japanese Black cows. The length of gestation of twin pregnancies tends to be less than that of single pregnancies (Penny *et al.*,1995). The affect of shorter gestation lengths on the birthweights of twins will depend on how much shoter the gestation is compared to full-term calves. The further from full-term birth the twins are born the lighter they are likely to be (see Chapter 2).

Although differences in gestation length may make a contribution to differences in birth weight they do not account entirely for them. Twins tend to have proportionately smaller placentas than singletons which would reduce the availability of nutrients and substrates required for optimal prenatal growth, thereby reducing their birth weights (Mellor,1983).

4.4.2 Placental Anatomy

The number of placentomes and degree of vascular anastomosis between the placentas of twin calves is likely to reflect the proximity of adjacent embryos at the time of implantation. Placentas with a smaller number of placentomes and higher degrees of vascular anastomosis were likely to have been from predominantly unicornuate pregnancies (both fetuses in the same uterine horn), whereas the reverse was more likely

with bicornuate pregnancies (one fetus in each uterine horn) (Testart and Du Mesnil du Buisson,1966).

Placentome Number

Although the mean and range in number of placentomes of the placentas examined were less than those reported in previous studies (Testart and Du Mesnil du Buisson,1966; Rowson *et al.*,1971; Sloss and Dufty,1980) similar trends were seen.

Twinning influences the number of placentomes and the way in which they develop. Sakakibara *et al.*,(1996) found that the number of placentomes from Holstein dams bearing twins, was approximately double that in those bearing single calves. Similar studies in sheep have shown that the number of placentomes per embryo generally decreases as litter size increases, because each additional chorionic envelope in the uterus reduces the number of potential implantation sites available for each individual (Mellor,1983). However, for any particular litter size the number of placentomes per individual varies widely (Mellor,1983).

When twin pregnancies are compared, variation in the number of placentomes appears to stem from the proximity of the implantation site of adjacent embryos. In the present study one type A and two type B placental sets had totals of 79, 78 and 73 placentomes respectively. In comparison, the three type E sets and a single type F placental set had total numbers of 38, 33, 36 and 45 placentomes respectively. It is likely that type A and B placentas are derived from bicornuate pregnancies and type E and F from increasingly unicornuate pregnancies. These findings are supported by those of Testart and Du Mesnil du Buisson (1966) and Rowson *et al.*,(1971) who found that a larger number of placentomes formed when the pregnancy was bicornuate rather than when it was unicornuate or single.

Giant Placentomes

Sloss and Dufty (1980) found that when twinning occurs, the total number, distribution, size and weight of the cotyledons present depended on whether the fetuses were located in the same or opposite uterine horns. Mellor (1983) noted that in sheep placentas as the number of placentomes decreased the weight of individual placentomes increased.

In unicornuate bovine pregnancies the development of 'giant' placentomes has been recorded (Testart and Du Mesnil du Buisson,1966). These placentomes are at least twice the size of those observed in single or bicornuate pregnancies (Gordon,1996). Such placentome size increases are assumed to represent a compensatory mechanism whereby placental exchange is maintained when placentome numbers are low (Mellor,1987). Without such compensation the availability of nutrients and oxygen to the fetuses may be compromised.

The presence of giant placentomes in placentas could explain the lack of correlation between birthweight and placentome number. Had the placental area been assessed rather than placentome number a relationship between these factors may have been present. French data have shown that placental area is likely to be greater with bicornuate twins and that this is likely to be a factor influencing the size of twin calves (Testart and Du Mesnil du Buisson,1966; Penny *et al.*,1995; Sinclair *et al.*,1995a,b,c). Given that the presence or absence of giant placentomes would influence both fetuses in a twin pregnancy, the correlation between the *difference* in placentome number and *difference* in birth weight is not unexpected and is perhaps more meaningful as a indicator of differences in nutrient supply to co-twins in the present study. For this reason total placentome weight appears to be a better index of placental size than placentome number (Mellor,1983).

The mean birth weights of calves from placentas exhibiting a high degree of placental vascular anastomosis were no less than those exhibiting no anastomosis. It is possible that sharing of placental blood between the twins from these placentas effectively increased the placental area and availability of nutrients and growth substrates to each twin. Another possibility is that the sharing of placental blood created a greater efficiency of nutrient uptake by the twins. That is, nutrients not taken up by one twin that may have normally been returned to the uterine veins, may instead be taken up by the co-twin.

Placental Weight

The mean weight of the fetal membranes of Hereford cattle reported by Dufty (1974) was greater than that of non-fused placentas and less than that of the joined placentas in

the present study. Contrary to the findings of the present study, several authors have reported statistically positive correlations between calf weight and the weight of fetal membranes (Sloss and Dufty,1980), total placentome weight (Anthony *et al.*,1985) and placental weight (Sakakibara *et al.*, 1996). Of these, total placentome weight is likely to be the more accurate measure of placental size (Mellor,1983). In twin placentas the relationship between calf birth weight and placental weight is affected by the implantation sites of the embryos. Sakakibara *et al.*,(1996) found that the placental weight of Holstein dams calving twins was heavier than that in those calving a singleton. It is likely that the variation in placental anatomy and therefore weight, created by twinning distrupts this correlation between placental weight and calf birth weight. For example, type E placentas were generally lighter than those of type B or C, even though the birth weights of the calves from each placental type did not differ corresponding.

The weight of the placenta and fetal membranes can be used only as an approximate index of placental size. Studies of sheep placentas have found that placental weight decreases by about 50% during the first 2 to 3 hours after fetal separation due to autolysis of placental tissue (Mellor,1983). Efforts were made to collect and freeze the placentas used in the present study as soon as possible after they were delivered. Even so, the amount of time between their delivery, collection and freezing varied and would have resulted in differing degrees of autolysis and therefore weight loss of each placenta. At the time of dissection differences in the 'freshness' of each placenta were apparent.

Different Placental Types

As already indicated by placentome numbers, type A and B placentas are likely to be from bicornuate pregnancies and types E and F from increasingly unicornuate pregnancies. Studies of the development of the fetal membranes lend further support to the concept that the different placental types described result from the varying degrees of proximity of adjacent embryos (Williams *et al.*,1963; Hafez and Rajakoski, 1964; Mellor, 1969b). Thus, placentas with a high degree of vascular anastomosis (and lower placentome numbers) are likely to be derived from unicornuate pregnancies, and vice versa.

In Type A placentas there was a complete absence of chorionic fusion. Three of the fifty-seven twin placentas examined by Lillie (1922) were apparently of this type. Type B placentas were likely to result from fusion of adjacent chorions with little or no contact or fusion of allantoic membranes (Hafez and Rajakoski,1964; Mellor,1969b). Again Lillie (1922) referred to five twin placentas which had a single chorion but were united by a narrow connection or strand. The diameters of the connection between the two placentas were not recorded in the present investigation. The increasing degree of vascular anastomosis of types C to F would be likely to reflect increasing contact and area of apposition of adjacent allantoic membranes (Hafez and Rajakoski,1964).

Variation in the Number of Umbilical Blood Vessels

Normally single bovine placentas appear to have two umbilical arteries and two umbilical veins or sometimes one vein. Of the seven type A placentas described, six (86%) had two umbilical arteries and two veins, and one had two arteries and a single vein. The H-junction joining the umbilical arteries of some placentas has previously been noted by Lillie (1922).

In the joined placentas (types B-F) a total of 14 sets of umbilical vessels were described. Of these, twelve (86%) had two umbilical arteries and veins each, one had two arteries and a single vein and one had one artery and one vein. No obvious difference in calf weights were seen in the placentas that had one normal set of umbilical vessels (two arteries and two veins) and the other with two arteries and a single vein. Examination of the placentas from the calves of cow #226 showed that one had a normal set of umbilical vessels and the other had a single umbilical artery and a single umbilical vein. These calves weighed 37.5 kg and 28 kg. Nevertheless, there was little difference in the number of placentomes or the weights of the two sides of this placenta. As each umbilical artery normally each umbilical artery supplies a different area of the placenta, it is probable that the calf with one umbilical artery had a reduced arterial blood supply as reflected by the difference in birth weights between the twins. However, this cannot be confirmed as we do not know which calf came from which set of umbilical vessels.

Variation in the number of umbilical vessels has previously been reported in humans. Human umbilical cords usually have two arteries and one vein, but the absence of one umbilical artery is observed in 1% of umbilical cords of singletons (Sisson, 1978). This incidence increases to 7.2% in twin placentas (Sisson,1978). Sisson (1978) speculated that the absence of one umbilical artery may be evidence of disturbed placental growth. The presence of a single vein and artery in the umbilical cord of one of the twin calves observed here may also be evidence of a disruption in the pattern of placental growth.

4.4.3 Packed Cell Volume

At birth the mean PCVs of single and twin calves were similar to those of single beef calves (Adams *et al.*,1992; Adams *et al.*,1993). Contrary to the findings of the present study, the PCVs at birth of twin beef calves reported by Adams *et al.*,(1993) were considerably lower than those of single beef calves at birth. It is possible that this difference arose from the small number of twin calves compared to single calves (8 vs. 30 calves) sampled by Adams *et al.*,(1993).

Between birth and 24 hours of age the PCVs of all calves decreased by between 1 and 20%. It has been suggested that this decrease is due to two contributing factors:

- absorption of the fluid components of colostrum which causes haemodilution by increasing the plasma volume (Adams *et al.*,1993),
- a decrease in sympathetic-adrenomedullary activity after paturition leading to splenic uptake of red blood cells.

By 24 hours the mean PCVs of both twin and single calves observed here were similar to values reported for single beef calves and greater than those reported for twin beef calves (Adams *et al.*,1992; Adams *et al.*,1993). They were also similar to those reported by Kume and Tanabe (1994) for twin dairy calves from multiparous dams, but lower than those of single calves from multiparous dams.

The Effect of Twinning on Packed Cell Volumes

Differences in the PCVs of co-twins were also reported in dairy calves during the 'first day of life' (Tennant *et al.*,1975). While Adams *et al.*,(1993) found no such difference between co-twins at birth a difference had become apparent by the time the twins were

24 hours of age. Adams *et al.*,(1993) found that they could not explain this disparity by the gender or order of delivery of individual calves. They suggested that "many subtle physiological processes important to the newborn's adaption to extra-uterine life occur differently in twin than single born calves" (Randall, 1978; Adams *et al.*,1993).

Dividing the twin sets on the basis of their placental type allowed the possible effects of vascular anastomosis on haemodilution to be explored. The small difference in PCV decrease between twins with placentas having no apparent vascular anastomosis (types A and B) suggested that the extent of haemodilution in each twin was similar. In contrast the existence of a difference in postnatal PCV decrease between co-twins having placentas exhibiting vascular anastomosis suggests that the extent of postnatal haemodilution in co-twins differed.

Possible Causes of the Disparity in PCV Decrease Between Co-twins

Possible causes of the disparity in PCV decrease between co-twins are as follows:

- differences in the volumes of colostrum consumed between co-twins,
- greater splenic contraction in one of a pair of twins, or
- blood movement within the shared circulation of fused placentas (Adams *et al.*,1993).

While it is possible that the volume of colostrum consumed between birth and 24 hours by co-twins differed, it is not likely that this difference would have occurred only between twins with placentas exhibiting significant vascular anastomosis (types C, D, E, F) and not in twin pairs with separate or nearly separate placental vasculatures (types A and B).

Disproportionate PCV decreases may also arise from splenic contraction. Splenic contraction occurs in response to sympathetic stimulation and causes the release of red blood cells into the circulation (Renkin and Michel, 1984). If splenic contraction occurred more in one twin than in the other, this may cause differences in PCV decrease. However as with the previous argument it is unlikely that this would occur only in the twins having placentas exhibiting vascular anastomosis, and not in the others.

Given that larger disparities in PCV decrease occurred between twins with placentas having higher degrees of placental vascular anastomosis, the blood movement allowed by such vascular anastomosis could contribute to the apparent differences in postnatal haemodilution that occur between these twins.

