INFLUENCE OF BULL EXPOSURE ON REPRODUCTIVE PERFORMANCE OF POSTPARTUM DAIRY COWS

KHAIRIYAH BINTI MAT

The thesis is submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

School of Agriculture, Food and Rural Development

Newcastle University

Newcastle upon Tyne, UK

November 2013

ABSTRACT

Influence of Bull Exposure on Reproductive Performance of Postpartum Dairy Cows

by

Khairiyah Mat

The biostimulation of exposure to a male can improve postpartum reproductive performance in females of many species. The aim of this research was to examine the biostimulatory effects of fenceline bull exposure during the early postpartum period in lactating dairy cows on the resumption of ovulatory activity, subsequent conception and calving interval, either in naturally cycling or oestrus syncronised cows. Three experiments were carried out in which Holstein-Friesian cows were allocated after parturition between two groups: cows that had unlimited time of access to fenceline bull exposure (BC) and a control group that was not exposed to a bull (NBC). Experiment 1, assessing the effects of bull exposure on cows at varied stages post partum, showed that the interval from start of exposure to resumption of ovarian activity, assessed by milk progesterone concentration, was not significantly affected by bull presence. Cows showed generally poor visible indicators of oestrus making correct insemination timing difficult consequently prolonged the calving interval. Experiment 2, investigating the biostimulation approach for freshly calved cows, showed an increase in pregnancy rate to the first service and consequent reduction in calving interval. This was especially marked in those cows previously treated with a progesterone intra-vaginal device (PRID). The average number of services per conception was lower in biostimulated cows, though there was no significant improvement of oestrus detection in these cows. Experiment 3, investigating cows that were freshly calved and then syncronised for oestrus using PRID treatment, showed a similar improvement in conception rate to first service, though compromised by generally poor reproductive performance. Similarly, oestrus detection rate was low even with PRID treatment. The outcomes from this study highlight the potential effects of a biostimulation approach as a strategy to improve reproductive performance in postpartum anoestrus dairy cows raised in intensive farming systems.

DECLARATION

I certify that no part of the material offered has been previously submitted for a degree or other qualification in this or any other university.

Khairiyah Mat

DEDICATION

To my unborn baby, the greatest gift in my life and my loyal company during my thesis writing. May you will always be blessed and grow up healthy, strong and wise and have a tremendous future. Secondly, to my late father Mat bin Man, may your soul rest in peace, you will always in my memory.

Khairiyah Mat

November 2013

ACKNOWLEDGEMENTS

Praise to the God, the Almighty for grant this priceless opportunity of a lifetime, the precious experience and providing me with health, strength and guidance to accomplish this study.

First and foremost, I would like to express my sincere gratitude to my supervisors, Dr. Andrew Beard and Prof. Sandra Edwards for supervising this study and providing much appreciated help, support and guidance throughout the time of research and writing of this thesis.

I would like to thank the technical team of the School of Agriculture, Food and Rural Development, Newcastle University, Brian, Jim, Chris and all the helping hands Angela, Peter and Richard, at the dairy unit, Cockle Park Farm, for allowing the research to happen, cooperating with the study and providing assistance during the experiments, very grateful for the kindness.

I would like to acknowledge Ministry of Higher Education, Malaysia and Universiti Malaysia Kelantan for their scholarship award, which allows me to commence this study in first instance.

My deepest gratitude to my husband, Ahmad Hisham for his unconditional love, understanding, encouragement, and never ending support in all my struggles and frustrations over a few years although being apart throughout this study. Last but not least, the most importantly, my sincere thanks to my mum Saadiah Said and all my family and friends for their love, support and encouragement. Without them I could not have made it here.

TABLE OF CONTENTS

LIST OF TABLES

Table 4.5: The proportion of animals, from groups either exposed or not exposed to the bull, showing short, normal, abnormal (delayed ovulation type I and II, persistent CL type I and II based on atypical ovarian hormone pattern) ovarian cyclicity post calving (day 0-80).

Table 4.6: The mean interval (days) from day of resumption ovulatory activity (OA) to first until eight/fifth services for both NBC and BC treatment groups for 31 animals that showed a natural first progesterone rise postpartum (these animals were not been given PRID treatment before resumption of OC).

Table 4.7: The mean interval (days) from day of calving to first until eight/fifth services for both NBC and BC treatment groups, for all 41 animals.

Table 4.8: The mean interval (days) from day of resumption of ovulatory activity (OA) until successful AI for the 33 animals which showed a progesterone (P4) rise.

Table 4.9: The mean interval (days) from calving day until successful AI for the 33 animals which showed a progesterone (P4) rise.

Table 4.10: The mean interval (days) from calving day until successful AI for all 41 cows.

Table 4.11: The pregnancy rates to first service for all animals from NBC and BC group, including cows that were treated with PRID.

Table 4.12: The pregnancy rates to second, third and fourth services for cows from NBC and BC group, including 10 cows that were treated with PRID during their first service.

Table 4.13: The pregnancy rate to first time service in cows by comparing the type of ovarian cyclicity post calving for both control and treatment group. As shown in table 8, 3 NBC cows and 12 BC cows were pregnant to first service, this table analyses the effects of normal or odd ovarian cycle to conception rates after first service.

Table 4.14: The mean interval (days) of calving for both NBC and BC compares for overall animal, those with PRID and without PRID treatment.

Chapter 5. Influence of bull exposure on conception at a synchronised oestrus in postpartum dairy cows

Table 5.1: The comparisons of body condition score (BCS), parity number, previous milk production, previous calving interval and interval from calving to PRID treatment between cows allocated to the two treatment groups.

Table 5.2: The comparisons of body condition score (BCS) and average milk production from calving to time of insemination between two treatment groups.

Table 5.3: Conception rate to services following PRID withdrawal.

LIST OF FIGURES

Chapter 3. Influence of bull exposure to postpartum anoestrous cows on resumption of ovulatory activity

was not associated with PRID treatment, as all cows resumed cyclicity normally (some of cows received PRIDs but only after the first rise in progesterone).

Figure 4.9: Different types of ovarian cycles (normal and abnormal types) in NBC and BC cows and heifers with representative milk progesterone profiles. These profiles were obtained from the point after they had calved. ***a.*** Normal ovarian cycles throughout the study period ***b.*** Abnormal ovarian cycle with delayed ovulation type I, with prolonged low progesterone concentration of 1.5ng/ml or less for 45 days postpartum or more. ***c.*** Abnormal ovarian cycle with delayed ovulation type II, with prolonged interval from first ovulation to second ovulation. ***d.*** Abnormal ovarian cycle with persistent corpus luteum type I, with milk progesterone concentrations of more than 1.5 ng/ml for 18 days or more. ***e.*** Abnormal ovarian cycle with persistent corpus luteum type II, with milk progesterone concentrations of more than 1.5 ng/ml for 17 days or more during the following postpartum oestrous cycles.

Figure 4.10: Histogram showing the mean (±SEM) time in minutes spent by cows in the BC group which interacted with the bull, measured starting from 2 days (day -1; n=4 and -2; n=7) before the day with lowest progesterone concentrations (day 0; n=8) and 2 days (day1; n=6 and 2; n=6) after that.

Figure 4.11: The cumulative percentage of all cows for both groups that conceived at different services.

Figure 4.12: The mean (±SEM) calving interval (days), comparing the interval for cows that were treated with bull exposure and without bull exposure for all animals used (NBC=20; BC=21), comparing cows that were not treated with PRID (NBC=11; BC=13) and also comparing the interval for cows that were treated with PRID (NBC=9; BC=8).

Figure 4.13: The progesterone concentrations for animals from both NBC and BC group during the oestrous cycle when they were served and conceived to the service. Profiles were obtained from the average concentrations of 2 cows from NBC and 4 cows of BC, the number of cows were marked along the profile to show the number of cows calculationg to the data at each point. Blue arrows mark the time of AI for cows in BC group and red arrows mark the time of AI for NBC group.

Figure 4.14: The progesterone concentrations for animals from both NBC and BC group on their oestrous cycle when they were served but failed to conceive. Profiles were obtained from the average concentrations of 5 cows from NBC and 2 cows of BC, the numbers of cows are marked along the profile to show average concentration for a number

of cows at a certain point. Blue arrows mark the time of AI for cows in the BC group and red arrows mark the time of AI for the NBC group. This shows that most cows that were not pregnant received AI at the wrong time for both treatment groups.

Figure 4.15: The progesterone concentrations for animals from both NBC and BC groups from the day that they were served and conceived at the first service.

Figure 4.16: The progesterone concentrations for animals from both NBC and BC groups from the day that they were served but failed to conceived to the first service.

Chapter 5. Influence of bull exposure on conception at a synchronised oestrus in postpartum dairy cows

Figure 5.1: The illustration of the experimental protocol.

Figure 5.2: The diagram of PRID protocol.

Figure 5.3: Mean (± SEM) number of steps shown by cows during the PRID treatment and following the oestrous syncrinosation protocol. Day 0 indicates a day after PRID withdrawal, results shows that there is no significant difference in the increase of steps during oestrus NBC and BC cows showing this indicator (NBC=2, BC=3).

Figure 5.4: The mean (±SEM) time in minutes spent by cows in the BC group that showed increased interaction with the bull (n=6), measured from 24 hours before PRIDs were removed from cows (on day -1) and continuing during the predicted period of oestrus until after cows received AI (day 2).

Figure 5.5: Mean (±SEM) of progesterone concentration from daily milk samples for all animals either pregnant or non-pregnant for a 12 day period starting from the day before PRID withdrawal until 11 days after PRID treatment in both the NBC and BC group (NBC=13, BC=15).

Figure 5.6: The mean (±SEM) of milk progesterone concentration for all animals, either pregnant or non-pregnant, from one day before PRID removal (starting day; Day 0) until 45 days in the treatment groups (NBC=13, BC=15).

Figure 5.7: The mean (±SEM) progesterone concentrations for cows that were pregnant, detected by sustained high progesterone concentrations for more than 30 days after insemination, in the treatment groups (NBC=2, BC=6). Day 1 indicates the day before PRID withdrawal.

Figure 5.8: The mean (±SEM) progesterone concentrations for cows which were not pregnant, determined by a rise in progesterone concentrations followed by a decrease to below 1.5ng/ml, for the treatment groups (NBC=11, BC=9). Day 1 indicates the day before PRID withdrawal.

Chapter 6. General discussion and conclusion

Figure 6.1: Diagram illustration of the factors that influence the interval of resumption of ovarian cyclicity from calving in postpartum dairy cows.

Figure 6.2: Diagrammatic representation of the progesterone concentrations in cows post caving. The first rise of progesterone occurs after the first ovulation, which normally silent oestrus. The interval during low progesterone concentrations is the first inter luteal phase (marked as Y), oestrus signs were observed during this period in this study.

Chapter 1

General Introduction

Chapter 1. General Introduction

1.1 Background of dairy production in UK

The dairy cows in the UK are commonly managed under intensive conditions with animals either housed all year round or outside for approximately six months for grazing during the spring and summer. The animals are milked two or three times a day depending on the farm routine, though on-demand robotic milking systems are also becoming more common. These intensive systems are being applied globally with the intention to increase the efficiency of milk production (Dairyco, 2012). The major concern in intensive dairy production is to increase the yield per cow, as this helps in reducing the cost of production (Sorensen *et al*., 2006). At the present time, by the association of effective management systems with modern selected breeds of dairy cow such as Holstein, very high milk yields can be achieved (Petit *et al*., 2002). For example, in 2011, the UK annual average milk yield was 7533 litres per cow per annum; this figure shows a large increase compared to 6346 litres per cow per annum in 2001. The comparisons of average milk yield in the UK from 1975 to 2011 are shown in Figure 1.1.

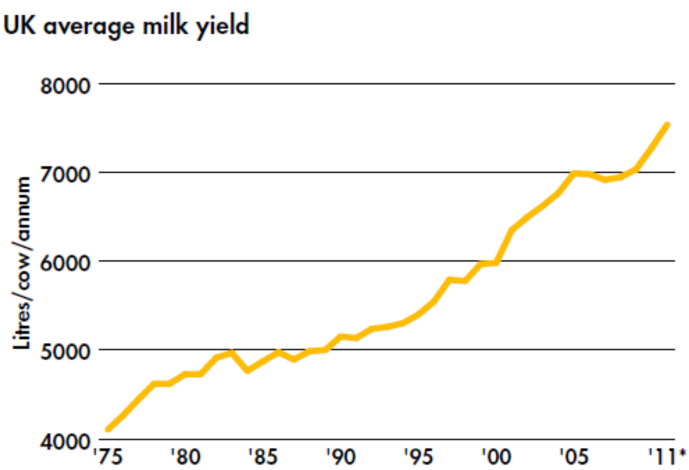


Figure 1.1: The average milk yield of dairy cows in the UK shows about 50% increase in production in from year 1975 to 2011 (Dairy statistics, Dairyco, 2012).

On the other hand, the dairy statistics report by Dairyco (2012) shows that the number of dairy farms in the UK has fallen compared to a few years ago, resulting in a reduction in the total number of dairy cows, as shown in Figure 1.2. This is probably due to increasing production cost as feed prices, oil prices, cost of land rent and other production costs have increased. The costs of dairy concentrate rations rose from £154 per tonne in 2006 to £234 per tonne in 2011. However, the average of herd size has also grown to 123 cows per farm in 2011 compared to only 83 cows back in 2001. Since the number of milk producers has reduced and the cost of production has increased, milk production has needed to be increased to fulfil the demand for consumption and maintain profit for producers. The bigger herd size and large proportion of dairy cows inseminated artificially has facilitated an increased rate of genetic improvement (Waariach *et al*., 2008) focussed on increasing milk production. Therefore, the use of a bull in many herds is no longer essential which also can reduce the cost of production by reducing the cost of running a bull (Vishwanath, 2003).

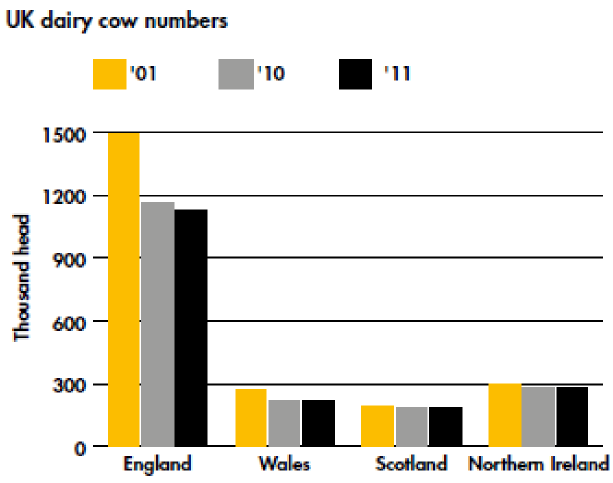


Figure 1.2: The numbers of UK dairy cows compared from 2001 to 2011 in England, Wales, Scotland and Northern Ireland (Dairy statistics, Dairyco, 2012).

1.1.1 *Managing dairy production*

In managing dairy production, there are many variables involved which interact in determining overall milk yields as well as the profit earned. These include nutrition, the breeding program, the environment provided by the housing system and also health management (Breen et al., 2009) as illustrated in Figure 1.3. Nutrition is one of the most critical aspects, as cows require good feed to deliver high milk production as well as to maintain their body condition and fertility (Wathes, 2010). Additionally, the breeding program is also crucial to produce offspring with high genetic merit for milk production and good reproductive performance (Dobson et al., 2007). As artificial insemination is normally used to breed dairy cows, it is essential for farmers to use good quality semen to achieve the production aims (Hafez, 1993). Housing systems with effective slurry management can improve animal welfare by providing a better environment. Health aspects are also important and need to be managed well to maintain good body condition, as suggested by International Dairy Federation (2011). The challenge in dairy production is the good management of these listed variables to increase milk yields and the efficiency of production.

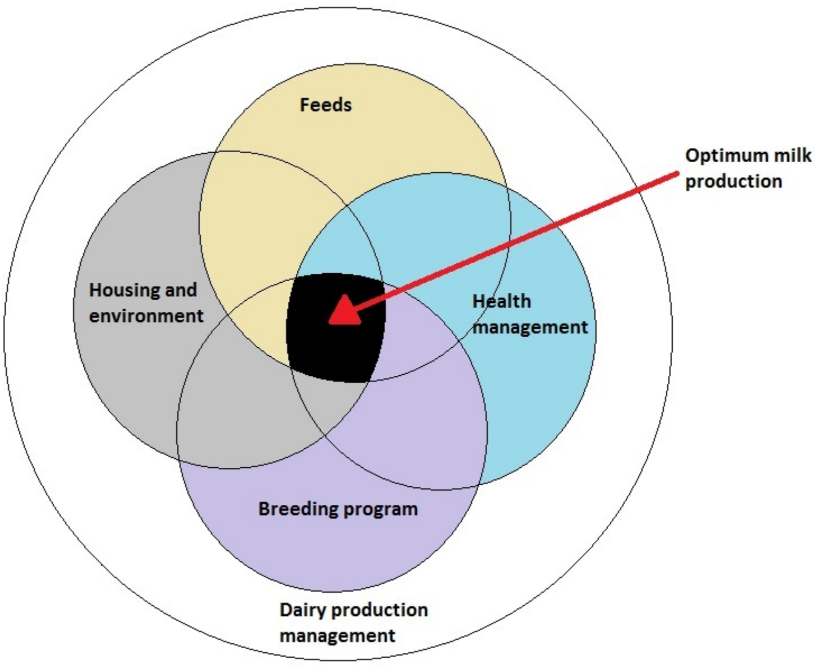


Figure 1.3: Illustration of variables involved in dairy production to obtain the optimum milk production.

1.1.2 *The dairy production cycle*

Within intensive dairy production systems, the optimum production cycle length for a dairy cow is suggested to be 365 days, or one year (James and Esslemont, 1979). This plan was established to be financially beneficial and to increase the lifetime production of a cow. The natural lifespan of a dairy cow is approximately 25 years; however dairy cows are rarely kept longer than five years (FAWC, 2009). Herd life is strongly correlated with production levels; normally lower production cows potentially live longer than high production cows, but if less profitable they are sent for slaughter at a younger age. Usually, dairy cows will have their first calf at two years old, and are milked after calving for approximately 305 days. Certain breeds produce more milk than others; different breeds produce within a range of around 4,000 to over 10,000 kg of milk per year (Dobson et al., 2007). Production levels peak at around 40 to 60 days after calving.

The postpartum period is the period following parturition in which lactation starts and reproductive cycles are re-established. The interval from parturition to first ovulation averages about 20 days and the time to first oestrus averages about 34 days (Ball and Peters, 2004). During the postpartum period, the farmer will try to get the cow back in calf within 85 days of calving. Production declines steadily after peak production at 40-60 days, until lactation is terminated at about 305 days after calving. At the end of the lactation period, cows will be dry for 60 days to prepare them for calving again. One year after the birth of her previous calf, a cow must calve again in order to keep to the cycle of one calf per year per cow. This system will give the optimum yield from the cow and will keep a good profit margin for the producer. Nevertheless, high production cows are more difficult to breed at a one year interval, normally 13 or 14 month calving intervals are more appropriate for this type of cow with concern to reduce losses of lifetime yield from prolonged calving intervals (Arbel et al., 2001). Dairy cows may continue to be economically productive for a number of lactations; ten lactations are possible depending on their conditions. However, in UK conditions the optimum lifespan to provide economic value for normal healthy cows is between 4.3 and 4.9 lactations (Stott, 1994). Replacement female calves will be kept for approximately 15 months before they are ready to be inseminated for the first time.

1.1.3 *Current cow production cycles*

The report from National Milk Records (NMR, 2012) shows that the average calving interval of dairy herds in the UK is around 427 days. This prolonged calving interval normally happens because of delayed rebreeding and can be a problem due to the increase in production costs. Cows will be milked for a longer period in late lactation with low milk production, have a longer dry period, require an increase in number of inseminations per pregnancy and may also incur infertility treatment costs. For example, CAFRE (2005) reviewed the comparison between 12, 15 and 18 month calving intervals. The report showed that for 12 month calving interval, the average annual yield for 365 days was 8000 litres and the dry period normally would be 8 weeks. Milk production was lower for a 15 month calving interval, with 7258 litres for 365 days, and the dry period was longer, in the range between 10 to 12 weeks. For an 18 month calving interval, milk yield for 365 days was much lower at 6522 litres and the dry period could be up to 13 to 20 weeks. These figures show that a prolonged calving interval may reduce total milk production per unit of time and thus not be cost effective.