4.4.4 Possible Blood Transfer Between Twins with Fused Placental Vasculatures

Transfer of blood from the placental vasculature of the first-born twin into the feto-placental circulation of the second-born twin, or loss of blood from the placental vasculature of the second-born represent theoretical explanations of some of the PCV changes observed here. The calculations conducted indicate that the magnitudes of such net blood gains or losses by the second-born twin in order to explain the different post-natal PCV changes in co-twins would be well within the volume limits of the feto-placental vascular system.

The placental blood volume is effectively surplus to requirements after rupture of the umbilical cord. Thus, it is seen as an advantage if the placental and umbilical blood is propelled into the newborn, but the newborn is not apparently harmed if this does not occur. All the estimated volumes of blood that were transferred or lost were less than the respective placental blood volumes of the calf from which loss occurred. Even if the volumes of blood lost had been under estimated by 20%, they would have still been less than the estimated placental blood volumes, so that the blood volumes of the calves themselves would not have been depleted.

Although placental vascular anastomosis is more common in cattle it does occur between the placentas of monochorional human twins (Sisson,1978). Transfer of blood between the feto-placental circulations of human twins (known as fetofetal transfusion or twin transfusion syndrome) has been noted as causing differences in PCV at birth (Sisson, 1978).

4.4.5 Type E Placentas

According to the previously noted calculations three of the second-born twins from type E placentas probably experienced loss of blood, while in the remaining two sets the

second-born twins probably gained blood. In the three (#259, 297, 294) in which blood loss was hypothesized to have occurred the umbilical vessels of one calf had torn at the end of the connecting vessels within the allantochorion (Fig 4.12) rather than within the umbilical cord (Fig 4.11). If the torn connecting vessels did not constrict as effectively as the umbilical vessels, blood may have been lost via them into the uterine cavity after they were torn with the birth of the first twin. Support for this idea lies in the observation that blood loss rather than transfer possibly occurred in three of the four type E placentas with ruptured connecting vessels.

The second-born calf of the remaining type E placenta (#123) with torn connecting vessels was estimated to have gained 10% of the placental blood from its co-twin. While this is not what would have been anticipated, it is noteworthy that unlike the three cases mentioned previously, this placenta was decaying at the time it was dissected and this may have affected the description made of this placenta at the time it was dissected.

The second-born calf of the type E placenta in which the connecting vessels were intact (#260) was estimated to have gained 25% of its co-twin's placental blood volume, supporting the idea that the large diameter connecting vessels allow blood transfer between co-twins.

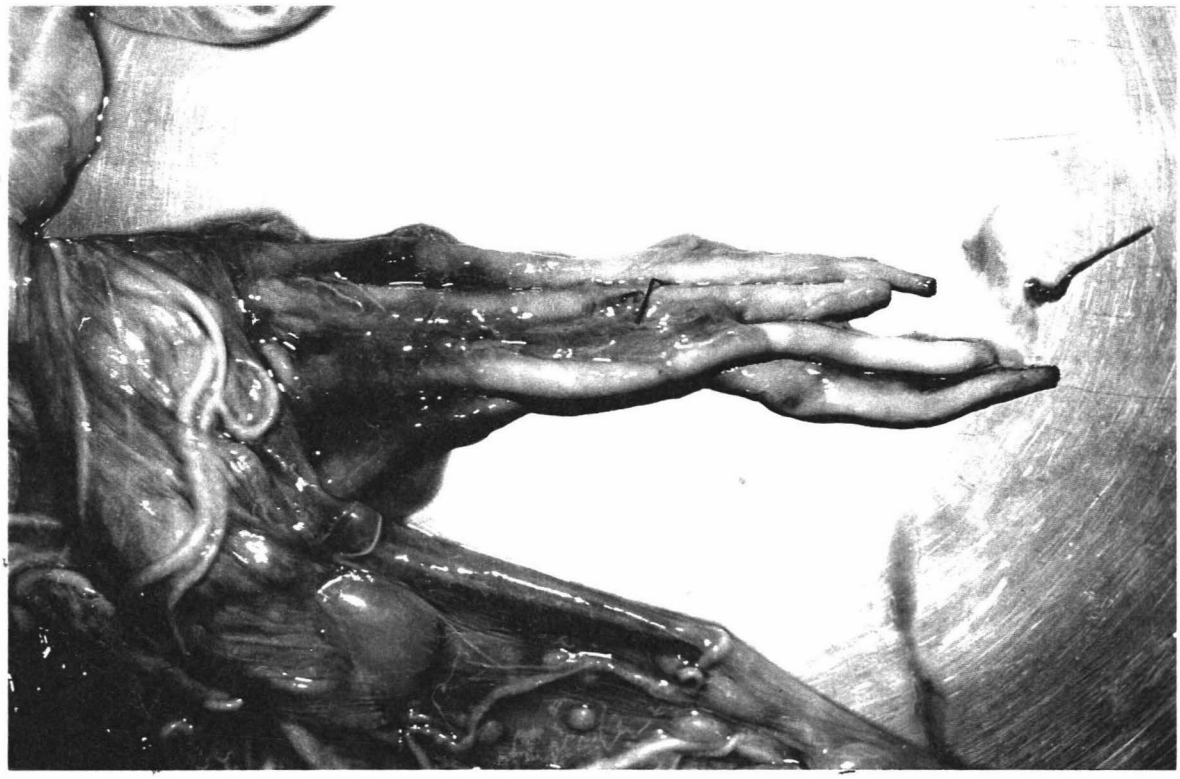

Fig 4.11: A normal set of umbilical vessels. Two umbilical arteries and two umbilical veins - all constricted.

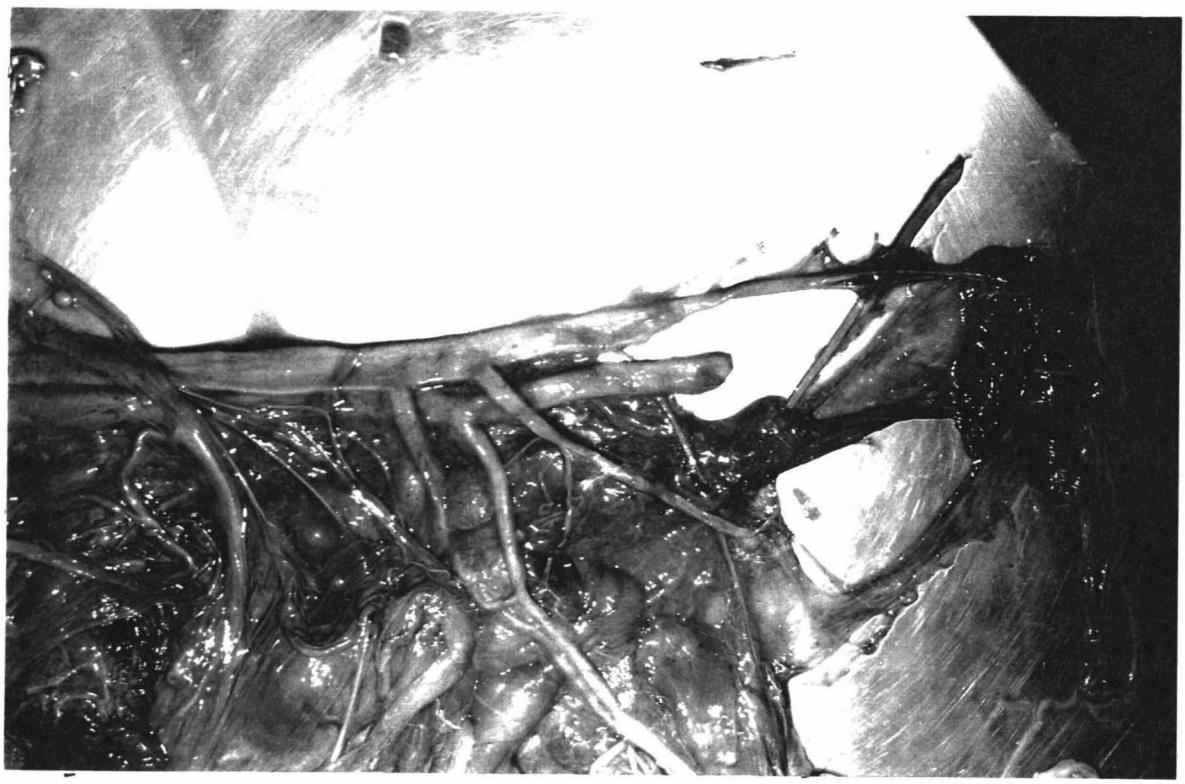

Fig 4.12: The ruptured large diameter connecting vessels of a Type E placental set. Note that the vessels are not apparently constricted.

4.5 General Discussion

This study has shown that fusion of the fetal membranes of twin calves allows anastomosis of placental blood vessels. Gross anatomical differences in the degree of membrane fusion and vascular anastomosis can be used to separate twin placentas into six categories. The PCV decrease that occurs between birth and 24 hours in neonatal calves often differs between co-twins if they are from placentas exhibiting a high degree of vascular anastomosis. Differences in those postnatal PCV decrease between twin calves may be due to the net movement of blood within the joined placental vasculature after the birth of the first-born twin.

Strength would have been added to the results of present study if the it had been known which twin came from which placenta in each placental set. The hypothesis that differences in the postnatal PCV decrease were due to blood flow from one placenta into the other was supported by estimation of blood volumes and the degree of vascular anastomosis. The validity of this hypothesis would be increased if it could be shown experimentally that net blood movement within the placental vasculature does occur during the birth of twins.

4.5.1 Future Research

Future research in this area could include:

- Packed cell volume measurements from both calves made regularly before, during and after birth to assess gradual changes in blood volume before during and after birth.
- Latex casts of the placental vasculature of fused placentas to confirm the presence of anastomosis between the arterial and venous circulations of fused placentas.
- Dye or marker injection like those performed by Lillie (1922) and Mellor (1969b) into the placental vessels of different placental types during the prenatal period to confirm the degree of anastomosis and determine the effect of anastomosis on the movement of blood within the fused placentas.
- Compare placentome weight to calf weight in twin births to confirm whether a relationship between these parameters exists in twin calves.

- Measure changes in cortisol concentration preceding birth of twin sets. Relate these to placental type and assess whether the degree of placental vascular anastomosis influences the pre-partum cortisol surge in twin calves.

Chapter Five:

Immunoglobulin Transfer in Neonatal Dairy Calves

Abstract

In the present study it was found that failure of passive transfer of colostral immunoglobulins as indicated by low serum gamma glutamyl transferase activities (below 200 U/l) occurred in approximately 45% of the calves studied. Calves that had not received colostrum were not easily distinguished from those that had on the basis of obvious physical or behavioural features. The amount of time that calves spent with their dams before being separated ranged from 74 to 1492 minutes. Thirty-three per cent of calves had not sucked within this time. Of the calves that did suck, 79% did so within 6 hours of their birth.

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Chapter 5: Immunoglobulin Transfer in Neonatal Dairy Calves

5.1 Introduction

At birth, calves move from the uterus into an environment containing pathogenic organisms. Calves do not receive maternal immunoglobulins (Igs) through intrauterine placental transfer (Besser and Gay.,1994) and do not produce their own Igs until they are exposed to antigenic stimuli. Thus, newborn calves are very susceptible to disease. Colostrum, the milk produced by the newly calved cow, is rich in immunoglobulins and for the first few weeks of life calves depend on the initial feeds of colostrum to provide them with protection from disease (Michanek and Ventorp,1989; Wedasingha,1992). Those that do not acquire passive immunity through Ig transfer from colostrum have low serum Ig levels and are about 9.5 times more likely to die prior to weaning than clinically normal calves that get colostrum (Perino *et al.*,1993).

5.1.1 The Role of Immunoglobulins in Disease Prevention in Young Calves

Common neonatal calf diseases associated with low Ig levels include omphalophlebitis, colisepticemia, enteritis and respiratory disease (Selman *et al.*,1970a; Besser and Gay, 1994). Of these, enteric and respiratory diseases are the two main hazards to calf health (Wedasingha,1992). Colostral immunoglobulins appear to have both systemic and local effects in reducing the incidence of these diseases. High serum concentrations of IgG and IgA are associated with reduced susceptability to enteric and respiratory diseases (Wedasingha,1992).

It is thought that reduced susceptibility to enteric diseases is provided by immunoglobulins active within the intestines (Wedasingha,1992). Viruses that cause diarrhoea (rotavirus and coronavirus) replicate within the intestinal epithelial cells and *E.coli* bacteria adhere to epithelial sufaces where they produce enterotoxins (Wedasingha,1992). Immunoglobulins within the intestines compete with micro-

organisms to adhere to intestinal epithelia suppressing their multiplication and the subsequent development of disease (Wedasingha,1992). Kearns *et al.*(1983) found diarrhoea on 72% of New Zealand dairy farms and these causing more than 50% of the total calf mortality.

5.1.2 Low Serum Immunoglobulin Concentrations

Immunoglobulin concentration in young calves has been reported in both European and American studies (Mohammed *et al.*,1991; Perino *et al.*,1993; Rea *et al.*,1996). Low serum immunoglobulin levels (less than 15 Zinc Sulphate Turbidity units) were reported in 34% of Friesian calves studied by Fallon and Hart (1987) in Ireland. In American studies the prevalence of comparably low serum immunoglobulin concentrations was reported as being 12.6-35% by Rea *et al.*(1996) and as 21% by Perino *et al.*(1996).

The attainment of an immune status sufficient to provide protection from disease is dependent on the uptake of adequate quantities of Igs. The three main variables affecting Ig uptake are the concentration of Igs in the colostrum which is consumed, the volume colostrum consumed and the degree of immunoglobulin absorption from the calf's intestine (Stott *et al.*,1979ab; MacKenzie, 1984; Ventorp and Michanek, 1991; Wedasingha, 1992).

5.1.3 Factors Affecting Colostral Immunoglobulin Concentration

During the 2-4 weeks prior to parturition Igs are transferred to colostrum from the blood. Their concentration in colostrum is assumed to be dependent on their concentration in the cow's blood (MacKenzie,1984). Immunoglobulins may be taken up by mammary glandular epithelial cells from the interstitial fluid or be synthesized by plasma cells within the gland (MacKenzie, 1984; Aldridge *et al.*, 1992).

The concentration of immunoglobulins in colostrum is influenced by a cow's breed, her number of previous lactations, body condition and the time after parturition (MacKenzie, 1984; Fallon and Harte,1987; Odde,1988; Mohammed *et al.*,1991). In both New Zealand and the USA, Jerseys have been shown to produce a smaller volume of colostrum with a higher Ig concentration than Friesians (MacKenzie,1984). The

amount of colostrum produced in a lactation increases up to the third or fourth lactation, due perhaps to continued antigenic stimulation (MacKenzie,1984; Aldridge *et al.*, 1991). However older cows have a larger mammary capacity with an associated increase in the number of functional secretory cells and greater efficiency of the Ig transport mechanism (Aldridge *et al.*, 1991). The colostrum yield of a cow and her daughters is positively correlated (MacKenzie,1984). A variation in colostrum yield of up to three fold has been shown between quarters of the same cow (MacKenzie,1984).

The body condition of the cow also affects colostrum yield. An extended period of restricted feed intake resulting in reduced body condition has been shown to reduce colostrum yield as a result of serum protein catabolism (Logan,1977; Odde,1988). This results in less Igs being available for transfer from the serum to the mammary gland and colostrum. Beef cows which lost 25% of their weight over the last months of pregnancy produced 50% less colostrum, but the concentration of Igs was not affected (Logan,1977).

Colostral Ig concentrations are at their maximum at the time of birth and remain constant for the next nine hours provided milk is not removed from the gland (MacKenzie,1984). After this time the concentration of Igs gradually decreases whether or not milk is removed (Edwards *et al.*,1982). When milking occurs the onset of copious milk secretion is stimulated and the immunoglobulin concentrations decrease rapidly.

5.1.4 Absorption of Colostral Immunoglobulins

To be fully effective immunoglobulins must be absorbed intact (MacKenzie,1984). Absorption of macromolecules such as immunoglobulins occurs through pinocytosis. Igs are taken up from colostrum in the calf's intestinal lumen into vesicles within the epithelium. The contents of the vesicles are then released into the interstitial space where they are transported by way of the lymphatic system into the systemic circulation (MacKenzie,1984).

The degree to which immunoglobulins are absorbed is affected by the age of the calf at first feeding and the mass of Ig ingested (Aldridge *et al.*, 1992). From the time of birth the intestines ability to absorb immunoglobulins decreases eventually reaching zero (Edwards *et al.*,1982). This process is called closure. By 9 hours of age the calf absorbs only half the Ig it would 8 hours earlier (Besser and Gay, 1994). Ig absorptive capacity declines at a progressively greater rate from 12 hours after birth, until closure is complete between 24 and 48 hours *post partum* (Stott *et al.*,1979ab; Michanek and Ventorp, 1989). If colostrum feeding is delayed closure is also delayed up to the time of spontaneous closure about 24 hours after birth (Stott *et al.*,1979ab). Once complete, the calf is no longer able to absorb Igs. Initially intestinal epithelial cells lose the ability to release the Igs into the interstitial space, and later the ability to take up proteins from the lumen is lost (MacKenzie,1984).

5.1.5 Transmission of Immunoglobulins

The absorption of immunoglobulins and attainment of an adequate passive immunity by the calf is affected by the time between birth and first sucking. Michanek and Ventorp (1989) showed that there was no difference in Ig transmission when calves received their first feed between 1 and 24 hours after birth. However those calves which received their first feed closer to birth, transmitted significantly more macromolecules on their second, third and fourth feeds. Under normal conditions, intestinal closure is initiated at the first substantial feed of colostrum (Aldridge *et al.*,1992). This is supported by Stott *et al.* (1979a) who showed that calves fed colostrum shortly after birth exhibited earlier closure.

Serum immunoglobulin concentrations are directly related to the mass of Ig ingested in the first feed (Aldridge *et al.*,1992). A large mass of Ig may be consumed through the intake of:

- a large volume of colostrum with an average Ig concentration; or
- a moderate volume of colostrum with a high Ig concentration.

Transmission of Ig is linearly related to concentration. If the same total amount of Ig is given in different volumes of colostrum, the calves fed smaller volumes of colostrum

with higher concentrations of immunoglobulins will have better transmission (Stott and Fellah, 1983). Calves which receive a greater immunoglobulin mass during the first feed will have a higher serum Ig concentration after closure (Aldridge *et al.*,1992).

5.1.6 The Effect of Cow and Calf Behaviour on Colostrum Intake

Both the Ig concentration in colostrum and the intestine's ability to absorb Igs decrease from the time after birth. Therefore the length of time between birth and sucking has a considerable effect on a calf's ability to acquire adequate passive immunity (Edwards *et al.*,1982; Ventorp and Michanek,1991). The time between birth and sucking varies greatly between calves. In a study of 21 Swedish Holstein cows and their calves Ventorp and Michanek (1991) found that it ranged between 50 minutes and 11 hours, 44 minutes.

Initiation of sucking and colostrum intake is the result of a series of behavioural interactions between a calf and its dam during the early postnatal period. After the onset of breathing the calf usually stands up and begins to search for a teat. Sucking success depends on the calf finding its mother and then locating the udder and teats (Ventorp and Michanek,1991). Delays in sucking are often due to a breakdown in the calf-cow interaction (MacKenzie,1984). They may be due to an inability to carry out the required sequence of postnatal interactions on the part of the cow or the calf. Edwards and Broom (1982) found that the incidence of licking and other maternal behaviour depends upon the psychological state of the mother, and even vigourous teat seeking cannot lead to sucking unless the dam stands to permit it (Edwards,1982).

Factors Influencing Calf Behaviour

Postnatal calf behaviour may be affected by breed, calf maturity, placental insufficiency, hypothermia, dystocia, hypoxia and environmental conditions (MacKenzie,1984; Mellor, 1988; Ventorp and Michanek,1991; Aldridge *et al.*,1992; Balle, 1997). Ventorp and Michanek (1991) found that the calves which were active earlier were usually earlier to suck. This is supported by Edwards and Broom (1979) who found a positive correlation between time from birth to first standing and time to first sucking. Any factor which causes poor calf vigour or predisposes it to weakness within the first 24

hours is likely to impede passive Ig transfer by increasing the time between birth and first sucking, or decreasing the volume of colostrum consumed (Edwards,1982; MacKenzie,1984; Aldridge *et al.*,1991).

Beef calves tend to stand and suck earlier than dairy calves (Selman *et al.*,1970b). Prematurity and immaturity may adversely affect a calf's coordination and consequently its ability to stand, walk, locate the teats and suck (Aldridge *et al.*,1992; see Chapter 2). Poor calf vigour is common after a difficult or prolonged delivery (Edwards,1982). Along with colostral Ig concentration and a decreasing ability of the calf's intestine to transfer Igs, cows show a marked decrease in calf-directed activities with time after parturition (Edwards and Broom,1982). Activity of the calf stimulates licking by the dam (Selman *et al.*,1970ab) and thus calf hypoactivity may reduce the attractiveness of the calf. Environmental temperature also affects the intensity of teat seeking, the time to standing and therefore the time to first sucking. Calves stand sooner and are more intense in their teat seeking when the air temperature is higher (within a range of -5°C to 25°C)(Edwards,1982).

Factors Influencing Maternal Behaviour

Delays in the onset of maternal behaviour may be the result of the cow being exhausted after a difficult or prolonged labour, or she may be affected by a poor environment or lack of maternal instinct (Ventorp and Michanek,1991). Maternal behaviour involves attraction to the newborn calf, receptivity and licking directed toward it and acceptance of the calf at the udder. Experience affects the cow's reaction to the calf. Hafez (1964) stated that nursing behaviour in older animals is facilitated by the reflexes conditioned during previous lactations and in primiparous animals is inhibited by the pain and shock of parturition. In support of this Edwards (1983) showed that cows are more permissive of sucking than heifers, with heifers also showing a higher incidence of abnormal maternal behaviour (Edwards and Broom,1982). However Edwards, (1982) found that delays to first sucking became more common as the parity of the dam increased.

Dairy cows have been selected for weak maternal bonding and docility during milking (Edwards,1983). They must let down milk in the absence of the calf. Given that calves are reared separately and are removed soon after birth, dairy cows are required to display minimum maternal aggression in response to the calf being handled (Edwards and Broom,1982). Selman *et al.* (1970a) comparing the behaviour of beef cows, dairy cows and dairy heifers, they found that beef cows licked their calves immediately after birth and continued to do so for longer than either dairy cows or heifers. Beef cows were also observed to accept their calf's teat seeking advances sooner than the other dams.

5.1.7 The Effect of Udder Conformation on First Sucking

The modern day dairy cow has been bred for specific characteristics resulting in changes in both its physical appearance and behaviour (Michanek and Ventorp,1991). These changes are evident when comparisons are made between dairy and beef breeds. As dairy cows have been bred for milk production the udder has grown disproportionately to the rest of the body. Edwards (1982) stated that the most important factor determining the time to first sucking was the udder conformation of the dam. Poor udder and/or teat conformation are thought to impair the calf's teat seeking success. The udder of an older dairy cow becomes pendulous as the mammary tissue bulk increases and suspensory ligaments become stretched. The teats hang further away from the cows abdomen and closer to the ground. Ventorp and Michanek (1992) found that a smaller distance from the udder to the ground led to increased variation and a significant difference in the time spent teat seeking and it also had a significant effect on the time to first sucking. It is likely that the compact udder of a beef cow facilitates the calf in sucking (Fallon and Harte, 1987) and thus decreases the time between birth and first sucking, with the relatively larger udder of the dairy cow hindering teat seeking efforts.

5.1.8 Measurement of a Calf's Immune Status

Gamma glutamyl transferase (GGT) is a membrane associated protein involved in amino acid transport (Thompson and Pauli,1981). In secretion products such as colostrum and milk there are high GGT activities. Thompson and Pauli (1981) found that the serum GGT activities of calves that had consumed colostrum were over 60 times higher than those found in calves that had not sucked. They suggested that GGT

was absorbed at the same time and through the same mechanisms as IgG and as a result serum GGT activity is a reliable indicator of whether a calf has consumed colostrum and thus acquired an immune status sufficient to protect it from neonatal disease (Thompson and Pauli,1981; Braun *et al.*,1982; Perino *et al.*,1993; Vermunt *et al.*, 1995). Perino *et al.* (1993) suggested that those calves with a serum GGT activity of 200U/l or greater were 9.5 times less likely to become sick or die than calves with lower GGT activities.