1.2 Dairy production in Malaysia

Similarly to other countries, in Malaysia dairy cattle are bred intensively for the ability to produce large quantities of milk, from which dairy products are produced. However, at the current time, the number of dairy cattle is insufficient in Malaysia to meet the demand for fresh milk which has increased every year. The dairy industry in Malaysia is therefore mainly dependent on imported milk and milk products. The self-sufficiency for dairy products is only 4.5%. In 2001, only 26,184 dairy cows were bred in Malaysia to produce milk however the demand for consumption is approximately 1,097.96 million litres of milk product. From 2000 to 2001, the demand for fresh milk increased by 5%. Therefore, to resolve this situation, milk was imported from Australia and New Zealand to meet consumer demand; in 2001, milk imported was increased by 21% with a value RM1.4 billion, approximately equal to £0.3 billion. The price of fresh milk varies, depending on the producer, between RM1.50 (£0.32) to RM4.00 (£0.86) per litre (DVS, 2008).

A dairy cow must be bred and produce calves; depending on market conditions, the cow will either be bred with a dairy bull or a beef bull. Heifers with dairy breeding may be kept as replacement cows for the dairy herd. If a replacement cow turns out to be a substandard producer of milk, she then goes to market. Generally, dairy cattle bred in Malaysia are of the species *Bos Indicus* as this genotype is well adapted to the tropical environment. The Mafriwal breed of dairy cattle has been developed by the Division of Veterinary Services (DVS) to meet the demands of the Malaysian dairy industry. The Mafriwal was produced by crossbreeding of the Friesian and the Sahiwal breeds, imported from Australia and New Zealand, to produce high milk yield under the normal environmental condition in Malaysia, which is high in ambient temperature (DVS, 2009). The average milk yield of this breed is approximately 2337 kg/lactation.

In an effort to boost the local dairy industry, recently the government has launched a new policy, with investments through the national development program, 10^{th} Malaysia Plan (2011-2015). This plan includes the development of the three major groups of government dairy farms, with 27,000 dairy cows along with downstream processing facilities, to increase milk self-sufficiency in Malaysia by the year 2020. In addition, the

Department of Education will reintroduce a school milk program to improve consumer perception of the local dairy industry while ensuring a market buyer (MoA, 2010).

1.3 Common problems in dairy production

Whilst great improvements in milk yield have been achieved, there is concern that higher production of milk has been accomplished at the expense of greater metabolic stress on the cows (Walker *et al*., 2008), as well as reducing reproductive performance (Yaniz, *et al*., 2006) and also animal welfare (Sorensen *et al*., 2006). There is a negative relationship between rising milk yields and decreases in fertility (Lopez-Gatius, 2003). High milk production is very physically demanding, since cows also have to maintain good body condition to be bred again and to carry the next calf. According to the data from previous studies by Royal *et al*. (2000) and Butler (2003), the massive increase in milk production does appear to bring negative effects in terms of decline in reproductive performance. Regardless of location around the world, this problem has been a major challenge in dairy production.

1.3.1 *Infertility*

Infertility has now been identified as one the common problems of dairy production in the UK, as well as in other countries. Fertility in dairy cows may be described as: ‘the ability of the animal to conceive and maintain pregnancy if served at the appropriate time in relation to ovulation’ (Darwash et al., 1997). UK figures for conception rate to first service have declined over time; as shown in a study by Royal *et al*. (2000), the rates were around only 40% in the period 1995-1998 compared to 60% in 1975-1982. Furthermore, poor fertility has become a major contributing factor to the high total annual culling rate (currently 23.8%) in UK dairy herds. As a consequence, this will lead to a rise in the cost of production as culled cows need to be replaced.

1.3.2 *Nutritional effects*

Generally, in the early postpartum period, cows will have a problem with energy balance caused by the increased energy output associated with high milk yield (Robinson *et al*. 2006). Consequently the cow loses body condition, which may affect the first postpartum oestrous cycle and the subsequent oestrus. A prolonged anoestrus period can be a sign of temporary depression of ovarian activity. The nutritional status directly affects the development and production of an oocyte, ovulation, oestrous cyclicity, fertilization rate, the development of the fertilized gamete and the whole period of gestation (Robinson *et al*, 2006). Lower body condition will affect the circulating levels of hormones which are the major stimulators of oestrous behaviour, and consequently affect fertility levels (Wright *et al*. 1992). Cows in negative energy balance may have extended periods of anovulation or anoestrus, and show poor oestrus expression which will cause difficulty in detecting oestrus (Scaramuzzi and Martin, 2008). When oestrus is not manifested in a cow during lactation for an extended period, it is challenging to inseminate the cow at the right time, which can prolong the calving interval. The nutritional approach has considerable potential to enhance the efficiency of ovarian activity (Royal et al., 2000).

1.4 The importance of maximizing the efficiency of dairy cattle reproductive performance

The Holstein breed has been used widely for high milk production in the UK, but these genetics have been associated with a higher rate of metabolic problems including lameness, mastitis and infertility (Biefeldt *et al*. 2005). As the reproductive performance declines, failure to achieve pregnancy will cause a prolonged calving interval, a decreased number of calves born per lifetime, lost lifetime milk yield and increased involuntary culling rates (Walker *et al*., 1996). For this reason, it is essential to seek factors and methods that enhance reproductive performance and fertility in order to improve total production in dairy cows.

Chapter 2

Literature Review

Chapter 2. Literature Review

2.1 The reproductive system of dairy cattle

The efficiency of reproductive performance has a major impact on profitability of dairy farms (Santos *et al.* 2009). It can be represented by a measure of the ability of a cow to become pregnant within a desired time. The fertility rates are strongly reliant on endocrine function (Sartori *et al.*, 2004), which can be influenced by the management system and nutrition provided (Boland and Lonergan, 2003). Pryce *et al.* (2004) explain that fertility rate can be measured by the age at puberty, conception rate to first insemination and maintenance of pregnancy, and the most appropriate duration of calving interval is approximately 365 days. Previous study found that fertility is better in heifers compared to lactating cows, as the conception to first service rates were observed to be 64% and 71% in heifers of high and average genetic merit, whereas conception rates were 39% and 45% for lactating cows of high and average genetic merit respectively (Pryce *et al.*, 1999).

2.1.1 *The oestrous cycle*

The bovine oestrous cycle is a dynamic process that involves hormonal control of the reproduction system, which is also associated with behavioural changes. Oestrous cycles begin after the animals reach puberty, defined as the age at the first expressed oestrus with ovulation, occurring when Gonadotropin Releasing Hormone (GnRH) is produced by hypothalamus at sufficient levels to regulate follicle growth, oocyte maturation, and ovulation (Taylor, 1995). There are several factors contributing to age at puberty, such as nutritional intake, genetic traits and other environmental factors (Robinson, 2006). Normally, for dairy cows, the average age at puberty is 11 to 13 months (Pryce *et al*, 2004). The normal length of the oestrous cycle is approximately 18 to 24 days (average 21 days) in non-pregnant cows and is controlled by an endocrine system involving several different hormones produced in the hypothalamus, anterior pituitary, ovary and uterus (Ball and Peters, 2004). The cow is a polyoestrous animal, which means that once the oestrous cycles have started they will continue throughout the year unless pregnancy occurs, when the cycle will stop to allow the embryo to develop. In a multiparous animal, the first observed oestrus following pregnancy occurs

3 to 6 weeks after calving; however the length of the cycle varies between cows, breed and herds. This postpartum period is also important for involution of the uterus (Butler 2001).

According to Ball and Peters (2004) the oestrous cycle in cattle can be divided into four phases which are oestrus: the period of sexual receptivity prior to ovulation (day 0); metoestrus: the postovulatory period (days 1-4); dioestrus: when an active corpus luteum is present (days 5-18); and prooestrus: the period of approximately 3 days before next oestrus (days 18-20). Partitioning of the cycle can also be described according to the two different types of ovarian structures in the oestrous cycle, determined as the follicular phase and luteal phase.

2.1.1.1 *Follicular phase*

The follicular phase refers to the period of the development of the mature follicles to form the oocyte of suitable quality for ovulation and capable to be fertilised (day 18-21 of oestrous cycle). The follicles grow in a wave pattern, which starts with the emergence of a group of follicles. Subsequently, the selected follicle continues to grow and becomes dominant; during this period the growth of any other follicles is held back. Multiple hormones are involved in influencing the development of the dominant follicle. At the establishment of the follicular wave, the increase in Follicle Stimulating Hormone (FSH) concentrations produced by anterior pituitary will lead to the recruitment of a group of follicles (Mihm *et al.*, 2002). After a group of follicles has been formed, the secretion of FSH falls and the dominant follicle growth and development is continued which indirectly triggers a surge of Luteinizing Hormone (LH) produced from anterior pituitary. The increasing levels of oestradiol influence the release of Gonadotropin Releasing Hormone (GnRH) and this affects the LH surge, which is associated with behavioural signs of heat as oestradiol increases in concentration (Blowey, 1999). The secretion of FSH remains at lower concentrations to prevent a new follicular wave during the development of the healthy dominant follicle. Subsequently, the dominant follicle ovulates or undergoes atresia, and then the secretion of FSH rises to induce a new follicular wave (Beam and Butler, 1997).

2.1.1.2 *Luteal phase*

Following ovulation, the ovulatory follicle changes function to develop luteal cells and the corpus luteum (CL) is formed. This is the luteal phase (day 1-17 of oestrous cycle); during this phase progesterone (P4) is the primary steroid product of the corpus luteum and is secreted in high concentrations (Okuda *et al.* 2001). The corpus luteum is the major structure on the ovaries during this phase of the oestrous cycle and it increase in size from the early oestrous cycle, with increasing progesterone production. The dominant follicle will not be ovulated while the progesterone level is high. Furthermore, high progesterone concentrations will inhibit the cow from expressing any behavioural signs of oestrus. If the ovum is not fertilised, the corpus luteum will disappear, along with a decrease in progesterone concentration, which allow the next ovulation to occur.

2.1.2 *Oestrus*

Oestrus is the physiological stage during which a cow is likely to stand to be mounted and is ready for insemination. This is the limited period of sexual receptivity, characterized by intense sexual motivation, when the female will seek the male (Thomas & Dobson, 1989). Moreover, standing oestrus is also referred to as standing heat, which acknowledges the most significant visual sign of oestrus occurrence (Roelofs *et al.*, 2007; Van Eerdenburg *et al.*, 1996). This standing behaviour occurs under the influence of patterns of secretion of hormones from the brain and reproductive organs.

2.1.3 *Hormonal control of oestrous cycle*

Dairy cattle reproduction is controlled by multiple hormones that are produced by several endocrine glands and secreted into the blood to be transported throughout the body to perform their respective functions (Figure 2.1).

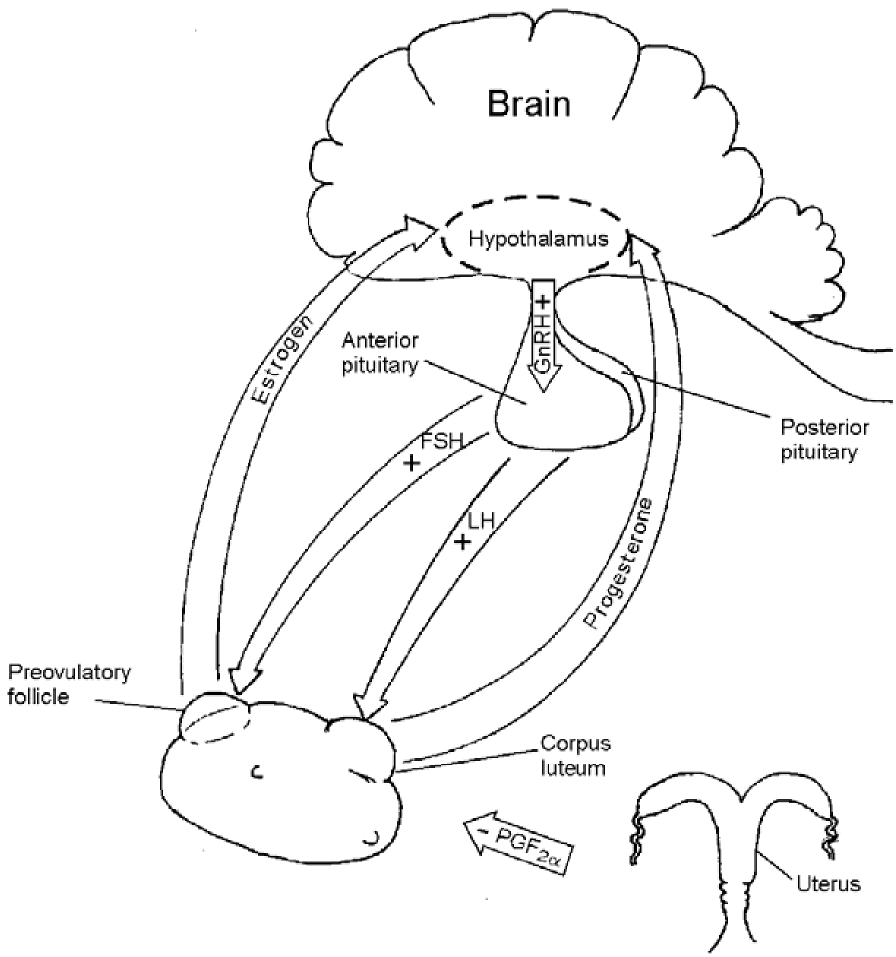


Figure 2.1: Schematic representation of the interrelationship between hypothalamus, anterior pituitary gland, ovary and uterus, in regulation of hormone secretion (Larson and Randle, 2008).

2.1.3.1 *Gonadotropin-Releasing Hormone*

Gonadotrophin-releasing hormone (GnRH) is synthesised in the hypothalamus, in response to other reproductive hormones (Figure 2.1). The surge of GnRH subsequently causes the release of the pituitary hormones, LH and FSH. High concentrations of progesterone will inhibit the action of GnRH, whilst absence of progesterone allows the release of the GnRH surge (Larson and Randle, 2008).

2.1.3.2 *Luteinizing Hormone*

This hormone plays a crucial role in cattle reproduction. It is produced and stored in the anterior pituitary gland and the release of this hormone is controlled by GnRH. LH is necessary to stimulate follicular development and luteinising of the mature ovulated follicle. It initiates ovulation of the ovulatory follicles and promotes growth and function of the corpus luteum. During low activity periods, 1 pulse is released into the bloodstream every 6-8 hours, but as the dominant follicle get closer to maturation the frequency of LH release increases to every 30 minutes. Every pulse of LH will stimulate the production of oestradiol secretion in the ovary (Ginther et al., 2001).

2.1.3.3 *Follicle Stimulating Hormone*

Follicle stimulating hormone (FSH) is also produced and stored in the anterior pituitary gland. Similar to the releasing mechanism of LH, the release of FSH into the bloodstream is also controlled by GnRH. One of the major purposes of FSH is to influence the maturation of follicles and it is necessary to stimulate follicular development (Blowey, 1999).

2.1.3.4 *Oestradiol*

Oestradiol is an ovarian hormone, produced by developing follicles. The dominant follicle that develops later in the cycle continues to mature then produces rising amounts of oestradiol (Hampton *et al.*, 2003). The presence of higher blood concentrations of oestradiol will caused changes that are associated with oestrus, including enlargement the vulva and mounting behaviour. The dominant follicle will mature and ovulate 6 to 18 hours after the end of standing oestrus (Brewster and Cole, 1940). Figure 2.2 shows the development of follicles and formation of the corpus luteum, as influenced by secretions of the reproductive hormones involved.

2.1.3.5 *Progesterone*

Progesterone is also an ovarian hormone, produced by the corpus luteum in the ovary. The presence of progesterone causes opposite effects to those of oestradiol. The main function is to prepare the uterus to accept the fertilised egg. High concentrations of progesterone suppress signs of oestrus and also suppress the release of the hormones FSH and LH (Ireland and Roche, 1982).

2.1.3.6 *Prostaglandin (PGF2α)*

Prostaglandin F2 alpha (PGF2α) is produced from the wall of uterus after 16 to 18 days absence of pregnancy from the time of ovulation. The secreted PGF2α will pass to the ovary and initiate breakdown of the corpus luteum, with resultant reduction of progesterone concentration. This will allow the release of GnRH from the hypothalamus to initiate the next cycle (Blowey, 2011).

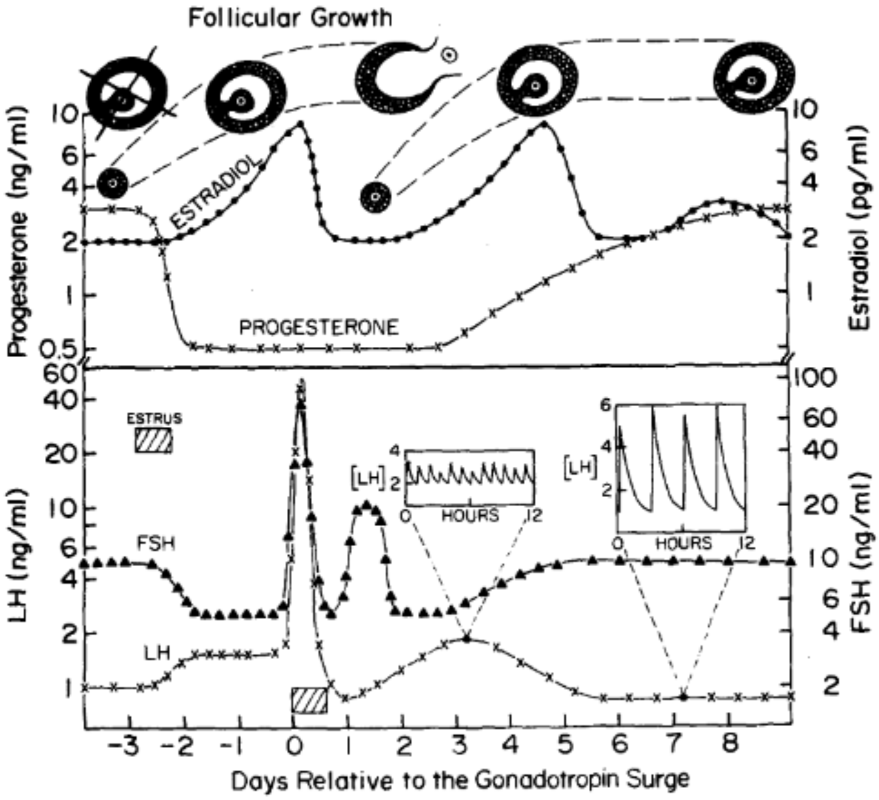


Figure 2.2: Schematic representation of the endocrine regulation of the bovine oestrous cycle, the development of follicles and formation of a corpus luteum is associated with the concentrations of LH, FSH and oestradiol (Hansel and Convey, 1983).

2.1.4 *Signs of oestrus*

During the oestrous period, cows will normally show several changes in their behaviour including chin resting, sniffing the vulva of other cow, flehmen, being mounted by other cows but not initially standing, mounting other cows and finally standing to be mounted; these are behavioural signs of oestrus (Roelofs *et al.*, 2005). There are a variety of factors that can influence the nature of oestrus expression displayed by cows (Orihuela, 2000). Normally, cows at the onset of oestrus will show both mounting and standing activities. Reolofs *et al.* (2008) stated that standing to be mounted is the most precise sign of oestrus and can be used to predict the appropriate time for insemination to increase pregnancy rate. Other signs of oestrus which are observed may not be very accurate for use to predict time for insemination compared to the behaviour of standing to be mounted. Behaviours such as mounting and standing to be mounted can be influenced by how many cows in the group are in oestrus at the same time, so that they become sexually active and increase interactions with other cows (Galina and Orihuela, 2007). Galina *et al.* (1996) explained that it requires at least two sexually active cows to interact together to exhibit oestrus behaviour. Additionally, Roelofs *et al.* (2005) explained that some oestrus behaviours are displayed more frequently in this situation compared to when only one cow is in oestrus. Restriction of the ability for cows to interact with each other could reduce the exhibition of oestrus behaviour expression; in intensive production systems it is very challenging to detect oestrus behaviour due to lower signs of oestrus which may be shown by cows in a packed area (Roelofs *et al.*, 2005). Behaviours such as sniffing and chin resting are not good indicators of oestrus, since not all cows will show these behaviours at every oestrus (Solano *et al.*, 2005). Other symptoms such as restlessness, alertness and loss of appetite may also be shown by cows during oestrus (Sarkar and Prakash, 2005). Moreover, rubbing and licking are other signs of oestrus sometimes presented by cows (Peralta *et al.*, 2005).

Erandus *et al.* (1992) described how the increase in vaginal mucus secretion during oestrus is strongly related to the concentration of serum oestradiol. Since the release of vaginal mucus has a strong correlation to the oestrous cycle, it can be an indicator for detecting oestrus. However, sometimes the discharge of mucus is not visible externally (Gordon, 1996). In contrast, other studies suggest that the discharge of vaginal mucus might also be caused by non-oestrus parameters and therefore claim that it is not appropriate for oestrus detection (Brehme *et al.*, 2001, cited in Firk *et al*., 2002).

There are various types of automatic detection device which have been developed to improve the efficiency of oestrus detection. An implanted telemetric sensor used to measure vaginal mucus resistance was introduced for automatic oestrus detection (Firk *et al.*, 2002). The reported efficiency of this telemetry application was 81% (17 over 21 cows) of oestrus detected with 3 animals were false positives (Redden *et al.*, 1993). Other than this, pedometers are normally used to measure changes in level of activity during oestrus. Cows tend to become restless and increase walking activity during oestrus. Some pedometers can record step counts, lying and lying bouts of individual cows, and data will be sent to a computer system to inform the farmer if the cow is in oestrus (Roelofs *et al.*, 2005).

According to Dobson *et al.* (2008) there have been no changes in the duration of total primary and secondary signs of oestrus over the past 30 to 50 years, though the percentage of animals displaying stand to be mounted behaviour and the duration of its expression have declined (Figure 2.3). Furthermore, it has been proven that high milk production increases the number of silent heats (Harrison *et al.*, 1990). Moreover, the decline in number of staff in dairy units has made it more challenging to detect oestrus and get successful artificial insemination (AI), in order to get cows pregnant when required (Dobson, *et al.*, 2008).

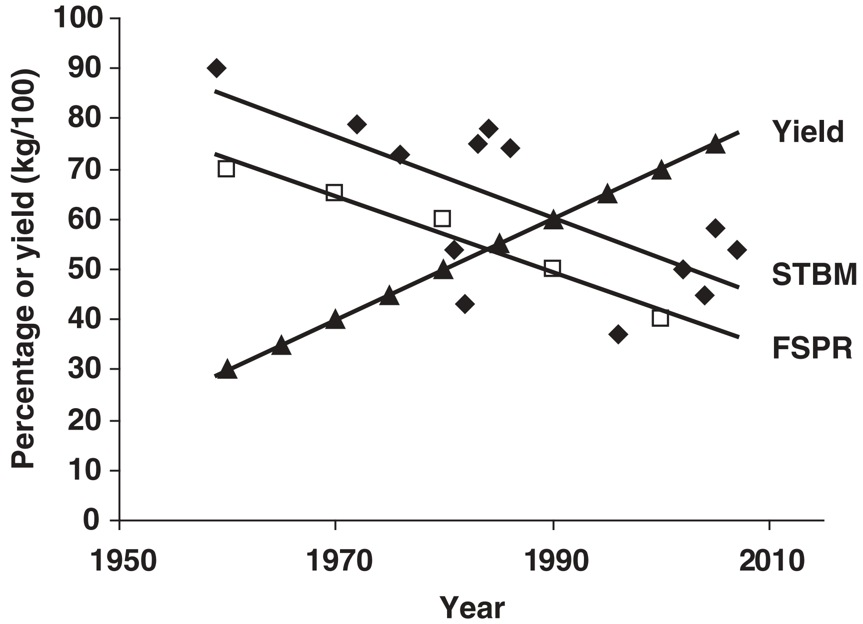


Figure 2.3: The observations of percentage of animals standing to be mounted (STBM ◆), and pregnancy rate to the first service (FSPR☐) in relation to the increase in average milk yield (Δ) in Holstein-Friesian dairy cows over the last 50 years (Dobson *et al.*, 2008).

2.1.5 *The postpartum period*

The interval from calving to the next conception strongly depends on the resumption of ovulatory activity, re-establishment of normal ovarian cycles post calving, the expression of oestrous behaviour at the appropriate time during the oestrous cycle, and the pregnancy rate following service (Peters, 1984). The interval from parturition until resumption of normal ovulatory activity is commonly shorter in dairy cattle compared to beef cattle (Wettemann, 1980) caused by genetic traits in these breeds. Progesterone is the main steroid synthesised by the corpus luteum, thus a concentration in milk of progesterone greater than 1.5 ng/ml can be a good sign of luteal function in the postpartum period, as it is associated with the presence of a corpus luteum (Gifford *et al.*, 1989). However, lack of follicular and luteal development during the postpartum period due to poor body condition after parturition results in an anoestrous condition (Wettemann, 1980).

Anoestrus is defined as state of complete ovarian inactivity, with no appearance of oestrus behaviour (Hafez, 1993). During this period, there will be no occurrence of ovulation and the ovary remains inactive due to inadequate LH pulses. This is not a disease but a period of recovery after pregnancy. However, prolonged anoestrus is a sign of infertility as, if the oestrous cycles fail to resume normally for a long period this will affect lifetime reproductive performance. There are multifactorial causes of extended postpartum anoestrus, including seasonal changes in the physical environment, nutritional deficiencies, lactation stress, high milk production, and aging (Hafez, 1993) as shown in Figure 2.4. Pregnancy reduces the sensitivity of the pituitary gland to GnRH (Schallenberger *et al.*, 1978), and the recovery of sensitivity starts to slowly increase after calving. The resumption of ovarian cyclicity depends on pulsetile LH secretion as influenced by GnRH (Peters and Lamming, 1983). However, inadequate concentration of oestradiol post calving may delay the induction of the preovulatory LH surge (Peters *et al.*, 1985). All of these deficiencies will lead to postpartum anoestrus.

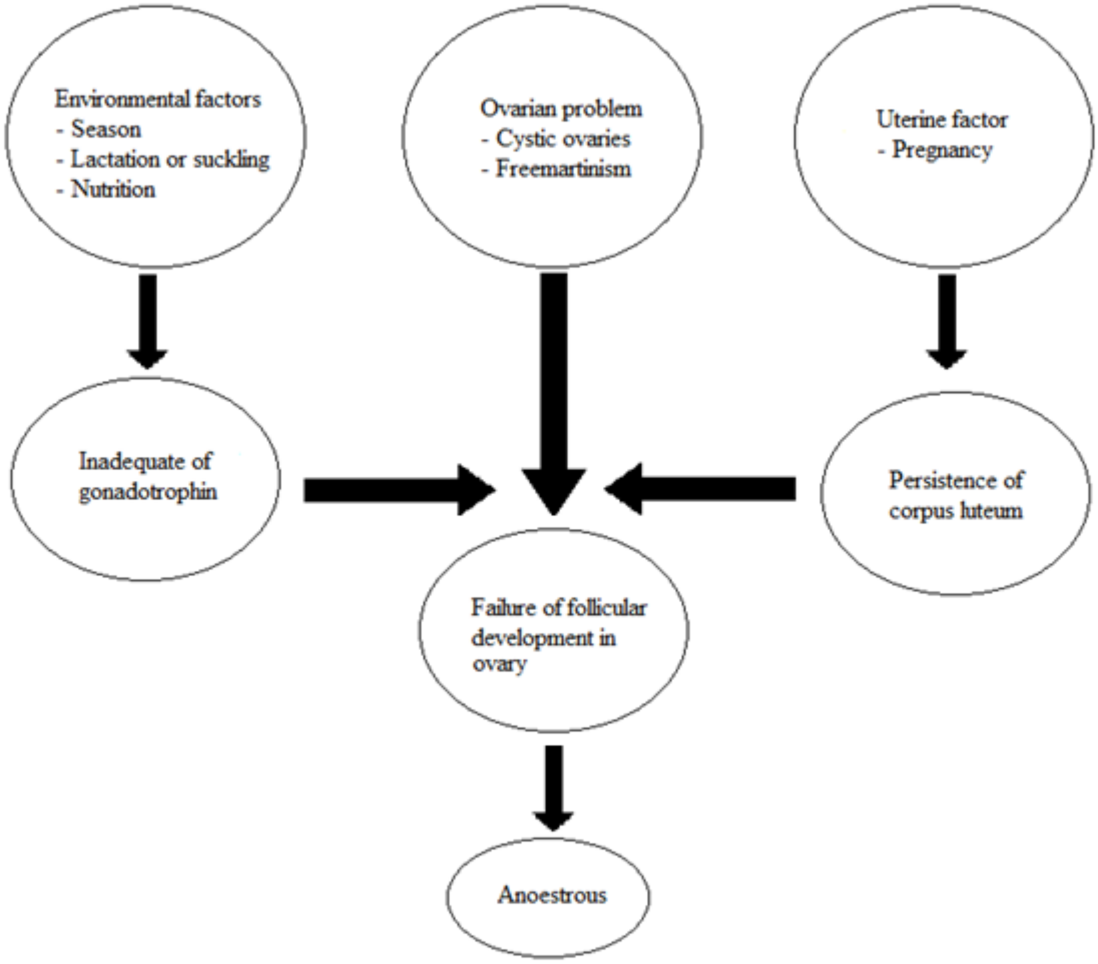


Figure 2.4: Schematic representation of a variety of possible causes leading to failure of follicular development in the ovary, and consequently anoestrous in cattle (adapted from Hafez, 1993).

After parturition, cows normally suffer from negative energy balance (NEB) during the early lactation stage (Wathes *et al.*, 2008), which will lead to a prolonged anoestrous period. It is well known that nutrition plays an important role and can be counted as a factor to determine the timing of the onset of oestrous cycling after calving. Especially for dairy cattle, more nutrients are required to synthesise energy for milk production and to maintain body condition during the early lactation period. The hypothalamic response in terms of release of LH may be influenced by the nutritional status of cows (Stumpf *et al.*, 1987). Thus, energy intakes during the postpartum period play an important role in reproductive performance. Insufficiency of nutrients can cause metabolic and endocrine changes leading to mobilisation of body tissue; this unfavourable condition causes inhibitory actions at the level of the brain, ovary and reproductive tract which prevent the cow establishing a pregnancy (Bauman and Currie, 1980).

Consequently, postpartum infertility in cows is often due to a prolonged period of anoestrus as ovulatory activity fails to resume normally. Furthermore, reduced oestrus expression by cows may also further decrease the percentage of successful inseminations (Fike *et al.*, 1996) as cows may be served at the wrong time. The duration of calving interval is influenced by many factors including breed, level of milk yield, age of animal, suckling or lactation status, nutritional level before and after calving, season and associated photoperiod, climate, health status and calving difficulty. Nutrition and milk production appear to be the most important among the listed factors.

2.2 Reproductive management

Currently, the fertility indicators of the rate of conception and pregnancy to the first service have fallen by approximately 1% each year in the UK (Royal *et al.*, 2000) and 0.45% in the USA (Beam and Butler, 1999). Number of service per conception has increased, as well as the proportion of cows with one or more abnormal hormone patterns in the past few years. Therefore, the main purpose of developments in mammalian reproductive technology is to preserve and sustain fertility (Lopez-Gatius, 2003) since infertility has become one of the major problems in animal production, especially for dairy cattle (Blowey, 2011).

2.2.1 *Hormonal manipulation*

Hormonal treatments are widely used on farm to stimulate the resumption of ovarian cyclicity post calving by oestrus synchronisation. Generally, there are two types of hormonal treatment given: either using gonadotropins to stimulate follicular growth or application of steroid treatments, which will stimulate the hypothalamus and pituitary and consequently increase the secretion of endogenous hormones. GnRH treatment has resulted in the induction of ovulation and initiation of normal regulation of the oestrous cycle in dairy cows (Britt *et al.*, 1974). Other than this, the most common treatment used to manipulate hormone secretion is a progesterone-releasing device, such as the Progesterone Releasing Intravaginal Device (PRID) and Controlled Internal Drug Release (CIDR). Oestrus synchronisation protocols concentrate on controlling the time of oestrus occurrence; the negative feedback of progesterone is useful to synchronise oestrus by suppressing production of LH and FSH. After 12 days, the removal of the progesterone device, with conjunction of corpus luteum regression, causes a sudden drop of progesterone concentrations. This will induce secretion of GnRH and produce LH pulses, which stimulate the development of the dominant follicle. Continuous progesterone treatment for 5 days during the postpartum period has increased the frequency of pulsatile secretion of LH (Macmillan *et al.*, 1995).

Overall, the effectiveness of these treatments depends on many factors including prepartum nutrition, body condition, energy intake by the cow, suckling, lactation and breed. On top of this, other factors that affect fertility include nutrients intake, management during the dry period, the skill and ability of technicians to detect oestrus and AI the animal, and environment (Lopez-Gatius, 2000; Roche *et al.*, 2000; Sturman *et al.*, 2000). Because of all these reasons, the effect of treatments may sometimes not be very successful to reduce the postpartum anoestrous interval in cattle (Wettemann et al., 1978).

2.3 The potency of male exposure in improving reproductive performance in females

Various techniques and strategies have been developed and implemented with the intention to improve reproductive performance in cattle, but most of the techniques involve the use of multiple hormones as treatments (Patterson *et al.*, 2003). Nevertheless, nowadays there is an increase in community awareness about livestock production and a demand for a “clean, green and ethical” process of production (Martin *et al.*, 2004). Therefore, other more natural approaches such as biostimulation have been implemented in farm management to reduce the use of hormonal treatments (Fiol *et al.*, 2010). However, the knowledge about biostimulation use to improve reproductive performance in farm animals is limited, especially in dairy cattle.

2.3.1 *Introduction to male effects on the female*

The presence of a sexually mature male animal has been shown to generate a positive effect on the onset of oestrus in sheep and goats during their breeding season and to increase the expression of oestrus in sows as it improves the postpartum ovarian activity and encourages oestrus expression (Langendijk *et al.*, 2000 and Rekwot *et al.*, 2001). If a bull is in a pen sited near to the cows, those cows that are in oestrus will normally move near to the bull (Gordon, 1996). Besides this, the duration of postpartum anoestrus in suckled beef cows was decreased when exposed to bulls or excretory products of bulls (Miller and Ungerfeld, 2008). Thus, the bull’s stimulatory effect on postpartum cows could be a useful treatment, as it is known to improve the proportion of cows that conceive (reviewed by Rekwot *et al.*, 2001).

2.3.2 *Possible cues in biostimulation effects*

In this context biostimulation is the term used to describe the stimulatory effect of a male on oestrus and ovulation through genital stimulation, olfactory pheromones, or other less well defined external cues such as tactile, visual and auditory (Chenoweth, 1983). Izard and Vanderbergh (1982) found that the interaction of a bull and cow influenced reproductive activity via olfactory cues. This biostimulation by a bull is delivered through the combination of olfactory, via pheromones, and other cues.

2.3.2.1 *Pheromones*

Pheromones refer to air-borne chemical substances that transfer specific information and consequently cause a specific behavioural reaction or physiological change in the recipients endocrine or reproductive system (Izard, 1983). Research shows that in cattle, priming pheromones from the male have an influence by hastening puberty, termination of seasonal anoestrus and reducing the postpartum anoestrus period (Izard, 1983, Fike *et al.*, 1996; Gifford *et al.*, 1989; Rekwot *et al.*, 2001; Berardinelli *et al.*, 2005; Miller and Ungerfeld, 2008; Tauck *et al.*, 2010). Currently, not much information is known about bull pheromones (Roelofs *et al.*, 2008), nevertheless the biostimulatory effect of the bull seems to be mediated by pheromones present in their excretory products (Berardinelli and Joshi, 2005). In cattle, urine, faeces or cutaneous glands are an expected source of pheromones that mediate the biostimulatory effect of bulls on resumption of the postpartum luteal activity in cows and hasten the onset of puberty in heifers (Rekwot *et al.*, 2001 and Fike *et al.*, 1996, Tauck and Berardinelli 2007).

2.3.2.2 *Non-pheromonal stimuli*

The stimulation through genital contact can favorably influence reproduction in cattle. In some study (Langley, 1978) better results of conception in cows that were inseminated by natural service compared to cows that were artificially inseminated (AI) have been found. Although possibly confounded by differences in semen quality this could be caused by genital stimulation to the cows by the bull either before or during service (Chenoweth, 1983). In addition, Fraser (1968) found that behaviors like nuzzling, nudging, and licking by the bull on the perineal area of the female have induced oestrous behaviours and could also prepare the female genital tract for optimal gamete transport. Furthermore, pregnancy rate in cattle has been improved by the effects of clitoral stimulation during AI by 6.3 to 7.5% (Randel et al., 1975). Other than this, visual and auditory cues are also known as sexual stimulators (Chenoweth, 1981). However Germain and Klemm (1989) claim that the stimulation by the bull is delivered strictly from olfactory cues, though, the effectiveness of stimulation by pheromones from the bull is critically associated with other non-olfactory cues. It is proven that bull biostimulation does influence reproductive activity in cows through all these cues (Zalesky *et al.*, 1984). Hence, biostimulation plays an important role in reproductive performance of animals as it influences endocrine changes on physiology and behaviours (Rekwot *et al.*, 2001).

2.3.2.3 *Reception of olfactory signals*

Figure 2.6 shows the location of the olfactory sensor in mammals; the chemical signal is received and analysed through the olfactory system, then producing attraction responses (Germain and Klemm, 1989). The olfactory signal is received via the main olfactory bulbs (MOB) and main olfactory epithelium (MOE), or through the accessory olfactory system via the vomeronasal organ (VNO) and accessory olfactory bulb (AOB). They are connected directly or indirectly to the hypothalamus, then stimulate GnRH secretion and in turn affect LH (Neills, 2006).

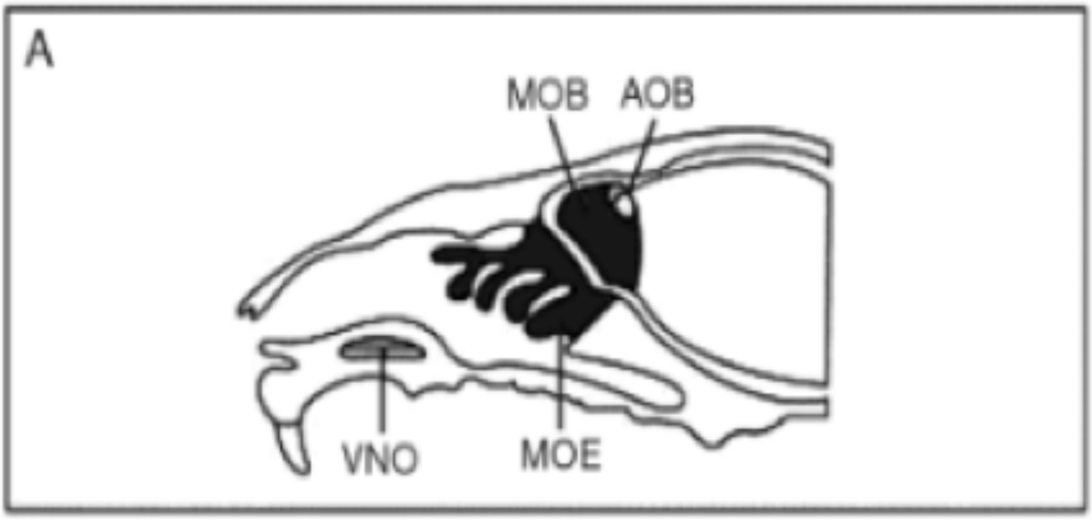


Figure 2.6: Schematic representation of the anatomy of the mammalian olfactory system showing location of the vomeronasal organ (VNO), the accessory olfactory bulb (AOB), the main olfactory epithelium (MOE) and the main olfactory bulb (MOB) (Neills, 2006).

2.3.3 *Biostimulatory effects in other species*

The male animal generates a sexual attraction that the oestrous female can identify and respond to. Exposing postpartum anoestrus suckled beef cows and heifers to bulls, either with close physical contact or by fenceline contact, can accelerate resumption of ovulatory activity (Fike *et al.*, 1996; Berardinelli *et al.*, 2005; Tauck *et al.*, 2010). However, the effect of male presence in cattle reproduction, especially in dairy cows, is not as clear as in sheep, goats or swine, as reviewed by Rekwot *et al.* (2001). Furthermore, insufficient work has been done to investigate the nature of the interaction between male and female in dairy cattle despite studies in many species of animal describing the female reproductive response to male exposure.

2.3.3.1 *Rodents*

In rodents, the role of biostimulation on endocrine response is well understood compared to other species. Puberty in mice is accelerated by pheromonal stimulation, which involves a hypothalamic-pituitary pathway (Izard and Vandenbergh, 1982). Studies by Bronson and Desjardins (1974) showed that serum luteinizing hormone (LH) concentrations increased in juvenile females within one hour of exposure to a male mouse, and remained elevated for several hours. This rise in LH secretion was followed by an increase in serum oestradiol within 3 to 6 hours after exposure to the male (Bronson and Desjardins, 1974). It has been demonstrated that caging female rats with fertile males could maintain the regular oestrous cyclicity and normal gonadotropin secretion for an extended period of time (Nass et al., 1982). Beside this, the female–female interaction in rodents can also affect ovarian function and age at puberty and these interactions are mediated by priming pheromones produced by grouped females (Izard, 1983).