5.1.9 About the Present Study

Although many studies have examined various aspects of colostrum intake and cow-calf behaviour in the early postnatal period (Selman *et al.*,1970ab; Edwards and Broom,1979; Stott *et al.*,1979abc; Edwards,1982; Edwards and Broom,1982; Edwards *et al.*,1992; Edwards 1983; Michanek and Ventorp,1989; Ventorp and Michanek, 1991; Ventorp and Michanek, 1992; Spinka and Illman,1992; Illman and Spinka,1993), to our knowledge all have been conducted in countries other than New Zealand and under conditions very different from those which are experienced in this country. In these countries dairy calves were born indoors, under shelter and in either individual or grouped pens. Cows are frequently over-wintered in barns and fed on grains.

New Zealand dairy farming is pastorally based, seasonal and semi-intensive. Cows feed mainly on grass, supplemented if need be by hay or silage and rarely concentrates. Cows are rotated from one paddock to the next according to their feed requirements and the availability of grass. During calving in late winter and spring, those cows in which calving is imminent may be drafted off from the main herd and run separately. In New Zealand calves are most commonly born outside, in the paddock or on a loafing pad.

A previous study by Vermunt *et al.* (1995) demonstrated that a large percentage of New Zealand dairy calves may not receive adequate colostrum. In the present study it was hoped that by observing calves and their dams immediately after parturition, the potential influence of the behaviour of both calf and cow on the time to first sucking and the calf's colostrum intake may be examined. Observations and measurements of various physical parameters at the time the calves were removed from their dams were

anticipated to identify characteristics which may be of use as practical indicators of those calves which are likely to have low serum Ig levels.

5.2 Materials and Methods

This study was divided into two parts:

Part A

Blood samples for GGT analysis were taken from 57 dairy calves at the time they were separated from the cows (at 'pick-up'). At the time of sampling, several physiological and behavioural parameters were recorded with a view to determining if some measurements might be identified that would indicate which calves had not sucked.

Part B

Observations were made of the behavioural interactions of 29 heifers and cows, and their calves, from birth until the time the calf was removed by the farmer ('pick-up'). At this time blood samples were taken and subsequently analysed for their GGT activities. Comparisons were made between the GGT activities and the sucking-related behaviour of the calves, with reference to the effect of cow-calf interactions on these values. Although 29 sets of data were recorded some were incomplete, leaving 21 complete data sets for evaluation.

5.2.1 Animals

The animals used for this study were purebred Friesian cows and heifers and their calves, bred and raised on Massey University farms. Of the calves in Part A 61% were male and of those born and observed for Part B 50 % were male. In part B seven heifers and fourteen cows were observed.

5.2.2 Observations

All observations were carried out on Dairy Farm 4 at Massey University.

5.2.3 Part A: Method

The newly born calves were separated from their dams by farm staff at mid-morning each day. Therefore the amount of time they spent with their dam before being separated depended on the time of day that they were born. Calves born in the afternoon would not be separated from their dams until the following morning. Those born in the morning would be likely to spend only a short time with their dam. Because of this,

most calves would have been less than 24 hours of age at the time they were separated from the cow and moved to rearing sheds. When the calves were placed in the sheds blood samples were taken by jugular venepuncture into 10 ml plain vacutainers, for GGT analysis. At this time several measurements were made to describe the physiological and behavioural state of each calf. The following parameters were recorded:

1) Gender.

2) Rectal temperature.

3) Skinfold thickness.

4) Coat condition - wet or dry.

5) Hoof membranes- whether or not the membranes were worn.

6) State of the umbilical cord remnant - wet, dry or shriveled.

7) Belly fill - subjectively assessed and recorded as: 1 = hollow flanks,

2 = straight flanks,

3 = rounded flanks.

8) Gum colour - pink, red, purple or a combination of these descriptors.

9) Teeth - the number of deciduous incisors that had erupted and the number palpable.

10) Sucking reflex - response to two fingers placed in the mouth recorded as present or absent.

11) Nose - recorded as wet or dry, warm or cool to the touch.

12) Eyes - dull or bright.

13) Eyelids - open, half open or closed.

14) Response to pain - whether or not the calf responded to the painful stimulus of a gentle tail twist.

15) Vocalisation - whether or not the calf vocalised during the process of taking a blood sample.

16) General vigour - recorded as 1 = lying flat, no resistance to handling

2 = mild resistance to handling

3 = strong resistance to handling

5.2.4 Part B: Method

Observations of periparturient cows and their calves were made in five 24 hour cycles starting on the 26th, 27th and 28th of July and the 3rd and 4th of August 1995. Each 24 hour cycle was divided into four consecutive 6 hour shifts, with all observations within each shift being conducted by one of four observers.

An aim of this study was to observe cow-calf interactions and behaviour under 'normal' farming conditions in New Zealand. As a result, the site from which observations were made was dependent on the requirements and therefore location of the periparturient herd. The herd was moved depending on the weather and the state of the paddocks. Cows are moved off wet paddocks in an attempt to minimise pugging and damage to the paddocks. Because the paddocks were wet and the ground soft during the most of the study, four of the 24 hour observation periods were made when the herd was on a sheltered, bark-lined loafing pad (Table 5.1). For the remaining observation period the cows and their calves were in a flat paddock. The observer either stood near the area enclosing the cows, or sat in a vehicle at the point with greatest visibility. It is not thought that the presence of an observer would have altered their behaviour significantly as the cows were well accustomed to people.

The behaviour of both the cow and calf was recorded from the time the calf's feet appeared at the cow's vulva until the calf was separated from the cow at pick-up. As a result the total observation period for different cow-calf units varied between 74 minutes and 24 hours. Key events and observations were defined before the study commenced. The nature and time of each behavioural change (e.g. from lying to

standing, start or stop sucking) was recorded on lined paper. Weather conditions were described and air temperature recorded at the time of birth of each calf and at hourly intervals.

Definitions

Birth — the entire calf passed out of the cow.

Standing — the bases of all four hooves were in contact with the ground supporting the calf's weight for at least 1 minute.

Teat seeking — the calf nuzzling and sucking at surfaces, usually along the cow's body.

Sucking — the action of the calf while obtaining milk. The calf has a teat in its mouth and is seen to be both sucking and swallowing. Milk foam may be seen at the mouth.

Udder conformation

1 = teat tips are above the cow's hock,

2 = teat tips are level with the cows hock,

3 = teat tips are below the cow's hock.

5.2.5 Blood Sampling and Serum GGT analysis

Blood samples were taken by venepuncture of either jugular vein into plain 10 ml vacutainers. Each calf was restrained and sampled by the same person. Blood samples were centrifuged, the serum was separated, and serum GGT activity was measured using a Cobas-Mira Analyser (Perino *et al.*,1993; Vermunt *et al.*,1995) on the day of collection.

5.2.6 Statistics

The data were analysed and described in terms of the mean and standard error of the mean (SEM) using Microsoft Excel V 5.0 (Microsoft Corporation, USA). Possible correlations between GGT activity of serum samples taken and the physical and behavioural parameters as well as between the different behaviours measured were analysed by linear regression (Microsoft Excel V 5.0)

5.3 Results

Part A

5.3.1 GGT Activities of Serum Samples Taken From Neonatal Calves at Pick-Up

The range of GGT levels of the 57 calves was 5 to 5560 U/l with a mean value of 1257 ± 199 U/l. Twenty- four calves (42%) had values below 200 U/l (Appendix 5.1).

5.3.2 Correlations With Other Parameters Measured

Belly fill showed a small but significant correlation with GGT activity ($r = 0.290$, $P < 0.05$). The flanks of calves with higher GGT activities tended to appear either straight or rounded rather than hollow.

There were no obvious relationships between GGT activity and gender, rectal temperature, skinfold thickness, coat condition, the presence of hoof membranes, state of the umbilical cord remnant, gum colour, tooth number, presence of the sucking reflex, nose condition, eye or eyelid state, response to pain, vocalisation or general vigour.

Part B

5.3.3 Conditions of Study

Weather conditions during the study varied from sunny and still to gusty wind and moderate rain. Air temperatures ranged from 4°C to 10°C (Table 5.1).

5.3.4 Time from Birth to 'Pick Up'

The period over which observations were made (birth until pick-up) ranged between 74 (1 hour 14 minutes) and 1492 minutes (24 hours and 54 minutes) with an average duration of 806 ± 89 minutes (13 hours and 26 minutes) (Table 5.1).

Table 5.1: General data and environmental conditions at the time of birth.

Cow -calf ID	Cow Age (years)	Udder Conformation	Calf Gender	Obs Time (mins)	Time of Birth (hours)*	Air Temp (°C)	Wind Strength	Calving Site
1-4	4	u	Male	194	1460	10	xx	LP
1-8	2	1	Male	593	0337	5	x	LP
2-1	5	u	Male	1258	1650	5	x	LP
2-2	2	1	Female	1227	1721	10	xx	LP
2-3	4	2	Female	1227	1818	10	x	LP
2-4	5	u	Female	1198	1750	7		LP
2-5	2	u	Male	947	2201	6	xx	LP
2-6	2	3	Female	638	0310	5	xx	LP
2-7	5	u	Female	349	0659	4	xx	LP
2-8	5	1	unknown	288	0900	4		LP
3-1	2	2	Male	1140	1600	9	xx	LP
3-3	2	1	Female	1057	1823	7	xx	LP
3-5	8	3	Male	74	0944	u		LP
4-1	6	2	Female	1492	1138	10	x	P
4-2	5	2	Female	693	1411	9	xx	P
4-3	2	1	Male	1192	1633	8	xxx	P
4-4	3	1	Female	1017	1733	7	xx	P
5-1	5	2	Female	756	2117	7	x	LP
5-2	5	3	Male	623	2330	6	xx	LP
5-3	4	u	Male	495	0038	8	x	LP
5-4	6	u	Male	466	0660	7	xx	LP

1 = teat tips above hock, 2 = teat tips level with hock, 3 = teat tips below hocks, u = unknown;* = 24 hour clock; x = light wind; xx = moderate wind; xxx = strong wind; LP = loafing pad, P = Paddock.

5.3.5 Calf Behaviour

Time from Birth to Standing

The mean time from birth to standing was 93 ± 26 minutes (1 hour 33 minutes) (Table 5.3). Calves born during the colder conditions of night tended to take longer to stand (Fig 5.1), however the correlation between the environmental temperature at the time of birth and the time between birth and standing was not significant. The calves born to heifers tended to take longer to stand than those born to cows (P = 0.076) and there was a mild negative correlation between cow age and the time to first standing (r=-0.428, n=19),however neither of these statistics were significant.

Sucking Behaviour

Of the 21 calves observed from birth, seven (33%) were not observed to have sucked by the time they were removed from the cows at pick-up (Tables 5.2 and 5.3). One of these calves (2-7) was separated from the cow (without sucking) within 6 hours of its birth (Tables 5.2 and 5.3). Two calves stayed with their dam for between 6 and 12 hours (2-6, 5-2) and the remaining four calves were separated after they had been with their dam for more than 12 hours (2-1, 3-3,4-3, 5-1,). The time between birth and first sucking was not influenced by udder conformation.

Table 5.2: The number of calves observed to suck in relation to the time between birth and pick up.

Time from Birth to 'Pick Up' (hours)	< 6	6-12	>12	Total
Number of Calves	4	6	11	21
Percentage (%)	19	29	52	100
Number of Calves Observed to have Sucked	3	4	7*	14
Number of calves not Observed to have Sucked	1	2	4	7

* = one calf was not observed to have sucked but had a GGT of 310 U/l indicating that it had.

Fourteen calves (66%) had sucked before being separated from their respective dams (Table 5.2 and 5.3). Of these eleven (52% of the total number of calves, 79% of the number that sucked before pick up) sucked within the first six hours after birth (Table

5.3). Two calves sucked between 6 and 12 hours of birth. The remaining calf sucked later than 12 hours after birth (Table 5.3).