2.3.3.2 *Sheep*

Ram presence has been identified as a stimulator for the occurrence of oestrous activity in ewes (Rekwot *et al.* 2001). Continuous ram exposure can enhance the proportion of ewes showing signs of normal oestrous cycles (O'Callaghan *et al.* 1994). A direct relationship between the duration of ram presence and the percentage of anovular ewes showing ovulation in response to the biostimulatory effects was identified, although a previous study found a decline in reproductive performance over time in ewes; return to anoestrus was high with continued exposure to rams. They concluded a possible development of habituation by ewes to the ram stimuli when exposed to the ram continuously. There was no difference in level of response in reproductive activity found in ewes exposed to rams continuously as compared to ewes isolated from ram contact. Thus, several weeks of isolation period is possibly required before ewes respond well to the biostimulatory effects of ram exposure (Cushwa *et al.* 1992).

2.3.3.3 *Swine*

In swine, a number of studies have found that high exposure to a boar does not improve the oestrus detection rate in weaned sows (Langendijk *et al.*, 2000; Hemsworth and Hansen, 1990 and Caton *et al.*, 1986), yet Kemp *et al.* (2005) reviewed that a certain level of boar stimuli is required for expression of oestrus in sows and gilts. Full expression of oestrus will be shown by sows at the maximum stimulation level of boar exposure that is applied (Langendijk *et al.*, 2000). However, continuous contact with a boar by housing sows adjacent to boars could reduce the oestrus detection rate. This indicates that sows habituate to higher levels of stimulation and express oestrus less.

A study by Tilbrook and Hemsworth (1990) compared the effects of different boar exposure treatments on the efficiency of detection of oestrus in gilts. They found that the efficiency of detection of oestrus in gilts was lower when the gilts were housed adjacent to boars and separated by a wire-mesh fence, compared to housing opposite to boars separated by a corridor, as shown in Table 2.1. This study supports the result from Caton *et al.* (1986), who found that the gilts housed adjacent to boars had a lower level of oestrus expression as compared to gilts exposed to a boar for 30, 15 or 5 minutes every day. In addition, continuous boar exposure caused shorter duration of oestrus in sows that were housed adjacent to boars compared to the sows that exposed to boars for a restricted period daily (Hemsworth and Hansen, 1990). Sows in oestrous can be attracted to the odour produced by a boar this is known as signalling pheromones (Izard, 1983), although there were several less well defined cues that may also be part of the cause of biostimulation.

Table 2.1. The effect of different treatments[l] on detection of oestrus in the gilts (Tilbrook and Hemsworth, 1990)

	a) Opposite	b) Adjacent	c) Olfactory	d) Auditory and Olfactory
Proportion of gilts detected in oestrus	23/24 (95.8%)	18/24 (75.0%)	24/24 (100%)	24/24 (100%)
Proportion of gilts detected in oestrus for more than one day	20/23 (87.0%)	7/18 (30.9%)	20/24 (83.3%)	20/24 (83.3%)
Mean (±SE) duration of oestrus (days)	2.3±0.2	1.1±0.2	2.7±0.2	2.5±0.2
Mean (±SE) interval between oestruses (days)	18.3±0.3	18.8±0.3	18.6±0.4	18.8±0.4

[l]Gilts were housed: a) opposite to boars and separated by a 1-m-wide corridor; b) adjacent to boars, separated by a wire mesh fence; c) isolated from boars with continuously exposed to boar pheromones (3α-androstenol and 5α-androstenone) for 5 min every 25 min for 8 h (olfactory); d) isolated from boars and concurrently exposed to the pheromones and a broadcast of the boar ‘courting song’ (auditory and olfactory).

2.3.4 *Biostimulation response in cattle*

The biostimulatory effects of bull exposure have initiated a favourable response by influencing hormone regulation and ovarian activity (Fike *et al.*, 1996; Berardinelli and Joshi, 2005; Berardinelli and Tauck, 2007 and Miller and Ungerfeld, 2008). A small number of previous studies have looked into the endocrine effects on ovarian function and expression of oestrus in response to exposure to bulls in lactating dairy cows. In postpartum suckled beef cattle, there are several studies that have found that bull exposure does influence reproductive activity in heifers and cows, as reviewed by Rekwot *et al.* (2001). Custer *et al.* (1990) suggest there is a possibility that the effect of the bull stimulation on resumption of ovarian cyclicity is mediated by the central nervous system, since LH release was stimulated immediately after exposure to the bull. However, the exact mechanism of biostimulation, and the transmission of the cues from bull to cow are less clear.

Fike *et al*. (1996) reported there was a slight effect of fenceline bull exposure on progesterone profile of cows, as cows that were exposed to a bull had increased progesterone concentration quicker than cows that were not exposed to the bull. This is similar to a study by Hornbuckle *et al*. (1995) in which progesterone concentration increased earlier during the postpartum period in beef cows that were exposed to the bull. This indication of resumption of ovarian cyclicity and the onset of the follicular phase by the decline of the progesterone level followed by a rise has been shown in previous studies (King *et al*., 1976, Darwash *et al*., 1999). Progesterone may promote increases of hypothalamic estradiol receptors during the luteal phase (Blache *et al*., 1994, cited in Roelofs *et al*., 2010), which consequently convey positive effects on expression of oestrus (Vailes *et al*., 1992). This indicates the importance of progesterone secretion in improving ovarian cyclicity, thus potentially increasing reproductive performance in dairy cows especially after calving. However the actual time of ovulation was not assessed in this trial.

2.3.4.1 ***Pre-pubertal effects***

Puberty is defined by the time that a heifer has her first ovulation. It is the result of many physiological events involving the hypothalamus, pituitary gland and ovaries. Age of puberty in heifers is influenced by many factors including age and breed of dam, breed of sire, environmental temperature, nutrition, body weight, growth rate and social environment (Schillo *et al.*, 1992). In cattle, the normal age to reach puberty is at 9 to 10 months for dairy breeds and 13 to 14 months for beef breeds. As the female reaches puberty, she starts cycling on a regular basis, and ideally is ready for her first insemination at 14 to 15 months of age. Delays in reaching puberty will cause a delay in age at first breeding and age of calving, increasing the expense of raising heifers.

The application of a biostimulation technique offers a potentially useful and practical tool to hasten puberty in heifers. In rodents, the presence of a male can accelerate the onset of puberty (Vandenbergh, 1974). Furthermore, Mavrogenis and Robinson (1976) revealed the same findings in swine; gilts that were exposed to a boar reached puberty at an earlier age compared to gilts that were not exposed to a boar. Thus, it is proven that biostimulation of exposing the female animals to a male helps accelerate puberty in other species. An experiment on the effects of exposure to bull urine on puberty in crossbred beef heifers showed that heifers treated with bull urine reached puberty earlier. This suggested that there is a priming pheromone in bull urine that can hasten the onset of puberty in beef heifers (Izard and Vandenbergh, 1982).

However, changes of behaviour in response to a biostimulation effect of bull urine were not observed in this experiment. Rekwot *et al.* (2001) reviewed that the presence of a vasectomised bull has been reported to hasten the onset of puberty in heifers and also the early resumption of ovarian cyclicity after the following parturition. In Nelore cattle, exposure of heifers to a bull during the prepubertal period decreased their age at first pregnancy (Oliveira *et al.*, 2009). The responsiveness to biostimulation of ovarian cyclicity in heifers can possibly be different depending on the nature of the exposure and the condition of interactions, such as the intensity and type of cue to which they are exposed. Moreover, this may only work under an adequate nutritional status (Izard and Vandenbergh, 1982).

2.3.4.2 ***Oestrous behaviour and hormone secretion***

Regarding the behavioural response of cows exposed to the bull, a study by Roelofs *et al.* (2008) showed that cows with bull exposure were attracted to the bull when they were in oestrus, as the frequencies of visiting the bull pen were increased during this period. However, other expressions of oestrus behaviour such as sniffing vulva, chin resting, flehmen, mounting other cows and standing to be mounted were not affected by bull exposure. The biostimulatory effect on circulating LH concentration is very small, and may be insufficient to increase the secretion of oestradiol, which would influence changes in exhibition of oestrus behaviour. Roelofs *et al.* (2007) however, found that fenceline bull exposure to anoestrous dairy cows during the early postpartum period did have an influence on LH release; there was an increase in basal and average LH concentration and the frequency of LH pulses.

2.3.4.3 ***Response of postpartum anoestrus animals***

The relationship between biostimulation from bull exposure and resumption of ovarian activity in anoestrus dairy cows has been investigated in very few studies (Rekwot *et al.*, 2001). Exposing postpartum anoestrus, suckled beef cows and heifers to bulls, either with close physical contact, fenceline contact, or by exposure to the excretory products of a bull has significant effects in accelerating the resumption of ovulatory activity (Fike *et al.*, 1996, Berardinelli and Joshi, 2005; Berardinelli *et al.*, 2005; Miller and Ungerfeld, 2008, Tauck *et al.*, 2010). Fike *et al.* (1996) suggest that the response of postpartum anoestrus cows to biostimulation effects from bull exposure was directly associated with the intensity of stimuli released. Fenceline and intermittent contact of cows with bulls could accelerate the resumption of ovarian cyclicity (Fike *et al.*, 1996; Fernandez *et al.*, 1996), however continuous or close physical contact with the bull is more effective (Bererdinelli and Tauck, 2007, Fernandez *et al.*, 1996).

In addition, exposure to the bull of newly calved dairy cows, starting from early in the postpartum period, results in earlier signs of oestrous than in control cows that were not exposed to the bull (Chenoweth, 1983). Results from a study by Miller and Ungerfeld (2008) found that the duration of anoestrus in postpartum beef cows was usually shortened when cows were exposed to bulls. In their experiment, the cows were assigned to two groups; the control group was exposed to one pair of bulls and the 'exchanged' group to two pairs of bulls that were exchanged weekly. They concluded that weekly exchange of bulls shortened the postpartum anoestrus period in suckled beef cows, with a higher cumulative frequency of cows in oestrus by week 4 and 5 to week 7, and that pregnancy rates were higher compared to continuous exposure to the same bull.

Nevertheless, the response to bull exposure is not necessarily the same for anoestrus dairy cows and is less clear compared to beef cattle. High milk production is a major factor that might contribute to a different reaction to bull exposure in anoestrus dairy cows. Shipka and Ellis (1999) found that there was no effect of bull exposure on long term reproductive performance of exposed cows; even worse, the ovarian reactivation period was extended in postpartum dairy cows that were exposed to a bull.

2.3.4.4 *Effects of biostimulation on conception rate*

The conception rate in cows could be increased following the acceleration of resumption of ovarian cyclicity from biostimulation by exposing cows to the bull. According to a study by Izard (1983), postpartum beef cows that were exposed to a vasectomised bull for about 3 to 4 hours, two times a day, conceived to a fertile mating earlier compared to cows without bull exposure. Furthermore, beef cows that were exposed to a vasectomised bull 30 days before the start of the breeding season, required 21 days within the breeding season for all to be mated, whereas cows without bull exposure required a longer period of 52 days for completion of breeding (Izard and Vandenbergh, 1982). The proportion of cows that were pregnant in less that 60 days after insemination was higher in cows with exposure to a vasectomised bull compared to cows without bull exposure (Izard, 1983). This finding is similar to the study on fertility by Ebert et al. (1972); the conception rate to first service was higher with 68% in cows that were exposed to the bull compared forty eight percent (48%) in the cows without bull exposure. However, the efficiency of the biostimulation effects on conception rate may depend on stimulation techniques, condition of the cows and other factors.

2.3.5 *Stimulation methods used in exposing the cows to a bull*

In many previous studies on biostimulation of postpartum cows by bull exposure, varied methodology was applied in order to define the mechanism of biostimulation and increase the efficiency.

2.3.5.1 *Continuous bull exposure*

In beef heifers, long term continuous exposure of heifers to a mature bull did not influence age at puberty (Roberson *et al.*, 1987), while Berardinelli *et al.* (1978) found that short term exposure of heifers to a mature bull also had no effect on age of puberty. However, recent research shows that mature bull exposure can influence age at puberty in beef heifers (Oliveira *et al.,* 2009). In an early study, beef cows that were exposed to the bulls during the early postpartum period had a reduced postpartum anoestrous interval. Bull exposure was continuous from the third day postpartum for cows on the bull exposure treatment. In this study, the first increase in progesterone, obtained from blood samples, indicated that the onset of oestrous cycles occurred at 43±2 days compared to 63±2 days ($P<0.01$) for the first year of study, and at 39±2 compared 61±3 days ($P<0.01$) postpartum for the second year for cows with bull presence and cows that were not exposed to the bulls, respectively (Zalesky *et al.*, 1984).

2.3.5.2 *Intermittent bull exposure*

Intermittent bull exposure, of 2 hours every third day, over 18 days, had no effect in decreasing the interval to first ovulation and first oestrus behaviour expressed in postpartum primiparous suckled beef cows (Fernandez et al., 1996). However, the characteristics of LH profiles were altered by intermittent bull exposure for a short period and higher mean of LH concentrations was detected in these cows. However, the approach should be done continuously over certain period of time to increase the efficiency of biostimulatory effect in anoestrous cows. Thus, these research results show that not necessarily all cues of bull stimulation and continuous exposure are required to influence female responses.

2.3.5.3 ***Fenceline bull exposure***

In a study by Fike *et al.* (1996), crossbred primiparous and multiparous beef cows at 30 days postpartum were assigned to two different treatments, which were fenceline exposure to bulls or isolated from bulls. Primiparous cows with fence line exposure had a shorter duration of postpartum anoestrus compared to the heifers group without bull exposure. However, there was no difference between the treatments in the multiparous group. Another study shows cows exposed to fenceline contact with bulls resumed ovarian cyclicity sooner than cows not exposed to bulls (Berardinelli and Tauck, 2007) and Roelofs *et al.* (2007) found that fenceline bull exposure during early postpartum had an acute effect on LH-release in anoestrous dairy cows, although LH concentration was on average higher on the day of exposure to the bull, compared to a day with no bull presence. In contrast, a study by Fike *et al.* (1996) found that fenceline bull exposure had no effect on improving pregnancy rates to AI, but was still a good approach to induce earlier postpartum resumption of oestrous cycles in primiparous cows. However, the mechanism of the biostimulatory effect of a bull on resumption on postpartum ovulatory activity is not well understood (Tauck *et al.*, 2006), especially for dairy cows, and needs further research.

2.3.5.4 ***Using the bull urine as a stimulus***

Bull urine can be used to mediate the biostimulation reaction in anoestrous cows. Exposing postpartum, anovular, suckled cows to bull urine for 24 h daily, in a manner by which pheromones were presented, stimulated resumption of ovarian cyclicity (Tauck *et al.*, 2006). However, pheromones produced by bulls may be less be effective if the cows are continuously housed with bulls. Several studies have been designed to test the biostimulatory effects on resumption of luteal activity by using bull urine that contains pheromones. A study by Tauck and Berardinelli (2007) found that the group of beef cows that were exposed to urine of a bull by controlled urinary delivery device tended to a have shorter intervals from calving to showing oestrus compared to cows that were exposed to urine of a steer. They concluded that a novel urinary pheremone of bulls was reflected in the improvement of fertility in the primiparous postpartum cow. The manner in which cows were exposed to bull urine may be a major reason for different findings from a study by Tauck *et al.* (2006), who found no difference in interval from urine exposure to resumption of luteal activity or proportions of cows that resumed luteal activity during their experiment. Tauck *et al.* (2006) used a controlled urine delivery device (CUDD) method, with continuous exposure to bull urine. They concluded that continuous exposure to bull urine was not an effective biostimulatory method for cows and that using a CUDD system did not improve the postpartum luteal activity. However, this method was also reported by Tauck and Berardinelli (2007) in conjunction with a progestin-based synchronisation protocol that included a controlled internal drug release device (CIDR), PGF2α, and fixed-time AI (TAI), who found a positive response in conception rate from cows that were exposed to the bull following this treatment.

2.3.6 *Factors affecting the efficiency of bull exposure*

A range of factors could be considered as contributors to different outcomes of previous studies of the effect of bull exposure on resumption of luteal activity in anoestrus cows. These include: exposure method used, the genetic base of cows, physical status such as lactating for dairy cows and suckling for beef cows, the facilities and environmental conditions. It is not understood how duration, intensity, and frequency of exposure may influence the effectiveness of bull presence in postpartum anoestrous lactating dairy cows. The study by Berardinelli and Tauck (2007) found that the response of anovular primiparous suckled beef cows to the biostimulatory effects of bull presence may depend on the intensity of exposure. Nevertheless, the role of different forms of bull exposure, levels of interaction and intensity require further investigation.

Age of a mature bull is not a concern, since biostimulatory effects of a young bull (aged between one and three years old) on the duration of postpartum anoestrus in beef cows were the same as for a mature bull (aged three years or more) (Cupp *et al.*, 1993).

2.4 Application of the biostimulation technique in dairy farms

In the current situation on most dairy farms in UK, the use of a bull is normally restricted to breeding purposes and to detect oestrus in most of dairy farm in the UK. Often, no bull is involved in dairy management since they have been replaced with advanced techniques such as artificial insemination and automatic oestrus detection. However, there is a potential for application of the biostimulation technique by exposure to a bull to improve reproductive performance in high producing dairy cows. For this reason, this study was developed to explore the possible potential of the biostimulation effect in high producing dairy cows during the postpartum period to improve their reproductive performance after parturition and reduce the calving interval, with the intention that this will improve lifetime performance and production for individual cows.

Chapter 3

Influence of bull exposure to postpartum anoestrous cows on resumption of ovulatory activity

Chapter 3. Influence of bull exposure to postpartum anoestrous cows on resumption of ovulatory activity

ABSTRACT

Twenty-seven multiparous dairy cows, entering the experiment at 21.0±13.3 days post calving and confirmed as being in an anoestrous state, were split into two matched treatment groups; no bull contact throughout the experiment (NBC; n=13), and fenceline bull contact (BC; n=14). The cows were housed in cubicles and the bull was placed at one end of the cubicle house within a pen which was separated from the cows in the bull contact (BC) group by a barred fence. The cows in the BC group had unlimited time of access to the bull throughout the trial period. A mature Aberdeen-Angus bull aged approximately 3 years old was used as the stimulus animal. The cows in the no bull contact (NBC) group were housed at the other end of the cubicle shed, distant from the bull pen. All cows were observed for oestrus behaviour and response to the bull every week. Milk samples were collected two times a week for milk progesterone assay. The first sustained rise in P_4 occurrence indicated when the ovarian cyclicity in cows had resumed after calving. Only 8 NBC and 12 BC cows resumed ovarian cyclicity within the 40 day trial period (P>0.05). Bull exposure had no effect on hastening oestrus in cows that did resume ovarian cyclicity (T=0.06, P>0.05). The average increase in progesterone concentration for cows in BC group was from 1.83 on day 1 to 4.38ng/ml on day 4 of bull exposure, which was faster compared to 0.45 on day 1 to 0.57 ng/ml on day 4 for cows in NBC; however this difference was not significant (P>0.05). There was no significant difference in oestrous expression in cows that were exposed to the bull in comparisons to the control group, as measured by signs of stand to be mounted (T=0.02, P>0.05), mounting other cows (T=0.06, P>0.05), chin resting (T=0.18, P>0.05) and vulval discharge. However the BC group showed changes in behaviour of visiting the bull on the day of oestrus. Exposure of cows to a bull during the early postpartum period had no absolute effect on oestrus occurrence, but the cows in BC group did interact more with the bull during the inter-luteal period.

3.1 Introduction

Dairy cows will often experience infertility problems after calving due to negative energy balance as a result of high milk production. Postpartum infertility in cows is often associated with a prolonged period of anoestrus and anovulation. Because of this, the ability to show proper expression of oestrus by cows may be reduced in the postpartum period and the percentage of successful pregnancies after insemination may also decrease (Fike *et al*., 1996). Infertility problems need to be addressed and one of the possible solutions is reducing the anoestrus period.

Miller and Ungerfeld (2008) found that the duration of postpartum anoestrus in beef cows was decreased when they were exposed to bulls or excretory products of bulls. If the bull is in a pen sited near to the cows, those cows that are in oestrus will normally move near to the bull (Gordon, 1996). This indicates that the presence of a male animal generates a positive effect on reproductive behaviour and possibly on the oestrous cycle in females. In studies of ovarian activity, the male presence has influenced the onset of oestrus in sheep and goats at the start of their breeding season (Rekwot *et al*., 2001). The bull's stimulatory effect on postpartum dairy cows could be a useful treatment as it is known to improve the proportion of beef cows that conceive (reviewed by Rekwot *et al*., 2001). However the effect depends on the nature of bull exposure.

Exposing postpartum anoestrous suckled beef cows and heifers to bulls, either with close physical contact or by fenceline contact, can accelerate resumption of ovulatory activity (Fike *et al*., 1996; Berardinelli *et al*., 2005; Tauck *et al*., 2010). Nevertheless, Rekwot *et al*. (2001) reviewed the physiology surrounding biostimulatory exposure to males is complex and not well understood in cattle generally, especially for dairy breeds, in comparisons to other species such as sheep, rodents and swine. Indeed, Roelofs *et al*. (2008) claimed that the role of bull exposure in lactating dairy cows is not as clearly defined as in beef cows and other species. Furthermore, the efficacy of the exposure to a male also depends on nutritional and metabolic factors in the females (Scaramuzzi and Martin, 2008).

Further evidence for potential benefit comes from studies on the effects of bull exposure on puberty in beef breed heifers, which found a favourable correlation between age at puberty and exposure to the bull. Heifers exposed to the bull pre-pubertal reached puberty earlier compared to the control group with no exposure to the bull (Roberson et al., 1991, Rekwot, et al., 2000).

The aim of this research is to develop an approach using biostimulation that will possibly be helpful in reducing the problem of poor reproductive performance, especially in dairy cattle. The main objective is to examine the effect of bull exposure particularly focussing on resumption of ovulatory activity in dairy cows during early lactation and the ease of oestrous detection in an intensive farming system. Thus, to attain this aim there are two main objectives:-

a) To measure the biostimulatory response in oestrous activity of dairy cows that are not or exposed to a bull (i.e. days to first oestrus detected during the trial, validated by milk progesterone profile).
b) To assess the changes in walking activity and level of oestrous expression presented in postpartum lactating dairy cows that are exposed to a bull.

The null hypotheses are:

a) The intervals from first day of trial to first oestrus detected are not different in both treatment groups (bull exposure, BC, and no bull exposure, NBC).
b) There is no difference in behavioural oestrus expression and walking activity of cows that are exposed or not exposed to the bull.

3.2 Material and methods

3.2.1 *Experimental protocol*

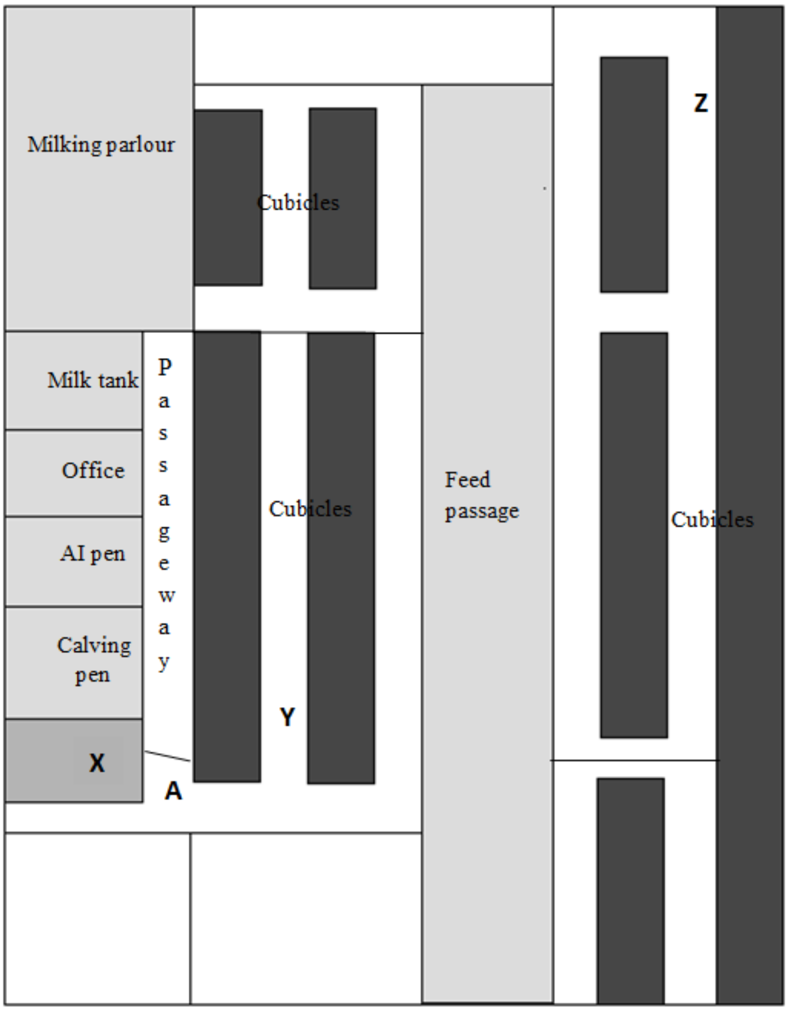
For this trial, 27 high-yielding, non-pregnant, Holstein-Friesian dairy cows were selected. All of these animals were within 1 to 55 days postpartum and they were confirmed as being in the anoestrus period, which means none of the animals were cycling at the beginning of the trial. The trial was implemented throughout February to May 2011, as all the cows were housed during this period. Every allocated cow entered the experiment as soon as they were selected for trial and confirmed in anoestrous. The cows were housed in a cubicle system and split into two groups, minimizing the differences in cow characteristics between groups as far as possible, which were no bull contact (NBC; n=13), and bull contact (BC; n=14).

3.2.2 *Housing*

At one end of the cubicle house the bull was placed within a pen with a barred fence which separated cows in the bull contact (BC) group from the bull. This placement was adapted from a previous study (Berardinelli and Tauck, 2007). This design was set up with the intention to provide as much exposure as possible to bull stimuli cues which include olfactory, visual, auditory and limited physical contact for cows in the BC treatment group.

A mature Aberdeen-Angus bull of 3 years of age at the beginning of the experimental period was used as the treatment animal. The bull was placed alone in the bull pen throughout the experiment and separated from cows by the fence with open bars which allowed limited physical contacts like licking and sniffing. Cows were milked twice a day morning and afternoon, and after milking BC cows were walked through the passageway to pass the bull.

At all other times, they had fenceline access to the bull pen at a meeting area. The cows in the no bull contact (NBC) group were placed at the other end of the cubicle shed, with as much distance as possible away from the bull. There was a concrete wall at the end of the cubicles so that the bull could not be seen at all by these cows. The cows in the control group had no direct exposure or possibility of interaction with the bull, however they may have received pheromones secreted in the air. The housing system is illustrated in Figure 3.1 and the bull exposure area shown in Figure 3.2.



A : Contact area
X : Bull pen
Y : Cubicles for postpartum anoestrus dairy cows in bull treatment group (BC)
Z : Cubicles for postpartum anoestrus dairy cow in no bull treatment (control) group (NBC)

Figure 3.1: Housing system and placement of the treatment and control group for the trial, there is a high cubicle wall along the passageway to the bull pen which makes the bull in the bull pen not visible to cows in control group.

Figure 3.2: Figures shows cows in the BC group in the trial standing and interacting with the bull at the meeting area.

3.2.2 *Animals and routines*

The experimental animals were housed in a dairy shed at the Cockle Park Research Farm (Coordinates: 55°12'49"N 1°41'3"W), which comprises a cubicle system equipped with rubber and sawdust flooring. Dairy cows were allocated to treatment depending on days postpartum and parity group. The details of each cow, such as calving number, date of previous calving, previous lactation milk yield, and body condition score were recorded at allocation.

Body condition score (BCS) of cows was assessed and recorded during the selection process and once a week during the study. The scoring system used was the standard measuring system with scores between 1 to 5, as shown in figure 3.3. The lowest score of BCS 1 represents a cow with poor body condition, with no fatty tissue under the skin, normally measured at tail head and loin, and the highest score of BCS 5 represents a cow with fat body condition (Hulsen, 2005).

Score	Condition	Detailed Description	Visual Guide
1	Poor	*Tail head* – deep cavity with no fatty tissue under skin. Skin fairly supple but coat condition often rough.	
		Loin – spine prominent and horizontal processes sharp.	
2	Moderate	*Tail head* – shallow cavity but pin bones prominent; some fat under skin. Skin supple.	
		Loin – horizontal processes can be identified individually with ends rounded.	

Score	Condition	Detailed Description	Visual Guide
2.5			
3	Good	*Tail head* – fat cover over whole area and skin smooth but pelvis can be felt. *Loin* – end of horizontal process can only be felt with pressure; only slight depression in loin.	
4	Fat	*Tail head* – completely filled and folds and patches of fat evident. *Loin* – cannot feel processes and will have completely rounded appearance.	
5	Grossly Fat	*Tail head* – buried in fatty tissue, pelvis impalpable even with firm pressure.	

Figure 3.3: Description of condition scoring of dairy cattle (DEFRA, 2013)

The animals were fed ad libitum on a total mixed ration (TMR) (Appendix 1) diet of home grown wheat, bought in soya and distillers grains which was formulated to meet the nutrient requirements dependent on their milk production level: either post calving, high milk or low milk production (near the dry period). The ration was provided by the stockperson every day. The animals were milked two times a day, at 06.00 and 14.00 in a Fullwood low-line 16:16 herringbone parlour. The oestrous behaviour was observed by the herdsman and also recorded continuously by video cameras mounted over each pen.

Normally all the cows in this farm are served by artificial insemination (AI) at the first oestrus that occurs after at least 35 days postpartum. Data on serving date and pregnancy outcome, determined by veterinary rectal palpation 60 days after AI and subsequent calving date, were recorded to determine any changes in pregnancy rate for cows that were exposed to the bull.

3.2.3 *Behavioural observations*

Detection of oestrus by observing the changes in activity level was performed using a pedometer system that interfaces with the parlour equipment (Fullwood Pedometer, Fullwood Ltd, Shropshire, UK). The pedometer is an electronic wireless motion sensor that monitors, records and reports details of animal activity. The pedometer is attached to one of the legs of each cow above the fetlock to continuously monitor stepping behaviour. For this trial, data on number of walking steps were obtained from data in the parlour computer system that recorded average number of steps every day. Since the data on number of steps was limited (the system only provided data on average number of steps for each day instead of data for every hour), the calculation of an increase in number of steps was done based on comparisons of median walking steps daily. The number of steps on the day of oestrus (as notified by the computer system and matched to the day of low progesterone concentrations) was compared with the median number of steps taken per day during the 10 days before oestrus. A ratio was calculated by dividing the number of steps on day of oestrus with the median number of steps of 10 days before oestrus. If this ratio exceeded a threshold of 5.0, this was defined as an actual increase in daily average number of steps and designated as an oestrus alert. The method of calculation is adapted from the study by Roelofs et al. (2005).

Beside this, the parlour was fully equipped with an automatic recording system for milk production, which was linked to specific software in the computer in the dairy office. This system will automatically recognise the changes in milk production and will notify if any cows have a drop in their milk production that might indicate onset of oestrus. These notifications according to the computer system were used to measure the number of cows that had changes in their milk production when they were actually in oestrus, as determined by milk progesterone profile.

The experimental observation of oestrous behaviour for both groups was carried out by visual observation twice a week; every Monday and Thursday, morning and afternoon for 60 min every session (09.00 to 10.00 and 13.00 to 14.00). The numbers of animals in behavioural oestrus were recorded during all observation periods. Expression of the oestrous behaviour defined as “stand to be mounted” is the primary sign of oestrus, while signs including flehmen, restlessness, sniffing the vulva of other cows, vulval discharge and mounting other cows are the secondary signs of oestrus. For this study, signs of oestrus that were recorded during visual observations were: stand to be mounted, mounting other cows, chin resting and vulval discharge. These signs were recorded to measure the intensity of oestrous expression. The intensity was measured by the number of times that primary or secondary signs of oestrus were observed, for example a high intensity of oestrus was scored when a high frequency of the primary sign was expressed. In addition, the “stand to be mounted” behaviours which were observed by the herdsman every day before the morning and afternoon milking session were also recorded.

The primary and secondary signs were assigned different numbers of points, as listed in table 3.1., which were recorded at each time a behavioural sign of oestrus was observed. The protocol for deriving the points of every oestrus sign is listed below:

a) Stand to be mounted

The score for "stand to be mounted" sign was derived from a combination of direct visual observation during observation period (2 days a week and 2 times in one day; 09.00 to 10.00 and 13.00 to 14.00) with the records of the daily stockperson observations during that cycle. The designated points were given for each time the cow showed "stand to be mounted" behaviour. If a particular cow stood to be mounted and then walked away before standing to be mounted again, this was counted as two occasions and points given again.

b) Mounting other cows

The scores were based on the direct visual observation periods and points were given for each occasion in that oestrus cycle a cow was detected mounting other cows. If she walked away and then repeated the behaviour, this was counted as two occasions and points were given again.

c) Chin resting

The same scoring procedure as for mounting other cows was used for assigning points for chin resting behaviour.

d) Vulva discharge

For vulva discharge, points were only assigned one time for any oestrus cycle.

As "stand to be mounted" behaviour is the primary sign of oestrus, a cow can be considered as confirmed in oestrus if this sign was displayed, otherwise the cow can be considered as in oestrus if the recorded total points of oestrus signs in one observation period was more than 100 points (Roelofs et al., 2005).

For cows in the BC group, behaviours related to interest in the bull were recorded. There were 2 cubicles near the bull pen marked as favourite cubicles of cows during the inter-luteal phase. Times that individual cows spent lying down in these cubicles were recorded during the same visual observation period (09.00 to 10.00 and 13.00 to 14.00).

Table 3.1: Scoring scale observed signs of oestrus (Roelofs et al., 2005)

Signs of oestrus	Points
Flehman	3
Restlessness	5
Sniffing the vulva of other cows	10
Mounted but not standing	10
Chin resting	15
Mounting other cows	35
Stand to be mounted	100

Besides direct visual observation, video cameras were installed to record the activity of cows for 24 hours per day. One video camera was attached to view the area of cows in the control group (cows without any bull contact), and another one was placed to view the area of cows in the treatment group (cows with bull exposure). However, the views captured by these cameras were very limited and thus unable to reliably detect oestrus signs. Therefore it was decided to use the camera in the BC group only to measure the frequencies with which cows in oestrus had contact with the bull. The contact with bull was defined as cows which approached and stood near the bull pen for more than 10 minutes at a visit. The video observations were analysed for changes in behaviour of contacting the bull over 3 two day periods (06.00 until 05.59); 2 days of lowest progesterone concentrations (below 1.5ng/ml) seen after the first decline in progesterone after the first confirmed elevation in progesterone (above 1.5 ng/ml) that marked the onset of ovarian cyclicity, 2 days before and 2 days after that.

3.2.4 *Milk sampling routine*

Milk samples were collected at afternoon milking twice a week, on Tuesday and Thursday, every week for six weeks. The samples were frozen at -20^{o}C after collection and were assayed as a single batch at the end of trial.

3.2.5 *Milk progesterone assay*

The progesterone test was used as the best indicator to decide the stage of oestrous cycle and the beginning of the first oestrous cycle after calving. As stated by Gifford *et al*. (1989), the onset of ovarian cyclicity is determined by the first milk progesterone concentration of 1.5ng/ml or above detected for approximately 1 week.

The frozen milk samples were thawed by placing them into a water bath at 30˚C and were analysed using a commercial milk progesterone test kit (Ridgeway Milk progesterone enzyme immunoassay, Ridgeway Science, Glouncestershire, UK). Seven plates containing 96 wells each were used to test milk samples from 26 cows from both treatment groups. The assay included the milk progesterone standards: 0ng/ml, 1ng/ml, 2ng/ml, 5ng/ml, 10 ng/ml, 20 ng/ml and 50ng/ml in duplicate. Two milk samples were used as a quality control in each assay. The standards, quality controls and samples were pipetted (10µl) into each well in duplicate. Then, 200µl of progesterone enzyme-conjugate were added to each of the wells. After that, the plate was placed onto the shaker for 1½ hours. In the meantime, substrate was prepared by dissolving it in 25ml substrate assay buffer. The wells were then emptied and washed three times with distilled water. 200µl of prepared substrate were added to all wells then left for 30 minutes, to wait for the colour changes. The samples that contained a low level of progesterone turned pink in colour, while samples with high level of progesterone remained colourless. The plates were then put on a precision microplate reader, and optical density used to read the absorbance at 570 λ. The concentrations of progesterone from the milk samples were read from the standard curve (figure 3.4). The mean of inter-assay coefficients of variation was 8.9% and the intra-assay coefficient of variation was 4.41%. The limit of detection of the assay was 0.22 ng/ml.

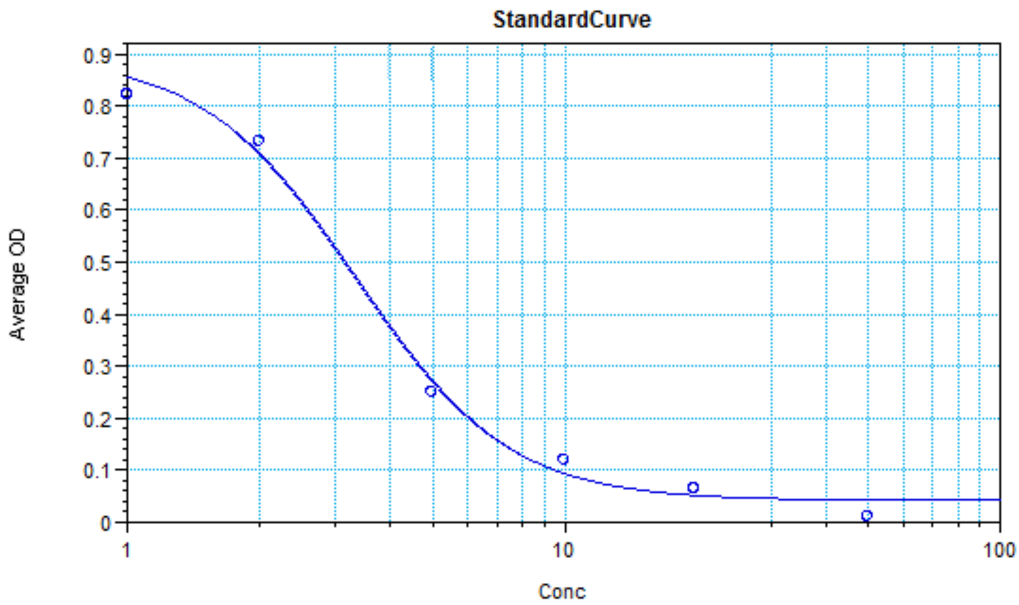


Figure 3.4. Example of a standard curve that was used to read milk progesterone concentrations.

3.2.6 *Data analysis*

The comparisons of the mean of body condition score, parity number, previous milk production and previous calving interval for both groups were analysed using one-way analysis of variance (ANOVA) (Minitab 16) with treatment groups as factor. The significance of differences in days to resumption of ovulatory activity and the changes in times spent visiting the bull on days close to oestrus were also tested by a one-way ANOVA test to find out if there was any significant difference between groups. Besides this, a repeated measures ANOVA test (Minitab 16), with day as the repeated measure within cow, was applied to analyse the progesterone data and changes in walking activity during oestrus for both treatment groups. Changes in behavioural expression during oestrus were analysed were tested using the chi-square test (SPSS 19) to identify any significant difference between groups in the percentage of cows exhibited particular signs of oestrus. The significance level was set at $P \leq 0.05$.

3.3 Results

3.3.1 *Comparison between control and treatment group at allocation*

The two groups were balanced on body condition score (BCS), average daily milk production from the previous lactation period, parity and also previous calving interval as shown in table 3.2. The comparisons show that there were no significant differences between groups for BCS, parity, milk production and previous calving interval which mean these two groups were equally balanced with no allocation bias.

Table 3.2: The mean (±SEM) of characteristics of the experimental animals at allocation

	NBC (n=13)	**BC (n=14)**	***T-test***	***P* value**
BCS	2.50±0.59	2.28±0.68	0.87	**0.394**
Parity	2.54±1.98	2.36±1.69	0.25	**0.801**
Milk production (litres/day)	29.12±7.57	34.23±3.44	1.63	**0.143**
Previous calving interval (days)	480.9±97.6	479.6±94.8	0.02	**0.981**
DPP at start of trial	20.2±12.4	23.4±14.3	0.62	**0.539**

3.3.2 *Progesterone concentrations*

Milk samples that were collected for 40 days based on 2 times weekly interval sampling were analysed. The onset of oestrous cycle was determined by measuring the concentrations of progesterone. Figure 3.5 illustrates an example of a progesterone concentration profile that shows increased progesterone level for 3 or more consecutive samples, to confirm that cows had resumed ovarian cyclicity after calving. After approximately 17 days of high progesterone level, the concentration goes down again; this represent the inter-luteal interval during which the next ovulation occurs.