The amount of time spent sucking ranged between 0 and 54 minutes with a mean value of 12 ± 3 minutes (Table 5.4). Of the calves that were observed to suck, all sucked for a total of at least 15 minutes and the mean time from birth to first sucking was 258 ± 83 minutes (4 hours and 18 minutes) with a range from 57 minutes to 19 hours, 4 minutes (Table 5.3). Calves born to heifers were observed to spend similar amounts of time sucking to those born older cows.

Table 5.3: Calf behaviour between birth and pick up

Observation Time (min)	Cow-calf ID Number	Time to stand (min)	Birth to sucking (min)	Total sucking time (min)	Serum GGT Activity (U/l)	Suck to sample (min)
74	3 5	66	66	4	u	10
194	1 4	18	188	6	u	4
288	2 8	15	160	5 *	11	n/a
349	2 7	0	n/a	0	7	n/a
466	5 4	75	102	22	17	300
495	5 3	54	412	22	1860	150
593	1 8	221	238	54	4310	150
623	5 2	229	180	0	23	n/a
638	2 6	195	n/a	0	10	n/a
693	4 2	17	38	20	1990	1073
756	5 1	22	n/a	0	15	n/a
947	2 5**	512	753	u	2550	60
1017	4 4	70	100	20	2310	990
1057	3 3	119	n/a	0	u	n/a
1140	3 1	99	405	15	u	735
1192	4 3	113	n/a	0	310	u
1198	2 4	49	1144	21*	9	n/a
1227	2 2	11	159	15	7380	720
1227	2 3	12	57	16	850	900
1258	2 1	34	n/a	0	12	n/a
1492	4 1	16	514	23	460	690

* = Sucking occurred after blood sample was taken

** = Dystocic birth

u = unknown

n/a = not applicable

5.3.6 GGT Activity

Serum GGT activity was measured in 17 calves and values ranged from 7 to 7380 U/l (Table 5.4). Eight calves had GGT activities below 200 U/l. Of these, five were not observed to have sucked, two sucked after the blood sample was taken (2-4, 2-8) and one was observed to suck, but did not show an increase in GGT level (5-4) (Table 5.4).

The amount of time calves spent sucking and the serum GGT activities attained showed a significant positive correlation ($r = 0.570$, $P < 0.05$; $n = 17$). Of the nine calves that had GGT activities greater than 200 U/l, eight were observed to suck for at least 15 minutes (Table 5.4). The remaining calf had a serum GGT activity of 310 U/l, but was not observed to have sucked. There were two periods of bad visibility due to darkness and rain while observations of this calf and cow pairing (4-3) were being made. During this time it is likely that the calf sucked without this being observed.

There was a positive correlation between the serum GGT activity of the calf at pick up and the age of its dam ($r = 0.577$, $P<0.05$, $n=16$).

Table 5.4: The amount of time spent calves spent sucking in relation to the age of the cow and the calf's serum GGT activity.

Calf #	Observed Time Spent Sucking (min)	GGT Activity (U/l)	Cow Age (years)
2-1	0	12	5
2-4	0	9	5
2-6	0	10	2
2-7	0	7	5
2-8	0	11	5
4-3	0	310*	2
5-1	0	15	5
5-2	0	23	5
2-2	15	7380	2
2-3	16	850	4
2-5	19	2550	2

4-2	20	1990	5
4-4	20	2310	3
5-3	22	1860	4
5-4	22	17*	6
4-1	23	460	6
1-8	54	4310	2

* = Unusual values

There was no correlation between the amount of time the calf spent sucking or the subsequent GGT activity, and the time from birth to standing, birth to first sucking, udder conformation, the proportion and amount of time either the calf or cow spent standing or the amount of time the calf spent teat seeking (Table 5.5).

Although the correlation between time from birth and first sucking and serum GGT activity was not significant, six of the eight calves with serum GGT activities of greater than 200 U/l first sucked within 6 hours of birth (Table 5.2). The remaining two calves with high GGT activities sucked between 6 and 12 hours of birth.

Table 5.5: Time spent sucking in relation to the proportion of the time between birth and pick up that the cow and calf spent in different behaviours

Total Suckling Time	Cow-calf ID	Cow Up %	Cow Down %	Calf Up %	Calf Down %	Teat Seeking %	Udder Conformation
0	2 1	35	65	23	77	12	u
0	2 6	84	16	62	38	17	3
0	2 7	20	80	0	100	0	u
0	3 3	37	63	18	82	3	1
0	4 3	25	75	17	83	1	1
0	5 1	18	82	71	29	13	2
0	5 2	100	0	58	42	5	3
4	3 5	30	70	14	86	3	3
5	2 8	92	8	95	5	48	1
6	1 4	35	65	35	65	11	u
15	2 2	43	57	31	69	7	1
15	3 1	47	53	37	63	4	2
16	2 3	48	52	18	82	7	2
20	4 2	70	30	61	39	19	2
20	4 4	97	3	61	39	4	1
21	2 4	66	34	85	15	17	u
22	5 3	71	29	83	17	11	u

22	5 4	100	0	72	28	11	u
23	4 1	83	17	63	37	7	2
54	1 8	87	13	39	61	3	1
u	2 5	35	65	14	86	2	u

u = unknown

5.3.7 General Observations of Cow Behaviour

All cows, except one that had a difficult labour (2-5), were seen to lick their calves soon after birth and all within 43 minutes. Twelve cows (57%) were seen to behave in a manner that facilitated sucking. These behaviours included moving the hind leg back to maximize access to the udder and licking the rear end of the calf as it approached the udder, thereby moving it towards the teats. In 15 (71%) cases other cows were seen to pay an active interest in the newborn calf.

5.4 Discussion

The major conclusions that arose from this study are as follows.

- Failure of passive transfer of immunoglobulins as indicated by low GGT levels occurred in approximately 45% of the calves studied (Parts A and B).
- Calves that had not received sufficient colostrum were not easy to distinguish from those that had on the basis of obvious physical or behavioural features.
- The time between birth and pick up was highly variable (ranging from 74 to 1492 minutes) and many calves (33%) had not sucked by the time they were separated from their dams.
- Of the calves that did suck, the majority (79%) did so within 6 hours of birth.
- Most calves that were observed to suck attained GGT levels greater than 200 U/l.
- All calves observed to suck did so for at least 15 minutes.
- There was no difference in the amount of time spent sucking of calves born to heifers compared to those born to older cows.

5.4.1 Serum GGT activities of Calves at Pick-Up

Approximately 45% (Part A - 42%, Part B - 47%) of the calves studied had serum GGT activities below the satisfactory level of 200 U/l as identified by Perino *et al.*(1993). This proportion is similar to that reported by Vermunt *et al.* (1995) and confirms that as in Europe and USA, failure of passive immunoglobulin transfer occurs in a relatively high number of dairy calves born under normal dairy farming conditions in New Zealand (Fallon and Hart,1987; Besser and Gay,1994).

Based on the physical and behavioural observations described in part A, calves that had not received adequate colostrum by the time they were separated from their dams were not easy to distinguish from those that had at 'pick-up'. The correlation between GGT activity and belly fill score ($r = 0.290$, $P < 0.05$) suggested that the flanks of those calves that had fed tended to appear either straight or rounded rather than hollow, however

belly fill alone could not be considered to be an accurate indicator of whether a calf had received sufficient colostrum.

5.4.2 The Effect of the Postnatal Behaviour of Both Calf and Cow on Serum GGT Activities at Pick-up.

Failure of passive immunoglobulin transfer may be due to intake of colostrum containing low Ig levels, an insufficient volume of colostrum being available or the calf not sucking soon enough after birth. Stott *et al.*(1979a) found that feeding at an increasing postnatal age (up to 24 hours) was associated with a linear decrease in the maximum serum immunoglobulin concentration and that an increasing volume of colostrum fed was associated with a proportionate increase in maximum serum Ig concentration. The aim of Part B was to discover whether any feature of the postnatal behaviour of either the calf or cow adversely affecting the calves' colostrum intake.

The concentrations of immunoglobulins in colostrum were not measured so this cannot be discussed with accuracy, however the effects of the two remaining variables, the volume of colostrum consumed (roughly estimated by the time spent sucking) and the time between birth and first sucking, may be examined. Although colostral Ig concentration is thought to influence the maximum serum Ig concentrations (Mohammed *et al.*,1991; Wedasingha, 1992; Aldridge *et al.*,1992; Besser and Gay,1994,), Stott *et al.*(1979b) found no correlation between pooled colostral Ig and calf serum Ig concentration. This suggests that of the three factors thought to influence serum Ig concentration, colostral Ig levels may have least effect.

5.4.3 The Volume of Colostrum Consumed

All calves that were observed to suck did so for at least 15 minutes, and eight out of nine attained GGT activities of greater than 200 U/l. In the remaining calf it is probable that there was no milk let-down by the cow. Although there was a postive correlation between GGT activity and time spent sucking it is likely that absorption of sufficient immunoglobulins depends on whether or not a calf sucks rather than the amount of time it spends sucking. It is probable that the efficacy of sucking in terms of the volume of milk swallowed per minute varies between calves. The correlation between time spent

sucking and GGT activity is likely to be due to the fact that those that spent time sucking attained high GGT activities and those that did not suck had low GGT activities.

The lack of correlation between the time spent sucking and any other features of the behaviour (such as the amount of time that the cow spent standing, calf spent standing, calf spent teat seeking, as well as the time from birth to standing and birth to sucking) was contrary to the study by Edwards (1982). This discrepancy may be due to the different conditions under which each study was conducted.

Edwards (1982) observed 133 cows and their calves under the sheltered, indoor calving conditions experienced in Europe. In contrast the present study included 21 cows and their calves in the outdoor exposed calving conditions experienced in New Zealand. While cow and calf behaviours are likely to influence the sucking time under the conditions of the present study, other factors such as the variable and sometimes adverse weather conditions may affect it more strongly. It is also possible that the sample size of the present study was too small to show up any behavioural effects or that behaviours other than those observed here would have shown a greater effect on sucking time.

5.4.4 Time Between Birth and Sucking

Edwards *et al.* (1982) found that the major factor affecting the serum Ig concentration attained by calves was the delay between birth and first sucking. Calves were left with their dams for a variable amount of time and their opportunity to obtain colostrum through natural sucking varied. In the present study 48% of calves had not sucked within 6 hours of the birth. This is a proportionately greater number of calves than the 33% (n = 53) reported by Edwards *et al.*, (1982). The proportion of calves not sucking within the first 6 hours is similar to the proportion found to have had a failure of passive immunoglobulin transfer as judged by blood samples taken at pick up in part A (42%). Of the 14 calves that did suck, 79% did so within 6 hours of their birth from which it can be suggested that if a calf is going to suck before pick up it will be most likely to do so within the first 6 hours.

These results suggest that approximately 45% of calves born under normal New Zealand dairy farming conditions may experience a considerable delay between birth and first sucking. This suggestion is supported by overseas research (Selman *et al.*, 1970; Edwards,1982; Petrie, 1984; Ventorp and Michenek, 1991) and is likely to explain the high incidence of low GGT levels. The progression to closure of the gut to colostral immunoglobulins occurs spontaneously with age at an increased rate from 12 hours after birth (Stott *et al.*,1979a). Calves that had not sucked within this time would have been less able to absorb immunoglobulins when they were finally fed, irrespective of the concentration or volume of the colostrum consumed.

In the present study udder conformation did not affect the time to first sucking, unlike the study by Edwards (1982). Although older cows did tend to have more pendulous udders this did not appear to affect the success of their calves in finding a teat and then sucking.

Of the calves that did suck, the duration between birth and first sucking ranged between 57 minutes and 19 hours 4 minutes. This range is greater than the range of between 50 minutes and 11 hours 44 minutes observed by Ventorp and Michanek (1991). Unlike the study by Edwards (1982), there was no correlation between the time to first stand and the time to first suck. Once again, the difference in results may be due to the smaller sample size and different conditions of the present study (the study by Edwards, (1982) included 161 calves).