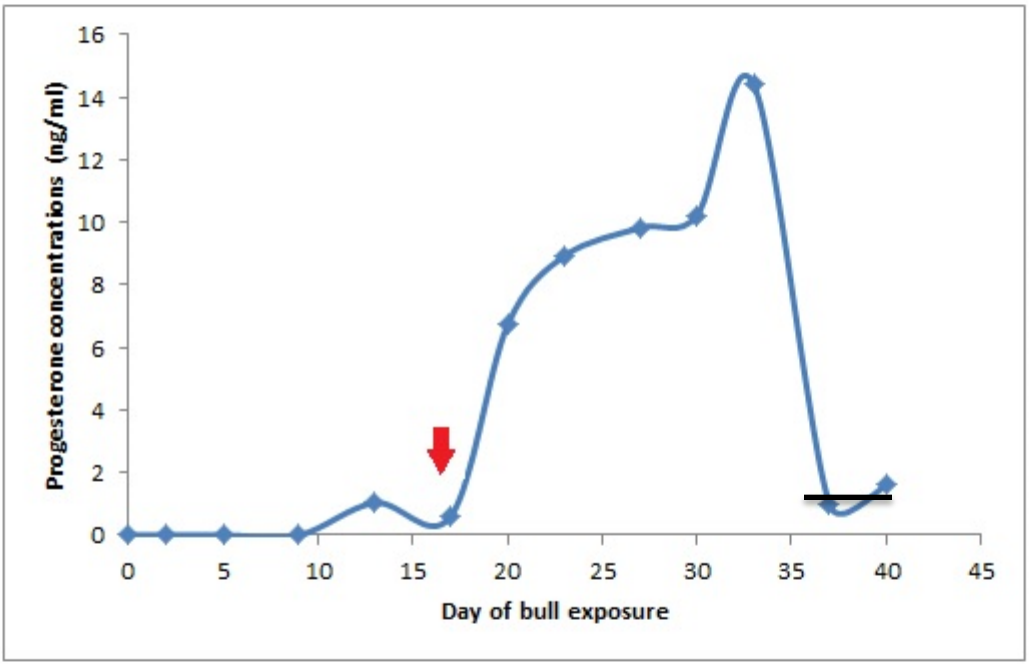


Figure 3.5: An example of a progesterone concentration profile of one cow from the BC group that resumed ovarian cyclicity after approximately 17 days of the trial. The arrow indicates the day that progesterone starts to rise after ovulation and corpus luteum formation, and the black line indicate the stage of inter-luteal interval with progesterone concentrations below 1.5 ng/ml.

The average progesterone profiles for both groups are shown in figure 3.6. The increase in average progesterone concentration for cows in the BC group was from 1.83 on day 1 to 4.38ng/ml on day 4 of bull exposure, which was faster than the change from 0.45 on day 1 to 0.57 ng/ml on day 4 for cows in the NBC group. However, this difference just failed to reach statistical significance (P=0.057). There was no significant difference (P>0.05) in average progesterone concentration between control and treatment groups throughout the trial. In addition, the pattern of progesterone concentration changes during oestrus in NBC and BC cows was not significantly different.

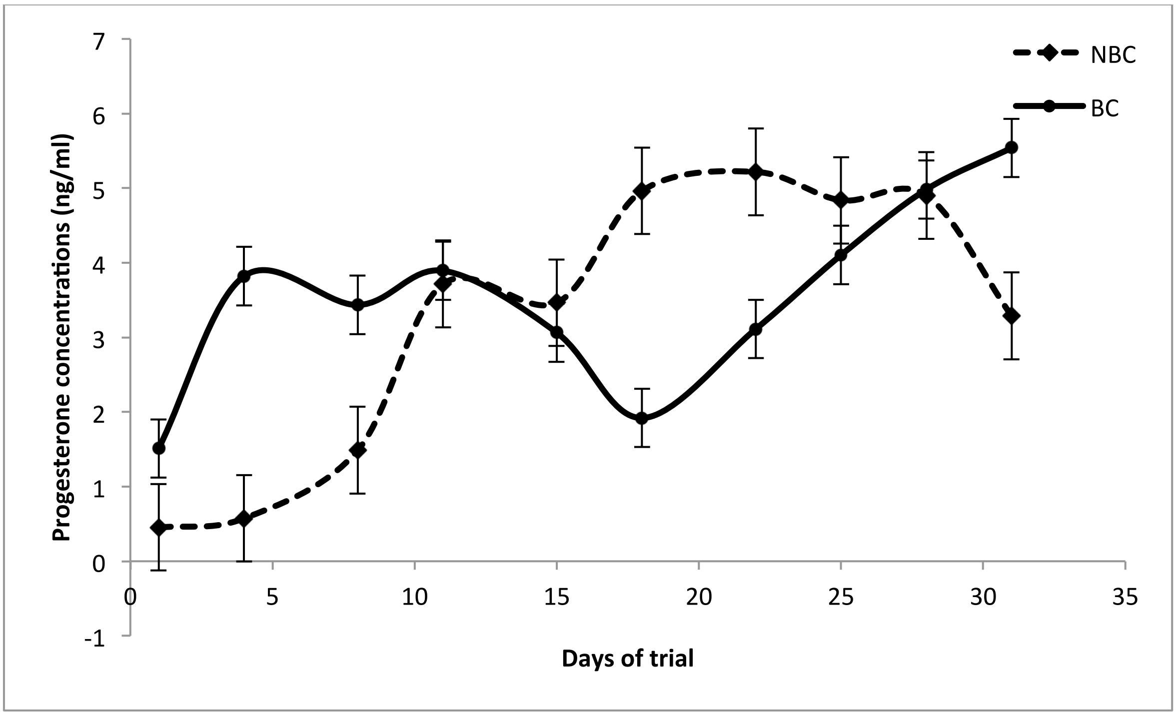


Figure 3.6: The comparison of mean (±SEM) progesterone concentrations for the BC and NBC treatment groups, starting from day 0 of trial until 31 days. The BC group were exposed to the bull throughout this period.

3.3.3 *Resumption of ovulatory activity*

From the allocated animals, 20 cows were detected to resume ovarian cyclicity post calving during the trial period (NBC=8, BC=12) as confirmed by milk progesterone concentrations of more than 1.5 ng/ml for three milk samples consecutively. The cumulative percentage of cows that resumed cyclicity in the no bull contact (NBC) and bull contact (BC) groups, determined by progesterone concentrations, is presented in figure 3.7. Table 3.3 shows the results of interval from calving to resumption of ovulatory activity for NBC and BC group. In total, 8 cows from the NBC group resumed ovarian cyclicity (OC) and all of these were detected in oestrus by 78 days postpartum. However, another 5 cows in this group were still not cycling at the end of the trial at more than 100 days postpartum. In contrast, in the BC group, resumption of ovulatory activity occurred in 12 cows and first oestrus was detected prior to 85 days postpartum. 2 cows did not resume ovarian cyclicity. Of the cows which did resume ovarian cyclicity, bull exposure therefore had no effect in hastening oestrus in dairy cows (t=0.24, P=0.816).

Table 3.3: The characteristics of cows that resumed ovarian cyclicity (OC) for both NBC and BC groups derived from progesterone profiles, time of artificial insemination and data of oestrus signs observed.

	NBC	BC	Test	*P value*
Proportion of cows that resumed OC	61.5% (8/13)	85.7% (12/14)	*Fisher's test* 2.05	**0.209**
Median interval from calving to resumption OC for all animals	41.5 (n=13)	45.5 (n=14)	*Mann-Whitney* 80.0	**0.787**
Mean interval from calving to resumption OC for those showing OC	46.5±17.3 (n=8)	48.6±20.5 (n=12)	*T-test* 0.26	**0.816**

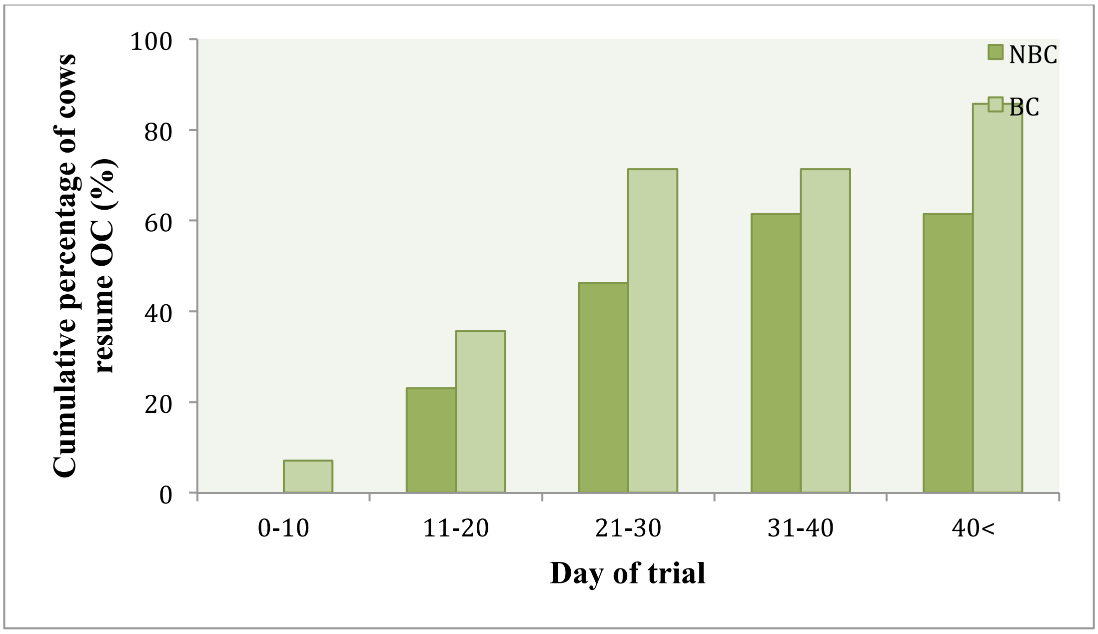


Figure 3.7: The cumulative percentage of cows from both groups (BC cows were exposed to the bull at different times postpartum) that showed resumption of ovulatory cyclicity (OC) throughout the trial[1] as determined by milk progesterone concentration[2] (NBC=13, BC=14).

For cows in BC group, the interval from start of bull exposure to resumption of ovarian cyclicity was analysed for cows at different periods of time post calving. Results show that there was no significant difference (F=1.53; P=0.267) in the interval from the start of bull exposure to resumption of OC related to the number of days postpartum (DPP) at the start of exposure. The interval from start of bull exposure to resumption of OC, as measured from progesterone concentrations, was 20.0±9.8 days for cows that were exposed to the bull soon after calving (less than 20 DPP) while for those with exposure starting between 21 to 30 DPP, the interval was 16.3±5.2 days and for more than 30 DPP was 28.8±14.3 days.

[1] Cows were at different days postpartum (DPP) at the start of trial, the exposure to the bull for all BC cows started at the same time at the start of the trial.
[2] There was no significant difference between groups in days taken to resume ovarian cyclicity and also in total number of cows resuming ovarian activity as measured from milk progesterone concentration.

3.3.4 *Changes during oestrus*

A slightly higher proportion of cows that had resumed ovarian cyclicity and were detected as in oestrus by milk progesterone concentrations showed oestrus behaviour in BC group compared to NBC; stand to be mounted (BC=58.3%, NBC=37.5%), mounting other cows, and chin resting (Table 3.4). This may suggest that bull exposure has slight effects on expression of oestrus behaviour which might be useful in detecting the resumption of ovulatory activity, however the difference was small and there was no statistically significant difference between groups (χ^2=1.307, P=0.253). In addition, the BC cows showed an interest in the bull during the inter-luteal interval. Half of the cows (6 cows) were detected to spend increased time visiting and interacting with the bull on the day with lowest progesterone concentrations measured during the inter-luteal interval, while 8 cows were detected with changes in lying behaviour indicating a preference for lying down in cubicles near to the bull pen during this period (Table 3.4).

There were some cows which had a drop in milk production or which increased their walking activity on the day of oestrus, but the proportion of cows that showed differences were small as shown in table 3.4. Besides this, bull exposure did not contribute to any greater changes in walking activity and milk production change of cows in BC group relative to the NBC group. The data of activity recorded by pedometers showed that there was an increase of walking activity (i.e. number of steps per day) during oestrus and the day prior to oestrus performed by 2 cows in NBC group and 2 cows in BC group. Of the 8 cows from BC group that were detected standing to be mounted from behavioural observations, 3 of these cows showed no changes in their walking activity recorded by pedometer on the day of oestrus. The rest of the cows did not show any difference in walking pattern throughout the study.

Table 3.4: Overall number of cows in the no bull contact (NBC) and bull contact (BC) groups which exhibited oestrus signs (as detected by visual observation, video recording and pedometer reading) during inter-luteal interval as determined by progesterone concentration.

Oestrus signs (% of cows)	Changes during inter-luteal phase (%)		*P* value
	NBC (n=8)	BC (n=12)	
Increase in walking activity	25.0% (n=2)	16.7% (n=2)	**P>0.05**
Stand to be mounted	37.5% (n=3)	58.3% (n=7)	**P>0.05**
Mounting other cows	50.0% (n=4)	66.7% (n=8)	**P>0.05**
Chin resting	50.0% (n=4)	41.7% (n=5)	**P>0.05**
Vulva discharge	37.5% (n=3)	16.7% (n=2)	**P>0.05**
<u>BC group only:</u>			
-Contact with bull (BC group)	-	50.0% (n=6)	**-**
-Lying in cubicle near bull pen	**-**	66.7% (n=8)	**-**
Drop in milk production	50.0% (n=4)	33.3% (n=4)	**P>0.05**

Table 3.5 shows the total points scored by cows in NCB and BC based on the number of times that they expressed oestrus signs. Overall, there was no significant difference in the frequencies of oestrus signs displayed by cows in BC group compared to cows in the NBC group.

Table 3.5: The calculated total points of oestrus behavioural signs displayed by cows (Table 3.1) in NBC and BC group.

Oestrus signs observed during inter-luteal interval	**Total points**			
	NBC	**BC**	***T-test***	***P* Value**
Stand to be mounted (STBM)	133±58 (n=3)	129±49 (n=7)	0.02	**0.896**
Mounting other cows	70±29 (n=4)	66±29 (n=8)	0.06	**0.811**
Chin resting	19±8 (n=4)	21±8 (n=5)	0.18	**0.685**
Vulva discharge	3±0 (n=3)	3±0 (n=2)	-	-

3.3.5 *Monitoring walking activity*

The pattern of walking activity measured using a pedometer showed that there was no significant difference (P=0.079) between cows in the different treatment groups in walking activity on the day of oestrus, as determined by the day of low progesterone concentration (<1.5 ng/ml before a rise) and associated with some oestrus behavioural expression in several cows. The mean number of walking steps per hour over 21 days (including an oestrus day and 10 days before and after oestrus) for both groups is represented in figure 3.8. Results show that walking activity of cows in the BC group was not affected by bull exposure during the study as the average was not different to cows without bull exposure. Lactation number was determined as a factor that affected walking activity of cows. Cows in their first or second lactation had increased walking activity during oestrus (approximately 99±48 recorded steps on a normal day to 170±114 steps on the day of oestrus), while there was no change (approximately 88±30 steps) in walking activity of cows of lactation three and above.

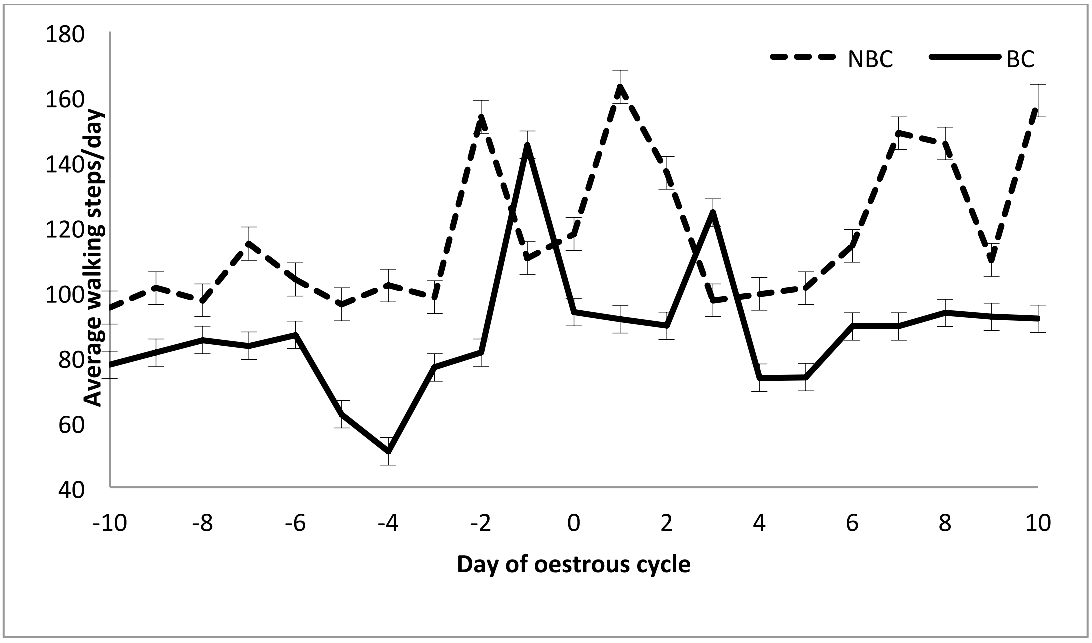


Figure 3.8: Mean (±SEM) number of recorded walking steps per day for cows in the no bull contact (NBC) and bull contact (BC) group over a 21 day period. Day 0 represents the day with the lowest progesterone concentration prior to a rise.

3.3.6 *Interactions between cows in the treatment group with the bull*

Of the 12 cows in the BC group that were detected to have experienced a follicular phase during the trial, 6 of them increased their interest in the bull on the day with the lowest progesterone concentrations during the inter-luteal interval (a period of low progesterone concentrations recorded below 1.5 ng/ml, ovulation occurs on one of the days during this period). The interactions of these 6 cows with the bull were observed for 48 hours during the period when low progesterone was recorded and compared to interactions in 48 hours before and after this period. There was a non-significant increase (P>0.05) in average visiting time on the day of oestrus (Figure 3.9).

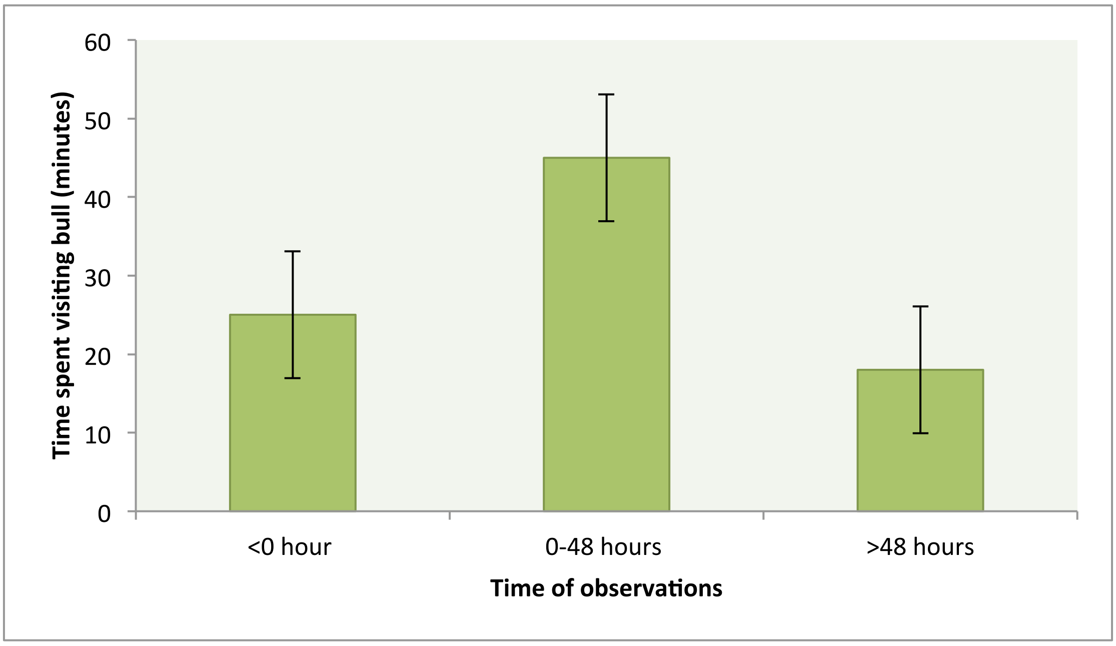


Figure 3.9: Mean time (±SEM) (minutes/48 hours) that cows in the BC treatment group showing bull interest spent visiting the bull during the 48 hours of lowest progesterone concentrations recorded, 48 hours before and 48 hours after this period (n=6).

Additionally, cows in oestrus also showed some changes in lying behaviour; 8 of them showed an increased preference to lie down in the two cubicles nearest to the bull pen during oestrus. In the morning period between 9 to 10 am and period between 12 to 1 pm, they showed an average lying time in these two particular cubicles of 92.1±26.2 minutes. Normally, after morning milking and feeding, they went to lie in these cubicles, then after a while they started to walk and feed again and after that they went back to the same cubicle to lie down. From all 12 cows, two of them showed no difference in behavioural changes either in bull visiting activity at the bull pen on the day of oestrus or preference for lying in the cubicles near the bull pen; these were both of parity >5 and showed no interest in the bull throughout the exposure period.

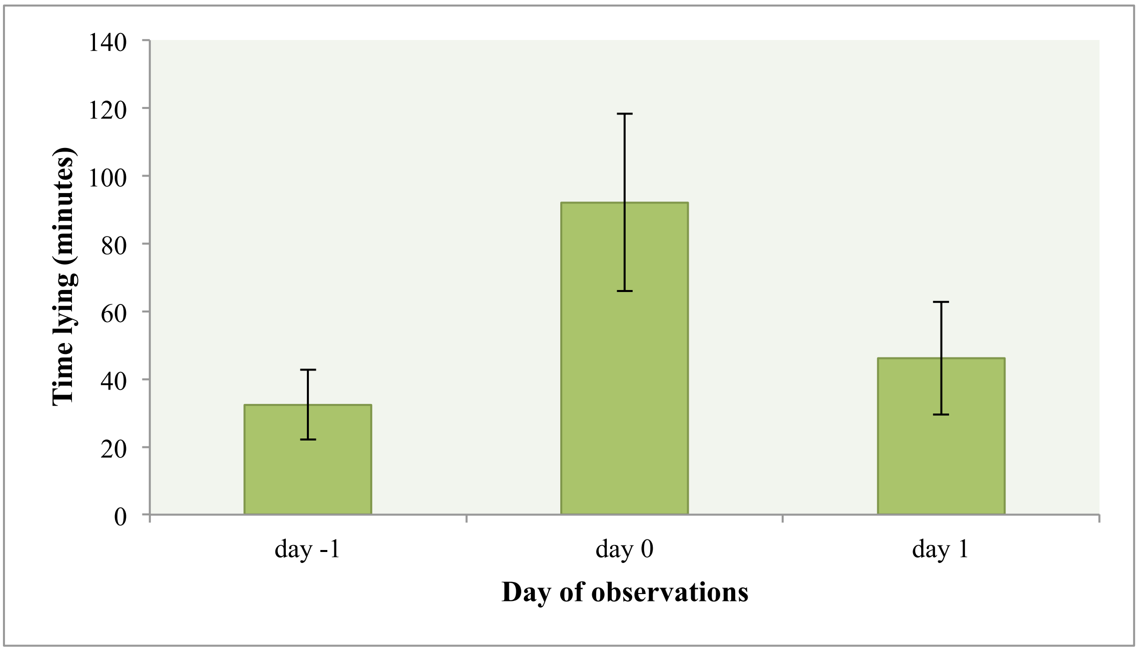


Figure 3.10: Mean time (±SEM) (minutes/2 hours) that cows showing a lying preference in the BC treatment group spent lying down in the 2 particular cubicles near the bull pen during the 2 hours observation on the day with lowest progesterone concentrations recorded, 2 hours on a day before and 2 hours on a day after (n=8).

3.3.7 *The calving interval of NBC and BC groups*

In this study, the results show that the mean calving interval was not different between cows in NBC group and BC cows as shown in table 3.6. Results were based on 8 cows from NBC and 9 cows in BC. 3 of 12 cows from BC were not included in this calculation as they were detected with subsequent problems and lost their calf during pregnancy. Overall, there was no significant effect of biostimulation on the calving interval for this trial.

Table 3.6: The mean interval (days) of calving for NBC and BC cows.

	NBC	**BC**	***T-test***	***P* Value**
Calving interval	462±26.1 (n=8)	480±83.1 (n=9)	0.32	**0.580**

3.4 Discussion

The main purpose of this study was to determine the effects of biostimulation by fenceline bull exposure on postpartum anoestrus dairy cows, which entered the experiment at various stages of the anoestrus period post calving. Responses assessed included the time taken to resume ovulatory activity from day 1 of bull exposure and the expression of various forms of oestrus behaviour. The findings showed that exposure to fenceline bull contact resulted in no significant difference in the average interval to resumption of ovulatory activity as compared to the control group that were not exposed to the bull. This indicates that biostimulatory effects by exposure to a bull during the anoestrus period is not necessarily effective in hastening resumption of ovarian activity to consequently affect the occurrence of first oestrus post calving. The absence of an effect might be explained by a number of factors; either the male effect does not operate in cattle in the same way as other species, or the effect is weakened in dairy breeds because of a greater influence of metabolic status on resumption of cyclicity, or the degree of biostimulation provided by the fenceline bull contact employed in this experiment was not great enough, or there is a critical period for the effect in relation to calving time which only a few animals experienced.

There have been some previous studies which demonstrated an effect of bull exposure on resumption of ovulatory activity and consequently a shortened postpartum anoestrus period (Fike et al., 1996; Berardinelli and Joshi, 2005), while several others found differently (Shipka and Ellis, 1998; Roelofs et al., 2008). Only a few studies used dairy breeds as their subject, and a variety of different methods for exposing a bull to the postpartum anoestrus cows have been employed (Berardinelli et al., 2005; Tauck et al., 2006; Miller and Ungerfeld, 2008). The mechanism by which bull exposure could accelerate resumption of ovulatory activity in post calving in dairy cows is still very unclear. In addition, in this study cows in the BC group that were exposed to a bull earlier in the postpartum period seemed to resume ovarian cyclicity earlier compared to cows that were exposed to the bull during the later postpartum period. Hence, this suggests that exposure to the bull earlier during the postpartum period may provide more effective biostimulation effects to the postpartum anoestrus cows. However, some confounding may have been present, as only cows still in anoestrus at the commencement of the trial were selected which, in the case of those with a longer period since calving, may have selected a population with poorer reproductive function.

Milk progesterone concentrations have been widely used as an indicator to detect resumption of ovulatory activity and approximate time of oestrus in previous studies (e.g. Gifford et al., 1989). A rise in progesterone concentration, exceeding 1.5 ng/ml in 3 consecutive samples, was used to objectively determine the resumption of ovulatory activity (Berardinelli and Joshi, 2005). Following this rise in progesterone, a sudden drop in progesterone concentrations in the interval between 18 to 24 days has been used as evidence of the approximate time of the next follicular phase and time to observe the behaviour indicative of oestrus. However, in this present study only a few cows expressed oestrous behaviour and showed an interest in the bull. In other cases, the onset of cyclicity was detected only based on progesterone concentrations. Accordingly, results from milk progesterone analysis indicate that resumption of ovulatory activity was the same for both groups. This suggest that the other signs monitored, including a drop in milk production and increase of walking activity, cannot be reliably used as measures to detect resumption of ovulatory activity and oestrus.

Previous studies have shown that increased walking activity associated with mounting behaviour has been a strong indicator of oestrus occurrence (Firk et al., 2000; Yaniz et al., 2006) and has been related to progesterone concentrations (King *et al*., 1976; Walton and King, 1986; Darwash *et al*., 1999). The increase in walking activity on the day of oestrus, recorded by a pedometer, may be helpful to detect oestrus in heifers and younger cows with lower lactation number. However, the results in this trial had reduced accuracy since some of the pedometers apparently failed to correctly record activity. The accuracy of pedometers varies between 22% and 100% (Lehrer *et al*, 1992), and is affected by technical limitations of the pedometer and the unfavourable management and environmental factors. In this study, the pedometers showed an obviously incorrect number of walking steps (e.g. less than 50 steps of total walking per day, despite the fact that cows were walking twice to the milking parlour and to the feed passage). Thus, whilst some results were in agreement with Roelofs *et al*. (2005), who demonstrated that cows tend to increase their walking activity during oestrus, a more reliable detection system is needed to substantiate this conclusion.

Oestrus detection by visual observation is focussed on the most obvious signs of oestrus such as standing heat (standing to be mounted), mounting, being mounted but not standing, chin resting, vulva sniffing and restlessness shown by cows. In this study, visual observation was performed twice a week as part of the experimental protocol, for two 1 hour sessions on each occasion. Additionally, observation of oestrus behaviour was performed daily by the stockperson before milking time. The precision and efficiency of direct observation as an oestrus detection method can be influenced by the frequency, duration and timing of the observation periods (Orihuela *et al*., 1983). Previous work suggests that the highest detection rates (94%) can be found with 2 periods of observation per day (60 min each time) during the quiet time (Roelofs *et al.*, 2010). The visual observation in this trial could therefore have been improved by increasing the frequency of observation to detect oestrus expression. Video recording also can be used to improve the observation but the detection rates depend on the quality of video. For this trial, video recording was used to observe the area near the bull pen, however the view was limited and thus oestrous expression could not be reliably observed when the cows were away from the bull pen area.

Results from the video recording showed that some cows were interested in visiting the bull, and tended to spend longer standing near the bull pen, on the day of oestrus. However these behavioural changes were not statistically significant. Similar behavioural changes were also detected in a study by Roelofs *et al*. (2008), where cows in oestrus were attracted to the bull and showed increased frequency of visiting the bull pen. However, there was no effect of bull exposure on the behavioural expression of oestrus observed by Shipka and Ellis (1998), which is similar to the findings from this trial. Information on which cues from the bull catch the attention of oestrus cows is not very clear. We only know that the biostimulation effect of the bull on resumption of ovarian cyclicity after calving seems to be mediated by pheromones present in excretory products of the bull (Berardinelli *et al*., 2005). According to Roelofs *et al*. (2008), fenceline bull exposure did not appear as a suitable aid for visual detection of oestrus.

The normal post calving period before the oestrous cycle starts is approximately 30 to 40 days in cows and heifers (Roelofs *et al*., 2006). Most of the cows and heifers in the current trial resumed ovarian cyclicity in the same range of postpartum days.

In this trial the treatment groups were exposed to the bull for only 35 days during anoestrus, hence the duration of exposure could be a reason for the poor results as no difference in cows exposed to the biostimulation effects were seen in resumption of ovarian cyclicity or in length of calving interval. Results from Berardinelli et al. (2005) suggest that insufficient intensity of stimulation might reduce the effect of the bull exposure; their study tested the effects of exchanging bulls to increase novelty and stimulus value. The design of the present study used a short period of fenceline bull exposure. The intensity of any biostimulatory pheromonal agents emitted from the bull might therefore have been insufficient to boost the ovarian activity (Berardinelli and Tauck, 2007). Furthermore, since the cows used in this treatment varied in their days of postpartum anoestrus at trial onset, their first ovarian cycle after resumption of ovulatory activity would also have been in different stages during the exposure period.

Berardinelli and Joshi (2005) discussed that the biostimulatory effects of a bull involves a temporary interaction mechanism between the sensory, neuroendocrine, and reproductive endocrine system in the postpartum anoestrous cow. The mechanism is very complex, but it clearly includes the hypothalamic mechanisms that control GnRH secretions and directly influence the pituitary hormones LH and FSH. These entire mechanisms link together to influence the results in accelerating resumption of ovarian cyclicity, and failure in any connection may reduce the effects of bull biostimulation on the postpartum anoestrous cows. The interacting factors are more complicated in dairy breeds, since their body condition and metabolic state is critically affected by high milk production during the early postpartum period.

In summary, exposure of cows to a bull during the anoestrus postpartum period had no absolute effect on the interval from calving to resumption of ovulatory activity after bull exposure. Furthermore, the biostumulatory effects of the bull were not very competent to increase oestrus expression. The method of exposure may contribute to the effects of bull biostimulation in anoestrus cows, and further research is needed to determine the appropriate bull exposure method. An assessment of the efficiency of bull exposure from immediately after calving to stimulate resumption of ovarian activity is important to reduce the anoestrus period and improve productivity of dairy cows. Thus a second trial was planned, using the lessons learnt in this first trial, to clarify the biostimulatory effect of bull contact on cows exposed to the bull from the very early postpartum period and allowing longer exposure to the bull.

Chapter 4

Influence of bull exposure on resumption of ovulatory activity and reproductive performance of postpartum anestrous cows

Chapter 4. Influence of bull exposure on resumption of ovulatory activity and reproductive performance of postpartum anestrous cows

ABSTRACT

In this study, 41 freshly calved high-yielding Holstein-Friesian dairy cows, with average parity 3.05±0.5, were allocated to two treatment groups; 20 cows with no bull contact (NBC) formed the control group and 21 matched cows formed the bull contact group (BC), exposed from 2 days postpartum onwards. Calving number, date of previous calving, body condition score, milk yield and date seen bulling were recorded. A sensor (Icetag) was attached to continuously monitor stepping behaviour and record changes in activity level. Visual observation was done to observe mounting activity indicative of oestrus. Milk samples were collected 3 times a week and analysed to determine progesterone concentrations to measure the resumption of ovarian cyclicity. Cows detected in oestrus were served by artificial insemination (AI) approximately 12 hours later. Cows that showed difficulty to conceive received a PRID treatment. The interval before cows resumed cyclicity was similar for cows either exposed or not exposed to bull stimulation; the average days postpartum for resumption of ovulatory activity was 30±2 dpp, based on progesterone concentration profiles. The postpartum increase in concentration of progesterone was slightly more rapid for BC cows compared to NBC cows, however the difference was small and not statistically different ($P=0.24$). The pregnancy rate to first insemination of BC cows was higher ($P=0.009$) compared to NBC cows, especially following a PRID treatment. The average number of insemination per conception was lower in BC cows compared to NBC with 2.0 and 3.4 inseminations, respectively ($P=0.010$). Consequently, the mean calving interval for BC cows was significantly shorter ($P=0.023$) compared to NBC cows with this effect coming mainly from the PRID treated cows ($P=0.043$). The biostimulatory effects provided by close physical contact with a bull through continuous fenceline exposure improved the breeding performance of cows, particularly cows treated with a PRID. Since the control group were in the same building, they may have received some

pheromonal effect but this would have been greatly attenuated compared to the treatment group.

4.1 Introduction

Selection of high-yielding dairy cows for milk production is linked to the recent decline in fertility (Royal *et al*., 2000; Pryce *et al*., 2004). The prolonged calving interval of high yielding cows is a significant problem for dairy cow production, as more time, effort and cost is required to get cows pregnant due to poor fertility. Studies in swine, sheep and beef cattle indicate that exposure to a male can improve postpartum reproductive performance (Berardinelli *et al*., 2005; Fike *et al*., 1996; Kemp *et al.,* 2005). However, the role of male presence in cattle reproduction, especially in dairy cows, is not as clear as in sheep, goats and swine. In the previous trial, the results showed that exposure of high producing dairy cows to continuous fenceline bull exposure commencing at some point between 3 and 50 days postpartum did not influence resumption of ovulatory activity. However several studies have proved the effectiveness of exposing beef cows to the bull in improving reproductive performance, including reduction in the anoestrous period and increase in pregnancy rates (Miller and Ungerfeld 2008; Tauck and Berardinelli, 2007). Berardinelli and Tauck (2007) suggest that the response of anovular primiparous cows to the biostimulatory effects of a bull may depend on the intensity of exposure, however the response in multiparous cows is minimal. In addition, cows showed an interest in the bull as they frequently visited the bull pen. As reported by Reolofs et al. (2008), some cows showed an increase in frequency of visiting the bull when they were in oestrus, and some also had a preference for lying down in cubicles near to the bull pen at this time. This suggests that there might be effects of bull exposure on cows during the postpartum period, but this might be very complex and requires a proper approach to make it effective and improve fertility in dairy cows. Thus, this study was designed as an extension of the first trial in order to determine how effective is the application of biostimulation (visual, auditory and by pheromones) by exposing high yielding dairy cows to a bull with fenceline contact from the time of calving. The effects on resumption of ovulatory activity and subsequent conception were assessed, to see if this approach can help in improving reproductive performance, specifically in reducing postpartum anoestrus and increasing pregnancy rate.

The major aim of the present study was to develop a technique that could be useful in commercial dairy farming in order to reduce the decline in reproductive performance which is linked to increasing milk production. To achieve this aim there were three main objectives, which were:-

a) To measure the changes in milk progesterone concentration in postpartum anoestrous lactating dairy cows that are exposed or not to a bull during the first rise in progesterone postpartum.
b) To determine the days taken for resumption of normal cyclicity following postpartum anoestrus in lactating dairy cows and days taken to conceive after the resumption of ovulatory activity in cows with or without bull exposure.
c) To evaluate the pregnancy rates and calving interval in cows with or without bull exposure.

4.2 Materials and methods

This study was performed on a group of high-yielding, non-pregnant, Holstein-Friesian dairy cows which were introduced into the experiment at 1 to 5 days postpartum throughout the autumn and winter period. The experiment lasted from November 2011 until April 2012, which is the period of winter housing for this herd. Every selected cow was allocated to the experiment as soon as they joined the main herd after calving. The treatments were the same as in the first experiment; the cows were divided into two groups: no bull contact (NBC=22) and bull contact (BC=23) as assigned in the first experiment. The arrangement of the dairy shed was also the same as before; at one end of the cubicle house the bull was placed within a closed pen with a barred fence which separated cows in the bull contact (BC) group from the bull. This allowed a maximum exposure to bull stimuli cues including olfactory, visual, auditory and limited physical contact, for cows in the BC treatment group.

For this experiment, a sexually mature Friesian-Holstein bull aged around 1.5 years was used as the stimulus animal. The bull was placed in the bull pen throughout the experiment, always being separated from the BC cows by the fence with open bars. The BC cows had an unlimited time of access to the bull pen except during milking. The BC cows also walked past the bull pen twice a day following milking. The cows in the no bull contact (NBC) group were placed at the other end of the cubicle shed as much distance as possible from the bull. There was no direct exposure or possibility of interaction with the bull for these cows, as in the first trial. Cows in the BC group were exposed to the bull from day 2 until approximately 80-150 days post calving.

4.2.1 *Animals and routines*

The dairy cows that were freshly calved during the period of the experiment were allocated to treatment groups to maintain a balance of calving date and parity group, and moved to the dairy shed at or shortly after 2 days postpartum. Similarly to first trial, relevant details of each cow including parity, calving date, previous lactation yield and calving interval were recorded. Body condition score (BCS) of cows was assessed and recorded during the selection process and once every two weeks during the study.

The feeding system was standard for the farm; cows were fed ad libitum on a total mixed ration (TMR) diet formulated to meet the nutrient requirements for post calving cows. The milking routine was according to normal farm routine, two times a day at 06.00 and 14.00. All cows were inseminated for the first time using artificial insemination (AI) from 40 days postpartum if they were showing signs of oestrus. Cows that were not showing oestrous behaviour received a PRID (NBC=9, BC=8) between 41 and 160 days postpartum. There were no cows that were given PRID had been inseminated previously during the postpartum period.

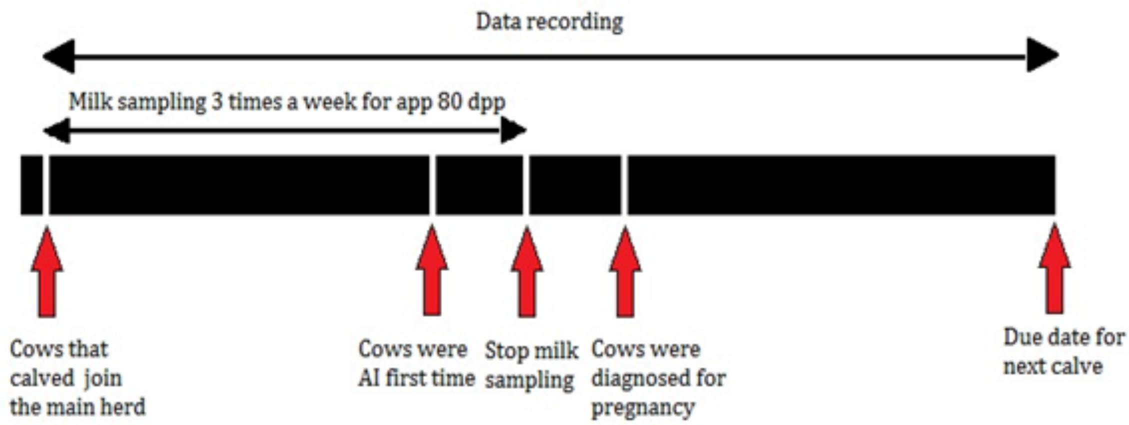


Figure 4.1: Diagram to illustrate the protocol of the second trial; providing bull exposure as a stimulus to the freshly calved cows.