While the negative correlation between time to first stand and air temperature observed by Edwards (1982) was not confirmed in this study, the tendency for calves to take longer to stand when born during the cooler night time hours may reflect a similar trend. Conditions of the present study varied from fine and still to gusty winds with persistent rain. As previously mentioned many of the previous studies have been carried out either indoors (Selman *et al.*,1970; Stott *et al.*,1979ab; Fallon and Harte,1987; Michanek and

Ventorp, 1989; Ventorp and Michanek, 1991; Michanek and Ventorp, 1992; Spinka and Illman, 1992) or under cover (Edwards and Broom, 1979; Edwards,1982; Edwards and Broom, 1982; Edwards *et al.*,1982; Edwards,1983; Illman and Spinka, 1993). This would moderate or remove the effect of air temperature on sucking behaviour and eliminate the effects of rain and wind.

Delays between birth and first sucking may have been influenced by the intense interest in the neonatal calf shown by other parturient cows. Cows other than the calf's dam were observed licking and sniffing the newborn calf and in some cases even allowed sucking. Edwards (1982) observed that a dam would sometimes desert its own calf in favour of a strange calf. This was observed once during the present study. Despite apparent complication imparted by periparturient cows and their calves being kept in groups, Edwards (1983) found that calves born in groups took no longer to suck than calves born in individual pens.

5.4.5 Discussion of General Behavioural Observations

Although breakdown of cow-calf interaction may extend the time between birth and sucking or prevent sucking from occurring, there was little evidence in this study that either the calves or cows were exhibiting overt behaviour that reduced or prevented sucking. Indeed, the incidence of cows encouraging sucking through their behaviour or adopting nursing postures (57%) was greater than that reported by Selman *et al.* (1970) who observed such behaviour in only 5 of 30 (17%) cases. While maternal rejection of her calf's teat-seeking advances (kicking at the calf, moving away in response to teat seeking behaviour) was observed in 50% of cow-calf pairs observed by Selman *et al.*(1970), there were no observed instances of such behaviour in the present study. Other studies have suggested that an absence of licking behaviour can contribute to a delay between birth and standing and therefore sucking. In the present study all cows were observed to begin licking their calves within 43 minutes after birth.

5.5 General Discussion

One of the aims of the present study was to observe sucking behaviour and hence colostrum intake of dairy cows and their calves under typical New Zealand farming conditions. These results have shown that most calves that are going to suck of their own accord (79%) when left with their dam under these conditions, will do so within 6 hours of their birth and attain an adequate serum immunoglobulin concentration. However a large proportion of calves (45%) did not suck between birth and pick up in the absence of any obvious signs of maternal rejection.

The AWAC codes state that it is essential that every calf recieves colostrum from its dam or another newly calved cow as soon as possible after it is born and certainly within the first 6 hours of its life (AWAC,1993). Clearly when dairy calves are left with their dams for up to 24 hours many will not receive colostrum. Given the importance of adequate colostrum intake human intervention may be advisable.

Based on the results of the present study it may be advantageous for calves that have been with the cow for up to 6 hours to be separated and be manually fed a sufficient volume of high quality colostrum. The volumes of colostrum considered to be adequate by Roy (1980), Jenny *et al.*(1981) and Wedasingha (1992) fall within the daily amounts of 100-120 ml colostrum/kg bodyweight recommended by the Animal Welfare Advisory Committee (1993, 1997). Although serum immunoglobulins are higher after natural sucking than after bottle of stomach tube feeding of (Stott *et al.*,1979c; Aldridge *et al.*,1992), the increased absorption gained when calves are manually fed earlier may be preferable to the uncertain outcome of leaving a calf with a cow for longer periods such that no natural sucking might take place before gut closure. Climatic conditions in New Zealand during winter and spring may further increase the delay between birth and sucking, thereby increasing the immunological benefits likely to be gained through such intervention.

5.5.1 Questioning the Importance of High Serum Immunoglobulin Concentrations in New Zealand Dairy Calves

Although attainment of high serum immunoglobulin concentrations is advantageous to young calves (Wedasingha,1992), failure to do so may not have the same importance in New Zealand, as overseas. The major cause of calf disease and deaths in New Zealand (and the Manawatu district) is diarrhoea, whereas pneumonia is more prevalent in the different conditions encountered on European dairy farms (Kearns *et al.*,1983; Webster *et al.*,1985; Peters,1987; Wedasingha, 1992). Given this consideration, the local protection provided by Igs within the intestine (Moon *et al.*,1978; Mylrea,1979; Saif and Smith,1985; Wedasingha,1992) may be of greater importance in the reduction of calf disease and mortality in New Zealand.

A calf that has not attained a high serum imunoglobulins concentrations may still benefit from the early intake of colostrum as a result of Igs within the intestine competing with these pathogens for adherence to intestinal epithelia (Wedasingha,1992) and the energy provided by its constituent lactose and lipids (see Chapters 2 and 3). Roy (1990) suggested that colostrum should be fed before microbes become established in the gut or otherwise the beneficial effects of Ig in colostrum feeding will be obscured. This suggestion supports the notion that colostrum feeding of young calves should occur early and continue during the immediate postnatal period.

5.5.2 Future Research

Future research into factors affecting immunoglobulin intake in New Zealand dairy calves could include the following:

- Accurate and regular measurement of environmental factors that may influence time to first sucking such as ground temperature, wind speed, air temperature, wind chill factor, rainfall and hours of sunlight.
- Making similar observations to those in Part B of the present study between birth and 6 hours after birth in a larger number of cow and calf pairs.
- Compare the time from birth to first sucking in calves born at night compared to those born during the day.

- Compare the 24 hour serum GGT activities of calves left with their mothers for 24 hours and those removed at 6 hours and fed 50-60 ml colostrum/ kg bodyweight at this time.
- Compare the time between birth and first sucking and subsequent serum GGT activities in dairy calves born in spring and those born in winter.
- Compare the long term health (incidences of diarrhoea or pneumonia) and mortality of calves left with their mothers for up to 24 hours with those fed colostrum by stomach tube at 6 hours of age.

Appendices

Appendix 2.1

Est. Gest Age	Calf #	Age at pick up	CRL(cm)	Temp(C)	Teeth Code	Resp.rate	GGT	Gum Colou	Feet	Umbilicus	Notes
280	30	1 day	86		11	84	6727	pink/red	worn	wet	
279	40	4 days	92	39	11	48	599	red/purp	worn	dry	
278	28	6 days		38.7	11	60	48	pink	worn	shrivelled	
276	41	1-2 days	87	38.5	10	84	880	red	worn	shrivelled	
274	29	6 days	89	39.4	10	78	39	pink	worn	shrivelled	
274	31	1 day	84	38.9	9	42	1363	pink	worn	wet	
271	2	1 day	79	39.2	10	72	2013	red/pink	worn	wet	
271	48	2-3 days	83	38.8	11	60	28	pink	worn	gone	
270	43	1-2 days	85	39	10	66	1274	purple	worn	shrivelled	
269	24	2 days	88	39.2	9	54	153	red	worn	wet	
269	26	1 day	89	39.51	9		33	purple	0.5w	wet	
269	27	0 days	83	39.16	11		1129	pink	worn	wet	
268	20	1-2 days	87	39.2	9	60	1316	red/pink	worn	wet	
268	25	2 days	79	38.8	11	48	430	pink	worn	dry	
267	12		82	39.8	9	42		pink	worn	dry	
267	89	2-3 days		39.87	7	62	128	pink	worn	shrivelled	
266	5	1 day	81	39	11	36	18	pink	worn	gone	
266	16	1-2 days	84	38.8	10	60	909	red/purp	worn	wet	
266	18	1-2 days	81	39.1	11	56	84	red	worn	dry	
266	19	1-2days	86	39.5	10	48	166	pink	worn	gone	
266	45	2-3 days	87	39.7	10	60	283	pink	worn	dry	
266	87	0 days		36.92	1	46	16	pink/red	unworn	wet	
265	21		83	39	10	30	179	red	worn	gone	
265	95	0 days	86	39.2	8	48	265	pink	worn	wet	
264	1	1 day	74	38	4	60	17	red	0.5w	wet	
264	23	2 days	89	39.1	11	48	462	pink	worn	dry	
264	34	1 day	80	38.8	11	48	5941	red	worn	wet	
264	36	1 day	83	38.8	10	54	5027	pink	worn	wet	
264	37	0 days		38.4	11		458	red	worn	wet	
264	46	2-3 days	86	38.9	11	48	61	pink	worn	gone	
264	49	2-3 days	79	39.5	11	60	50	pink/red	worn	gone	
264	91	2-3 days		38.2	10		213	pink	worn	shrivelled	
263	44	1 day		39					worn		
262	3	1 day	75	37.7	10	30	32	red	worn	wet	
262	35	1 day	82	38.9	10	72	2921	red/purp	worn	wet	
262	98	0 days	86	38.2	11	42	60	pink	worn	gone	bawler
261	22	2 days	84	38.8	5	30	9	pink	worn	wet	
261	32	1 day	83	38.6	4	48	198		worn	wet	
260	10		82	38.8	10	30		pink	worn	dry	
260	39	0 days			11		168	red/purp	worn	wet	
260	42	1-2 days	89	39.3	11	36	18	pink	worn	dry	
260	88	0 days		34.05	5	60	7	pink	unworn	gone	irreg. resp. mouth cold
260	96	0 days	81	38.7	5	100	15	pink/red	worn	wet	
259	7	1 day	80	39.3	4	84	85	pink	worn	wet	
259	11		79	39.2	4	78	235	red	worn	wet	
259	93	1 day		38.2	4		19	pink	worn	wet	
258	4	1 day	80	38.8	11	72	14	red/pink	worn	wet	
258	33	1 day	83	38.8	4	36		red/pink	worn	wet	
258	38	0 days			10		223	red/purp	worn	wet	
258	90	2-3 days		39.34	11		74	pink	worn	shrivelled	
257	6	1 day	71	37.6	5	30	13	red/purp	worn	wet	
257	86	0 days		37.3	4	30	11	purp	0.5 w	wet	Forced Resp Noises
257	94	1 day		38	7		18	red	worn	wet	
257	100	1 day	75	38.2	4	66	16	red/pink	worn	wet	
256	9	1 day	69	37.6	1	48	11	purp/red	worn	wet	
256	13		72	39.7	10	30	295	pink/red	worn	wet	
256	17	1-2 days	69	38.7	2	30	651	red/purp	worn	gone	bawler
256	47	1-2 days	73	38.2	2	60	11	pink	worn	wet-dry e	
256	84	1-2 days		39.4	2	30	535	dark purp	worn	dryish	
254	92	1 day		38.8	5		22	red	0.5w	wet	
253	8	1 day	70	37.3	4	30	9	red	worn	wet	bawler
253	83	1 day		38.8	5	50	619	red/purp	worn	Wet-dry e	
253	97	0 days	74	39.2	4	30	21	pink/red	0.5w	wet	bawler
252	14		66	38.7	7	66		red	worn	wet	bawler
250	15	1 day	75	37.6	1	30	8	red	worn	wet	hyper reactive
247	50	1-2 days	68	37.9	2	18	49	red	worn	shrivelled	
246	85	1-2 days		37.4	5	30	468	purp	0.5 w	dry	Forced Respiration

Actual Time		0600h	0700h	0800h	0900h	1000h	1100h	1200h	1300h	1400h	1500h	1600h	1700h	1800h	1900h	2000h	2100h	2200h	2300h	0000h	0100h	0200h	0300h	0400h	0500h	0600h	0700h	0800h	0900h	1000h	1100h	1200h	1300h	1400h	1500h
Experimental Time (h)		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Group																																			
4D:4F-CONTROL	Blood Sample	+			+			+			+			+			+			+						+			+			+	+	+	+
	PCV	+			+			+			+			+			+			+						+			+			+			+
	Glucose	+			+			+			+			+			+			+						+			+			+			+
	Triglyceride																															+			
	B-Hydroxybutyrate																															+			
	Urea																			+												+			+
	Total Plasma Proteins																			+												+			+
	Temperature Taken																+															+			
	Fed 25ml/kg			+				+				+																+				Fed 50 ml/kg			
4D:4F-FASTED	Blood Sample	+			+			+			+			+			+			+						+			+			+	+	+	+
	PCV	+			+			+			+			+			+			+						+			+			+			+
	Glucose	+			+			+			+			+			+			+						+			+			+			+
	Triglyceride																															+			
	B-Hydroxybutyrate																															+			
	Urea																			+												+			+
	Total Plasma Proteins																			+												+			+
	Temperature Taken																+															+			
	Fed 25ml/kg			+				+				+																+				Fed 50 ml/kg			
10D:4F-CONTROL	Blood Sample	+			+			+			+			+			+			+						+			+			+	+	+	+
	PCV	+			+			+			+			+			+			+						+			+			+			+
	Glucose	+			+			+			+			+			+			+						+			+			+			+
	Triglyceride																															+			
	B-Hydroxybutyrate																															+			
	Urea																			+												+			+
	Total Plasma Proteins																			+												+			+
	Temperature Taken																+															+			
	Fed 25ml/kg			+				+				+																+				Fed 50 ml/kg			
10D:4F-FASTED	Blood Sample	+			+			+			+			+			+			+						+			+			+	+	+	+
	PCV	+			+			+			+			+			+			+						+			+			+			+
	Glucose	+			+			+			+			+			+			+						+			+			+			+
	Triglyceride																															+			
	B-Hydroxybutyrate																															+			
	Urea																			+												+			+
	Total Plasma Proteins																			+												+			+
	Temperature Take																+															+			
	Fed 25ml/kg																															Fed 50 ml/kg			
4D:2F-CONTROL	Blood Sample	+			+			+			+			+			+			+						+			+			+	+	+	+
	PCV	+			+			+			+			+			+			+						+			+			+			+
	Glucose	+			+			+			+			+			+			+						+			+			+			+
	Triglyceride																															+			
	B-Hydroxybutyrate																															+			
	Urea																			+												+			+
	Total Plasma Proteins																			+												+			+
	Temperature Taken																+															+			
	Fed 50ml/kg											+																+							
4D:2F-FASTED	Blood Sample	+			+			+			+			+			+			+						+			+			+	+	+	+
	PCV	+			+			+			+			+			+			+						+			+			+			+
	Glucose	+			+			+			+			+			+			+						+			+			+			+
	Triglyceride																															+			
	B-Hydroxybutyrate																															+			
	Urea																			+												+			+
	Total Plasma Proteins																			+												+			+
	Temperature Taken																+															+			
	Fed 50ml/kg																															Fed 50 ml/kg			
10D:2F-CONTROL	Blood Sample	+			+			+			+			+			+			+						+			+			+	+	+	+
	PCV	+			+			+			+			+			+			+						+			+			+			+
	Glucose	+			+			+			+			+			+			+						+			+			+			+
	Triglyceride																															+			
	B-Hydroxybutyrate																															+			
	Urea																			+												+			+
	Total Plasma Proteins																			+												+			+
	Temperature Taken																+															+			
	Fed 50ml/kg											+																+							
10D:2F-FASTED	Blood Sample	+			+			+			+			+			+			+						+			+			+	+	+	+
	PCV	+			+			+			+			+			+			+						+			+			+			+
	Glucose	+			+			+			+			+			+			+						+			+			+			+
	Triglyceride																															+			
	B-Hydroxybutyrate																															+			
	Urea																			+												+			+
	Total Plasma Proteins																			+												+			+
	Temperature Taken																+															+			
	Fed 50ml/kg											+																				Fed 50 ml/kg			

Appendix 3.2

Absolute Glucose Concentrations							
Initial Sample	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.05	0.05	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
12 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.01	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	0.001	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	0.05
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
24 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.01	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	0.05	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.001	0.01	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
30 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.001	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	0.01	NA	NA	0.05	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.001	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	0.001
10D:2F-Fasted	R	R	R	R	R	R	NA
nsd = no significant difference							
NA = Not Applicable							

R= Repeat

Appendix 3.3

Change in plasma glucose							
12 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.001	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	0.05	NA	NA
10D:4F-Control	R	NA	0.001	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	0.05
4D:2F-Control	R	R	R	NA	0.01	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
24 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.05	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	0.05	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.05	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
30 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.01	0.05	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	0.05	NA	NA	0.01	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.001	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	0.001
10D:2F-Fasted	R	R	R	R	R	R	NA

Appendix 3.4

Pooled Data				
Absolute Plasma Glucose Concentration				
Initial Sample	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	nsd	nsd	NA
4F-Fasted Groups	R	NA	NA	nsd
2F-Control Groups	R	R	NA	nsd
2F-Fasted Groups	R	R	R	NA
12 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.001	nsd	NA
4F-Fasted Groups	R	NA	NA	0.05
2F-Control Groups	R	R	NA	0.01
2F-Fasted Groups	R	R	R	NA
24 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.001	nsd	NA
4F-Fasted Groups	R	NA	NA	nsd
2F-Control Groups	R	R	NA	0.001
2F-Fasted Groups	R	R	R	NA
30 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.001	0.01	NA
4F-Fasted Groups	R	NA	NA	0.05
2F-Control Groups	R	R	NA	0.001
2F-Fasted Groups	R	R	R	NA
Change in Plasma Glucose Concentration				
12 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.001	nsd	NA
4F-Fasted Groups	R	NA	NA	0.01
2F-Control Groups	R	R	NA	0.01
2F-Fasted Groups	R	R	R	NA
24 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.01	nsd	NA
4F-Fasted Groups	R	NA	NA	nsd
2F-Control Groups	R	R	NA	0.01
2F-Fasted Groups	R	R	R	NA
30 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.001	0.01	NA
4F-Fasted Groups	R	NA	NA	nsd
2F-Control Groups	R	R	NA	0.001
2F-Fasted Groups	R	R	R	NA

Appendix 3.5

	Fed Groups vs Fasted Groups			
	0	30	Change to 30	
Triglycerides	nsd	nsd	nsd	
B-Hydroxybutyrate	nsd	0.001	0.001	
Urea	nsd	0.001	0.001	
	0	12	24	30
Absolute Glucose Concer	nsd	0.001	0.001	0.001
Change in Glucose Conc	NA	0.001	0.001	0.001

Appendix 3.6

Change in Triglyceride concentration							
Initial Sample	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.01	0.01	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
30 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
Change over 3(	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.01	0.05	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA

Appendix 3.7

B-Hydroxybutyrate							
Initial Sample	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	0.05	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	0.05	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
30 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.01	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	0.05	NA	NA
10D:4F-Control	R	NA	0.05	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	
4D:2F-Control	R	R	R	NA	0.001	0.05	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	0.01
10D:2F-Fasted	R	R	R	R	R	R	NA
Change over 3	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.01	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.001	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	0.001
10D:2F-Fasted	R	R	R	R	R	R	NA

Appendix 3.8

Absolute Plasma Triglyceride Concentration				
0	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	nsd	nsd	NA
4F-Fasted Groups	R	NA	NA	nsd
2F-Control Groups	R	R	NA	nsd
2F-Fasted Groups	R	R	R	NA
30 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	nsd	nsd	NA
4F-Fasted Groups	R	NA	NA	nsd
2F-Control Groups	R	R	NA	nsd
2F-Fasted Groups	R	R	R	NA
Change in Plasma Triglyceride Concentration				
30 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	nsd	nsd	NA
4F-Fasted Groups	R	NA	NA	nsd
2F-Control Groups	R	R	NA	nsd
2F-Fasted Groups	R	R	R	NA
Absolute Plasma B-Hydroxybutyrate Concentration				
0 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	nsd	nsd	NA
4F-Fasted Groups	R	NA	NA	nsd
2F-Control Groups	R	R	NA	nsd
2F-Fasted Groups	R	R	R	NA
30 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.001	nsd	NA
4F-Fasted Groups	R	NA	NA	0.01
2F-Control Groups	R	R	NA	0.001
2F-Fasted Groups	R	R	R	NA
Change in B-Hydroxybutyrate Concentration				
30 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.001	nsd	NA
4F-Fasted Groups	R	NA	NA	0.05
2F-Control Groups	R	R	NA	0.001
2F-Fasted Groups	R	R	R	NA

Appendix 3.9

Urea							
Initial Sample	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	0.01	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	0.05
10D:2F-Fasted	R	R	R	R	R	R	NA
30 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.05	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	0.05	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.01	0.05	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
Change over 3	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.01	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	0.01	NA	NA
10D:4F-Control	R	NA	0.05	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	0.05	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA

Appendix 3.10

Absolute Plasma Urea Concentration				
0 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	nsd	nsd	NA
4F-Fasted Groups	R	NA	NA	nsd
2F-Control Groups	R	R	NA	nsd
2F-Fasted Groups	R	R	R	NA
30 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.01	nsd	NA
4F-Fasted Groups	R	NA	NA	0.05
2F-Control Groups	R	R	NA	nsd
2F-Fasted Groups	R	R	R	NA
Change in Plasma Urea Concentration				
30 hours	4F-Control Groups	4F-Fasted Groups	2F-Control Groups	2F-Fasted Groups
4F-Control Groups	NA	0.001	nsd	NA
4F-Fasted Groups	R	NA	NA	0.05
2F-Control Groups	R	R	NA	0.05
2F-Fasted Groups	R	R	R	NA

Appendix 3.11

Total Plasma Proteins							
Initial Sample	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	0.05	NA	0.05	NA	NA	NA
4D:4F-Fasted	NA	NA	0.05	NA	0.05	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	0.05	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
30 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	0.05	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	0.05	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
Change over 3	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	0.05	NA	0.05	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA

Appendix 3.12

Change in Temperature							
15 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	0.05	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	0.05	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
30 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	0.05	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	0.05	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA

Appendix 3.13

Temperature							
Initial Sample	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
15 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	0.05	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA
30 hours	4D:4F-Fasted	10D:4F-Control	10D:4F-Fasted	4D:2F-Control	4D:2F-Fasted	10D:2F-Control	10D:2F-Fasted
4D:4F-Control	nsd	nsd	NA	nsd	NA	NA	NA
4D:4F-Fasted	NA	NA	nsd	NA	nsd	NA	NA
10D:4F-Control	R	NA	nsd	NA	NA	nsd	NA
10:4F-Fasted	R	R	NA	NA	NA	NA	nsd
4D:2F-Control	R	R	R	NA	nsd	nsd	NA
4D:2F-Fasted	R	R	R	R	NA	NA	nsd
10D:2F-Control	R	R	R	R	R	NA	nsd
10D:2F-Fasted	R	R	R	R	R	R	NA

Cow Numbe	Birth		24 hours		Birth Weight		Time between births	Diff. Twin 1-2 PCV change 0-24			
1995	PCV - Twin 1	PCV-Twin2	PCV-Twin 1	PCV- Twin 2	Twin 1	Twin 2	Twin 1 & twin2 (min)	0 hours	24 hours	Twin 1	Twin 2
Type F											
144	26.5	31.5	13	23.5	28.6	28.4	4	-5	-10.5	13.5	8
23	34	41.5	20	36.5	32.6	34.6	23	-7.5	-16.5	14	5
Type E											
294	49	41	48	32	34.7	31.8	16	8	16	1	9
297	44	45	41.5	33	33	20.3		-1	8.5	2.5	12
259	43	48	39	35	28.5	35.5	110	-5	4	4	13
123	40	49	25	36	41	31	80	-9	-11	15	13
268	50	46.5	38	41	29.8	25.4	18	3.5	-3	12	5.5
Type D											
53		34		14	33.8	39				0	20
264	30.5	28.5	24	20	29	28.4		2	4	6.5	8.5
Type C											
226	33	42	15.5	29.5	28	37.5	86	-9	-14	17.5	12.5
253	52	53.5	36	39.5	36	21.3	34	-1.5	-3.5	16	14
Type B											
274	42.5	44	35	35.5	27.7	28.7	0	-1.5	-0.5	7.5	8.5
83	34	37	26	27.5	30.1	30.8	5	-3	-1.5	8	9.5
Type A											
20	42.5	42.5	32	34	30.3	32.2	48	0	-2	10.5	8.5
21	42	47	30.5		35.7	30.9		-5		11.5	
41	43	49	35	41	37.5	31.1	40	-6	-6	8	8
42	37.5	44		33	33	33.2	0	-6.5			11
66	40	29.5	34	23	39.3	31.1	72	10.5	11	6	6.5

Appendix 4.2

Method for Calculating the Volume of Blood Transfused from the First to the Second Born Twin.

First- born twin = t1

Second-born twin = t2

1) Calculate the change in PCV for Twin 1 from 0 to 24 hours:

PCV (t1,0hours) - PCV (t1, 24 hours) = PCV Change over 24 hours (A)

Repeat for Twin 2 = (B)

2) Calculate the difference in PCV change between Twin 1 (t1) and Twin 2 (t2).

(A) - (B) = Difference in PCV change (C)\

3) Determine the plasma volume of t1(Dalton,1964)

0.073 x Body weight t1 = Plasma volume of t1 (D)

4) Calculate the proportion of plasma per unit blood of t1 at 24 hours

100 - PCV (t1, 24 hours) /100 = Proportion of plasma in t1 (E)

5) Calculate the blood volume of t1 at 24 hours

(D) ÷ (E) = Blood Volume of t1 at 24 hours (F)

6) Repeat steps 3 - 5 for t2

(G) = Blood volume of t2

7) Estimate the PCV of t2 had no transfusion occurred

PCV (t2, 24 hours) - (C) = PCV t2 - no transfusion (H)

8) Determine the volume of red blood cells that t2 would have had at 24 hours had no transfusion occurred

(G) x (H) = Volume of red blood cells, t2, no transfusion (I)

9) Determine the actual volume of red blood cells t2 had a 24 hours

(G) x PCV (t2, 24 hours) = Volume of red blood cells, t2, 24 hours (J)

10) Calculate the volume of red blood cells transferred from t1 to t2

(J) - (I) = Volume of red blood cells transferred (K)

11) Determine the volume of blood transfused from t1 to t2

(K) ÷ PCV (t1, 0 hours) = Volume of blood transfused from t1 to t2

12) Calculate the plasma volume of the feto-placental circulation of t1 before birth (Caton *et al.*,1975):

0.1 x Body weight of t1 = Plasma volume of feto-placental circulation (M)

13) Determine the proportion of plasma per unit blood of t1 before birth

100 - PCV (t1, 0 hours)/100 = Proportion of plasma (t1) (N)

14) Determine the blood volume of the feto-placental circulation of t1 before birth

(M) ÷ (N) = Blood Volume of feto-placental circulation (t1) (O)

15) Determine the placental volume of t1

(O) - (F) = Placental volume of t1 (P)

16) Calculate the proportion of the placental blood volume transfused from the placental vasculature of t2 during birth

(L) ÷ (P) = Proportion of placental blood volume transfused (Q)

Appendix 4.3

Method for Calculating the Volume of Blood Lost From the Placental Circulation of the Second Born Twin

First-born twin = t1

Second-born twin = t2

1) Calculate the change in PCV from birth to 24 hours:

PCV (t1, birth) - PCV(t1, 24 hours) = PCV change over 24 hours (A)

Repeat for Twin 2 = (B)

2) Calculate the difference in PCV change between Twin 1 and Twin 2:

(A) - (B) = Difference in PCV change (C)

3) Determine the plasma volume of Twin 2 (Dalton,1964):

Body weight (t1) x 0.073 = Plasma volume Twin 2 (D)

4) Determine the proportion of plasma per unit blood of Twin 2 at 24 hours:

100 - PCV(t2, 24 hours)/100 = Proportion of plasma (E)

5) Determine the volume of blood of Twin 2 at 24 hours:

(D) ÷ (E) = (F)

6) Estimate the PCV of Twin 2 at 24 hours had no blood loss occurred:

PCV(t2,24 hours) + (C) = PCV of Twin 2 - no blood loss (G)

7) Determine the volume of red cells that Twin 2 would have had at 24 hours had no blood loss occurred:

(F) x (G) = Estimated volume RBC t2, 24 hours - no blood loss (H)

8) Determine the actual volume of red blood cells Twin 2 had at 24 hours:

(F) x PCV(t2, 24 hours) = Actual red cell volume t2, 24 hours (I)

9) Determine the volume of red blood cells lost by Twin 2 during birth:

(H) - (I) = Volume of red cells lost (J)

10) Calculate the volume of blood lost by Twin 2 during birth:

(J) ÷ PCV (t2, 0 hours) = Blood volume lost (K)

11) Determine the feto-placental plasma volume of twin 2 before birth (Caton *et al.*,1975):

0.1 x Bodyweight Twin 2 = Feto-placental plasma volume (L)

12) Determine the proportion of plasma per unit blood of Twin 2 before birth:

100 - PCV(t2, 0 hours)/100 = Proportion of plasma (M)

13) Determine the feto-placental blood volume of Twin 2:

(L) ÷ (M) = Feto-placental blood t2 (N)

14) Determine the placental blood volume of Twin 2:

(N) - (F) = Placental Blood Volume (O)

15) Determine the proportion of twin 2's placental blood volume lost during birth:

(K) ÷ (O) = Proportion of t2 placental blood volume lost (P)

Appendix 5.1

Part A: GGT 's from calves at pick-up

Calf #	GGT	Gender	Temperature	Skinfold	Coat	Feet	Umbilicus	Belly Fill	Gum Colour	Teeth	Vigour	Nose wet/dry	Nose warm/cool
1	8	m	38.47	5.5	dry	worn	wet	1	red	8	2	d	w
2	44	m	38.39	6.5	damp	worn	wet	1	pink	6 2	1.5	d	c
3	600	m	38.89	7	dry	worn	gone	2	pink	6 2	2	d	c
4	3610	f	38.99	5	dry	worn	wet	2	red	8	2	w	w
5	1640	f	38.89	6	dry	worn	wet	3	pink	8	2	w	c
6	900	f	39.02	5	dry	worn	wet	3	pink	8	2	w	c
7	185	f	38.68	6	dry	worn	wet	3	pale pink	8	2	w	c
8	1590	m	39.68	5	dry	worn	wet	3	red	8	2.5	w	w
9	610	m	39.08	5	wet	worn	gone	2	red	6 2	2	w	c
10	12	f	39.06	7	dry	worn	wet	2	purple	6 2	2	w	c
11	280	m	40.17	4	dry	worn	wet	3	purp/red	8	2.5	w	w
12	8	m	38.46	6	wet	worn	gone	2	purp/red	8	2	w	c
13	27	m	38.17	8	wet	not	wet	1	red	8	1.5	w	c
14	4340	f	38.72	5	wet	worn	wet	2	pink	8	2	d	c
15	10	f	38.63	6	wet	worn	wet	1	red	8	2	w	c
16	1800	f	38.35	5	wet	worn	wet	2	pink	8	2	w	c
17	2200	m	39.13	6	wet	worn	wet	2	purp/red	8	2	d	w
18	5	f	38.99	5	dry	worn	wet	3	pink	8	2.5	d	w
19	26	m	39.35	7	wet	worn	wet	2	red	6 2	2.5	w	w
20	13	m	38.83	5	dry	worn	wet-dry end	2	purp	8	2	w	c
21	3470	m	38.89	6	dry	worn	wet	2	pink	6 2	1.5	d	w
22	1130	m	38.99	5	dry	worn	wet	1	pink	8	2	d	c
23	1650	f	39.09	5	dry	worn	wet	2	purple	8	1.5	d	w
24	500	f	39.09	5	damp	worn	wet	1	red	6 2	1	d	w
25	20	m	38.56	7	wet	worn	wet	1	purple	8	1.5	w	w
26		m	38.37	5.5	wet	worn	wet	2	purp/red	8	1.5	w	w
27	30	m	38.21	6	wet	worn	wet	2	pink	8	2	d	w
28	190	m	38.06	5	wet	half	gone	1	red	6 2	1.5	w	w
29	30	m	38.78	4	wet	half	wet	1	pale pink	6 2	1.5	w	w
30	850	m	38.93	7	damp	worn	wet	2	red	6 2	1.5	d	c
31	5560	m	38.75	7	dry	worn	wet	2	purp/red	6 2	1.5	w	c
32	16	f	39.04	4	damp	worn	gone	1	purp/red	6 2	2	d	c
33	45	f	39.34	6.5	dry	worn	wet	2	purp/red	8	2	d	w
34	6	m	38.62	6.5	dry	worn	wet	2	purple	8	2	w	c
35	2780	f	39.02	5	dry	worn	wet	2	purp/red	8	1.5	d	w
36	3110	m	39.05	7	wet	worn	gone	2	purple	8	2	w	w
37	7	m	38.87	6	damp	worn	wet	2	purp/red	6 2	2	w	c
38	3010	f	39.1	5	dry	worn	wet	2	pink	8	2	d	w
39	3940	m	39.07	5	dry	worn	wet	2	red	8	3	w	c
40	20	m	38.79	8	dry	worn	wet	1	purp/red	8	1.5	d	c
41	1620	m	39.92	6.5	dry	worn	wet	2.5	purple	8	2	w	c
42	20	m	37.98	4	dry	worn	wet	1	red	8	2	d	c
43	1230	m	38.68	6	wet	worn	wet	1	red	8	1.5	w	c
44	14	m	38.46	5	wet	half	wet	1	pink	6 2	1.5	w	c
45	1630	m	38.99	6	damp	worn	wet	2	purple	8	1.5	w	c
46	240	f	39.26	5.5	dry	worn	dry	3	pink	8	2	w	w
47	5	m	39.5	6.5	dry	worn	shrivelled	1	purple	8	2	d	w
48	28	m	38.51	6	dry	worn	wet	2	pink	6 2	2	w	c
49	3720	f	39.28	7	wet	worn	wet	3	pink	8	3	w	c
50	3640	f	39.29	5	wet	worn	wet	2	red	8	2	w	c
51	3390	m	39.42	7	wet	worn	gone	2	red	8	2	w	c
52	3810	m	38.7	7	wet	worn	wet	2	red	4 2	3	w	w
53	1620	f	38.16	8	wet	worn	wet	2	pink	8	2	w	c
54	860	f	39.2	5	wet	worn	wet	2	red	8	2	w	c
55	180	f	39.28	5	wet	worn	wet	1	pink	8	2	w	c
56	1260	f	39.25	7	wet	worn	wet	2	pink	8	1.5	w	c
57	2990	m	39.11	7	wet	worn	wet	2	purp/red	8	2	w	c

Cow and Calf Behaviour

Data set	Cow-calf ID	Obs time(mins)	Cow Up(min)	Cow Up %	Cow Down (min)	Cow Down %	Calf Up (min)	Calf Up %	Calf Down (min)	Calf Down %	Calf teat seeking (min)	TS %
1	1 4	194	68	35	126	65	68	35	126	65	21	11
2	1 8	593	516	87	77	13	231	39	362	61	15	3
3	2 1	1258	437	35	821	65	295	23	963	77	151	12
4	2 2	1227	531	43	696	57	375	31	852	69	92	7
5	2 3	1227	590	48	637	52	225	18	1002	82	80	7
6	2 4	1198	796	66	402	34	1013	85	185	15	202	17
7	2 5	947	329	35	618	65	136	14	811	86	19	2
8	2 6	638	534	84	104	16	394	62	244	38	110	17
9	2 7	349	69	20	280	80	0	0	349	100	0	0
10	2 8	288	264	92	24	8	273	95	15	5	139	48
11	3 1	1140	540	47	600	53	418	37	722	63	43	4
12	3 3	1057	393	37	664	63	190	18	867	82	27	3
13	3 5	74	22	30	52	70	10	14	64	86	2	3
14	4 1	1492	1243	83	249	17	938	63	554	37	111	7
15	4 2	693	485	70	208	30	424	61	269	39	132	19
16	4 3	1192	299	25	893	75	199	17	993	83	8	1
17	4 4	1017	987	97	30	3	618	61	399	39	37	4
18	5 1	756	136	18	620	82	538	71	218	29	97	13
19	5 2	623	621	100	2	0	359	58	264	42	31	5
20	5 3	495	351	71	144	29	411	83	84	17	55	11
21	5 4	466	466	100	0	0	335	72	131	28	51	11

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