4.2.2 *Activity measurements and prediction of oestrus*

In order to detect a suitable time to inseminate the cows, observation of the changes in activity level was performed using a pedometer system (IceQube, Ice Robotics Ltd, Roslin, UK) that records and reports details of animal activity for 23 cows as subset from the 41 cows (NBC=11, BC=12). The pedometer was attached to one leg of each cow above the fetlock and, once it has been attached to the animal's leg, it continuously monitored stepping behaviour. An increase in the number of steps, as measured by the pedometer, was calculated based on the median number of steps (Roelofs et al., 2005). The numbers of steps taken in each 4 hour time period, starting at 0600 on the day of oestrus as predicted from the milk progesterone profiles, were compared with the number of steps taken in the same time period during the 10 days before and 10 days after. A ratio was calculated by dividing the number of steps in the 4 hour time period on the day of oestrus by the median number of steps in the comparative 10 day period and, if this ratio exceeded a threshold of 5.0, this was defined as an actual increase in number of steps and designated as an oestrus alert based on pedometer readings.

Mounting activity is a primary sign of oestrus; thus observation of behavioural signs was used to predict oestrus in cows for both treatment groups. Other behaviours, as listed in Figure 4.2 below, were also observed. The visual observation was done during two sessions, morning and afternoon for 60 min every session, on the days of milk sampling. Any bulling behaviour shown by the animals during the observation period was recorded. In addition, the herdsman recorded any mounting activity during other times outside the observation period. A video camera was set up to cover the contact area of bull and cows, and recorded any activity at the meeting area for 24 hours per day throughout the trial. This recording was used to measure cows' behaviour and frequencies of contact with the bull, for cows in BC treatment group.

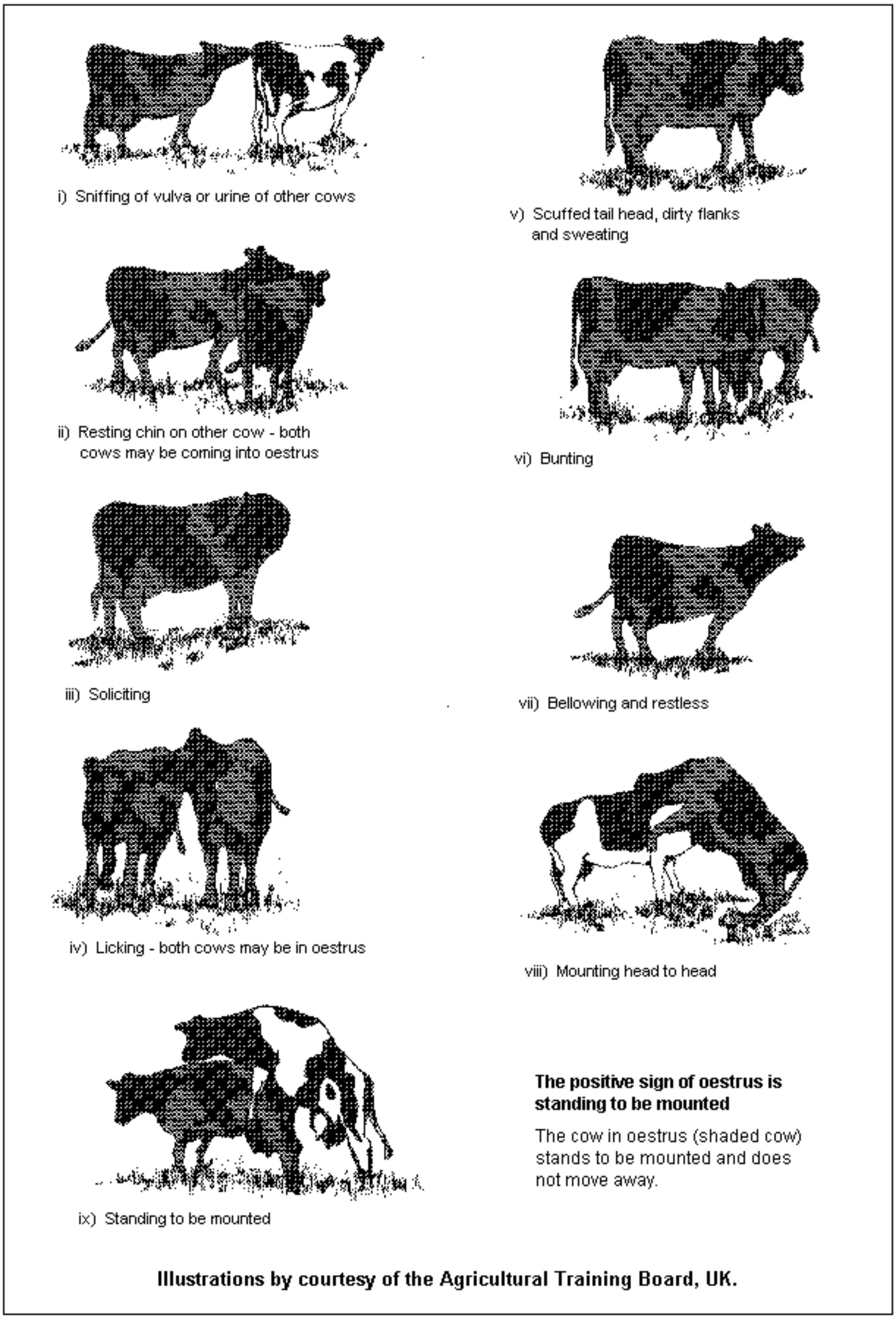


Figure 4.2: Some behavioural signs indicative of a cow (shaded) coming into oestrus or already in oestrus (Dairy Herd Fertility Reference Book 259, 1984)

4.2.3 *Milk sample collection*

Milk samples were collected, from the time that cows were moved into the dairy shed after calving, on 3 days each week until the end of trial, which were every Monday, Wednesday and Friday. Samples were collected during the afternoon milking session at 2 pm. Milk samples, of at least 5ml, were collected into clean tubes, securely capped and stored in the freezer at -20^{o}C. All samples were stored until the end of trial and were analysed in the laboratory of Newcastle University.

4.2.3.1 *Milk progesterone assay*

The cyclic status was determined by the progesterone level in the collected milk samples. Progesterone concentrations were determined using the commercial milk progesterone test kit (Ridgeway Milk progesterone enzyme linked immunoassay, Ridgeway Science, Glouncestershire, UK) in the laboratory and the procedures were the same as for the previous experiment.

The frozen milk samples were thawed by placing them into a water bath at 30˚C for 10 minutes. 24 plates that contained 96 wells each were used to test milk samples from 41 cows and heifers from both treatment groups (NBC=20; BC=21). The assay included the milk progesterone standards: 0ng/ml, 1ng/ml, 2ng/ml, 5ng/ml, 10 ng/ml, 20 ng/ml and 50ng/ml in duplicate. Further explanation on the methods of analysis is outlined in chapter 3. The mean inter-assay coefficient of variation was 17.1% and intra-assay was 2.9%. The limit of detection of the assay was 0.22 ng/ml.

4.2.3.2 *Endocrine parameters of ovarian status*

An individual progesterone profile was obtained for each cow from results of the milk progesterone assay. The progesterone concentration, based on the profiles, was used to determine the resumption of ovarian cyclicity and the normality of the cycles. The ovarian status can be determined by using the endocrine level as a parameter, as illustrated in Figure 4.3 by Royal *et al*. (2000). Resumption of normal ovarian cyclicity can be predicted when there is a rise in progesterone followed by sudden fall, to allow ovulation and another progesterone rise after approximately 5 to 10 days (Crowe, 2008). The incidence of abnormal ovarian patterns can also be identified and the effects on efficiency of response to bull stimulation measured.

Prolonged anovulation postpartum was defined by measuring a milk progesterone concentration of 1.5ng/ml or less for 45 days postpartum or more. The second type of delayed ovulation is a prolonged inter luteal interval, defined as a milk progesterone of 1.5 ng/ml or less for 12 days or more. Another type of abnormal ovarian cycle is delayed luteolysis with persistence of the corpus luteum, determined as a milk progesterone of more than 1.5 ng/ml for more than 19 days during first postpartum oestrous cycle, or persistent corpus luteum type II defined as milk progesterone concentration of more than 1.5 ng/ml for 19 days postpartum or more during the following postpartum oestrous cycles.

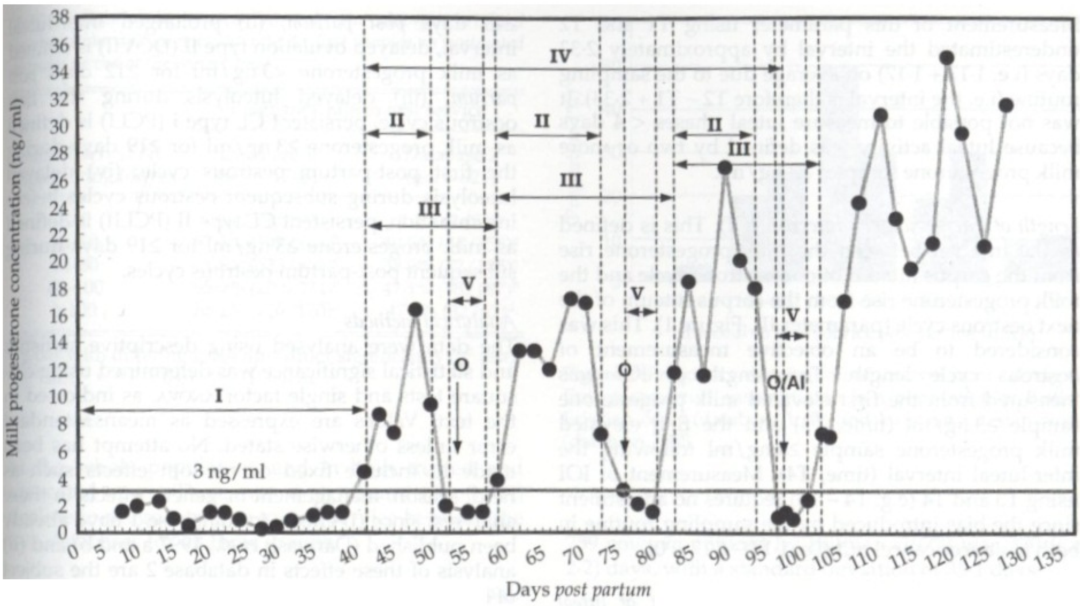


Figure 4.3: Reproductive parameters monitored using milk progesterone profiling (Royal *et al*., 2000) showing phases of ovarian cyclicity; stage I is the interval to resume ovarian cyclicity postpartum, II is the luteal phase, III the inter-ovulatory interval, IV the interval between resumption of ovulatory cyclicity and first AI postpartum, V is length of inter-luteal interval. AI is when the cow is inseminated by AI and O is period of oestrus.

4.2.4 *Data analysis*

All the data were analysed at the end of trial; statistical tests were applied to analyse the normally distributed data sets of progesterone concentrations, interval from calving to resumption of ovulatory activity, interval from resumption of ovulatory activity to successful insemination, and next calving interval. The pregnancy rate was measured and the categorical results were tested to see the difference between groups. Intervals from calving to resumption of ovulatory activity, as measured by progesterone concentration profiles, were tested to find out if there was any significant difference between groups by a one-way ANOVA test, using the Minitab version 16 software package. The mean milk progesterone concentrations of the first oestrus cycle, which was defined as the 21 days from the beginning of the first rise in progesterone concentrations from individual cow profiles, were calculated and compared using repeated measures ANOVA using SPSS version 19. Frequencies of time spent visiting the bull were measured for cows in the BC group to identify any changes in time spent visiting during the inter-luteal period. The difference in interval from day of resumption of ovulatory activity to conception, between groups was also assessed using one-way ANOVA (Minitab 16). The pregnancy rate after insemination was tested by using a chi-square test to identify any significant differences between groups in the proportion of cows to conceive at each insemination. Pregnancy diagnosis was carried out by rectal palpation 60 days after all cows were served to confirm if they were in calf, and the calving date was then predicted based on 280 days gestation period. The real calving interval was recorded and calculated after they calved again, and was then compared between groups using one-way ANOVA. Results were also compared between cows that resumed cyclicity normally and conceived, and cows that conceived after PRID treatment. The significance level was set at $P \leq 0.05$ for all statistical tests.

4.3 Results

4.3.1 *Comparison between groups at allocation*

The distribution of animals in the two treatment groups was compared to make sure that they were balanced on body condition scoring (BCS) at selection, average daily milk production for the previous lactation period, parity, and previous calving interval as shown in Table 4.1. The animals were divided into a no bull contact group (NBC) (n=22), which did not have any contact or exposure with the bull, and a bull contact group (BC) (n=23), which had fenceline contact for 24 hours every day throughout the trial period. However, 4 cows (NBC=2, BC=2) were discarded from data analysis following allocation to the experiment due to health problems (mastitis, feet problems and uterine infections). The results show that there were no significant differences between groups for BCS, parity, milk production and previous calving interval.

Table 4.1: The mean (±SEM) of characteristics of the experimental animals at allocation

	NBC (20)	BC (21)	SEM	*T-test*	*P* value
BCS	2.09±0.44	2.16±0.59	0.08	0.20	**0.657**
Parity	3.10±1.73	3.00±1.56	0.26	0.04	**0.851**
Previous milk production (litres/day)	34.1±4.78	31.5±6.46	0.89	2.12	**0.154**
Previous calving interval (days)	468±134.8	487±160.5	25.18	0.14	**0.708**

4.3.2 *The correlation of milk production, parity, BCS and calving interval*

In additional to comparisons above, the test of correlation was done using all cows at allocation to find out if there was any relationship between average of daily milk production from previous lactation, parity and BCS at selection of animals and previous calving interval. Table 4.2 shows the correlation between factors. Results show that there was no significant correlation between the listed factors in the animals, except for the body condition score and calving interval. The results show that previous calving interval in this group was highly negatively correlated with the body condition score ($P<0.001$). This may suggest that a longer previous calving interval may be associated with better body condition, and these factors therefore need to be taken into account in the statistical analysis.

Table 4.2: The results for correlation test of previous milk production, parity, BCS and previous calving interval for all cows at allocation.

Correlations: PARITY, MILK PROD, BCS, CALV INTERVAL

	PARITY	MILK PROD	BCS
MILK PROD	0.186		
	0.244		
BCS	0.020	-0.242	
	0.902	0.128	
CALV INTERVAL	-0.033	0.168	-0.955
	0.838	0.293	0.000

4.3.3 *PRID treatment*

Cows that were detected with problems to get in calf again were given PRID treatment, as advised by the farm veterinarian after being diagnosed by an ultrasound test. As shown in figure 4.4, some cows from both groups were give a PRID between 41 to 60 days postpartum. However, as days postpartum increased; more cows in BC were given PRIDs earlier compared to NBC cows. Cows in BC were treated with PRIDs up to 120 days postpartum while cows in NBC were still receiving PRID treatment until 160 days postpartum. However, there was no significant difference between mean time of PRID treatment between groups (NBC=104 DPP, BC=78 DPP; P=0.152).

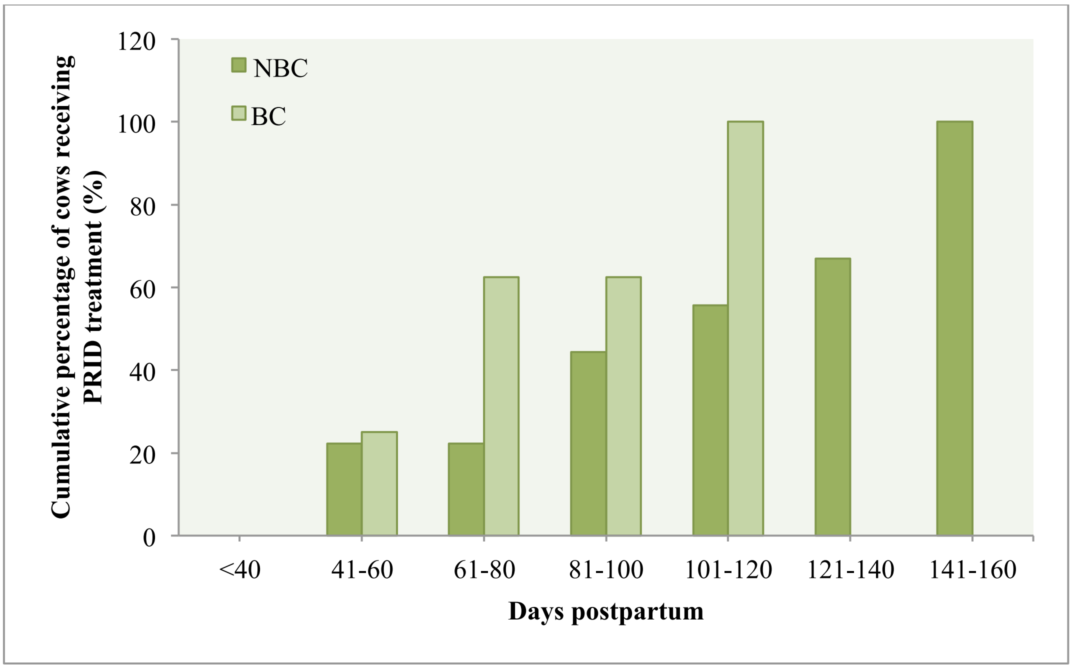


Figure 4.4: The cumulative percentage of all cows from both groups that received PRID treatment with time post partum (n=17; NBC=9, BC=8)

4.3.4 *Resumption of ovulatory activity*

From all 41 cows, only 33 of them showed a rise in milk progesterone within 80 days postpartum. These cows (NBC=15, BC=18) resumed ovarian cyclicity before 80 days postpartum, while the other 8 cows (NBC=5, BC=3) had subsequently resumed ovarian cyclicity by 100 days postpartum, as indicated by their insemination record and data recorded at the farm. There was no difference in the proportion of cows that resumed ovarian cyclicity post calving between groups. Heats were detected by visual observation in the shed before milking for 25% of cows in the NBC group and 38% in BC group.

17 of the 41 cows were treated with a progesterone releasing intravaginal device (PRID) and 12 of them had resumed ovarian cyclicity before the PRID treatment, as determined subsequently by milk progesterone profiles. In total, 36 cows resumed ovarian cyclicity post calving naturally. Only 5 cows that were treated with PRID had a rise in progesterone and resumed ovarian cyclicity with the aid of the PRID.

Table 4.3: The total number of cows that resumed ovarian cyclicity [OC] for both treatment groups derived from progesterone profiles, time of artificial insemination (AI), and recorded oestrus data.

	NBC (n=20)	**BC (n=21)**	**SEM**	***Test***	***P* value**
Proportion of cows that resumed OC (P4+AI+record)	100%	100%	-	chi-sq	-
Proportion of cows that resumed OC based on P4 before day 80 (n=33)	75.0% (15)	85.7% (18)	-	chi-sq	0.454
Proportion of cows that resumed OC naturally before day 80 (n=31)	70.0% (14)	81.0% (17)	-	chi-sq	1.000
Proportion of cows treated with PRID	45.0% (9)	38.1% (8)	-	chi-sq	0.756
Resumed cyclicity after PRID treatment	10.0% (2)	14.3% (3)	-	chi-sq	1.000
Interval from calving to resumption of OC based on P4 (days)* (n=33)	31.3±14.5 (15)	29.4±12.4 (18)	2.35	***T-test*** 0.16	0.691
Interval from calving to resumption of OC naturally based on P4 (days)(n=31)	28.6±10.2 (14)	28.4± 12.0 (17)	2.03	***T-test*** 0.00	0.969
Observed heat	25.0% (5)	38.1% (8)	-	chi-sq	0.505
Changes in walking activity (n=23)**	36.4% (4/11)	33.3% (4/12)	-	chi-sq	1.000

*Resumption of ovulatory activity was determined by concentration of P4.

**Only 23 cows were observed for changes in walking activity as a subset for whole group

In the NBC group, 15 cows showed an increase in milk progesterone and 18 cows from the BC group did so (Table 4.3). The mean interval taken to resume ovulatory activity from calving was based on these cows that had a progesterone rise. There was no significant difference in interval, either for cows that were exposed to the bull or not and for cows treated with PRID or not.

The results show that the cumulative percentage of cows resuming ovarian activity over time was not significantly different for group BC compared to NBC, as shown in Figure 4.5. Figure 4.6 shows the cumulative percentage of cows that resumed ovarian activity naturally without the PRID treatment. Figure 4.7 shows the cumulative percentage resuming ovarian activity for cows that were treated with PRID.

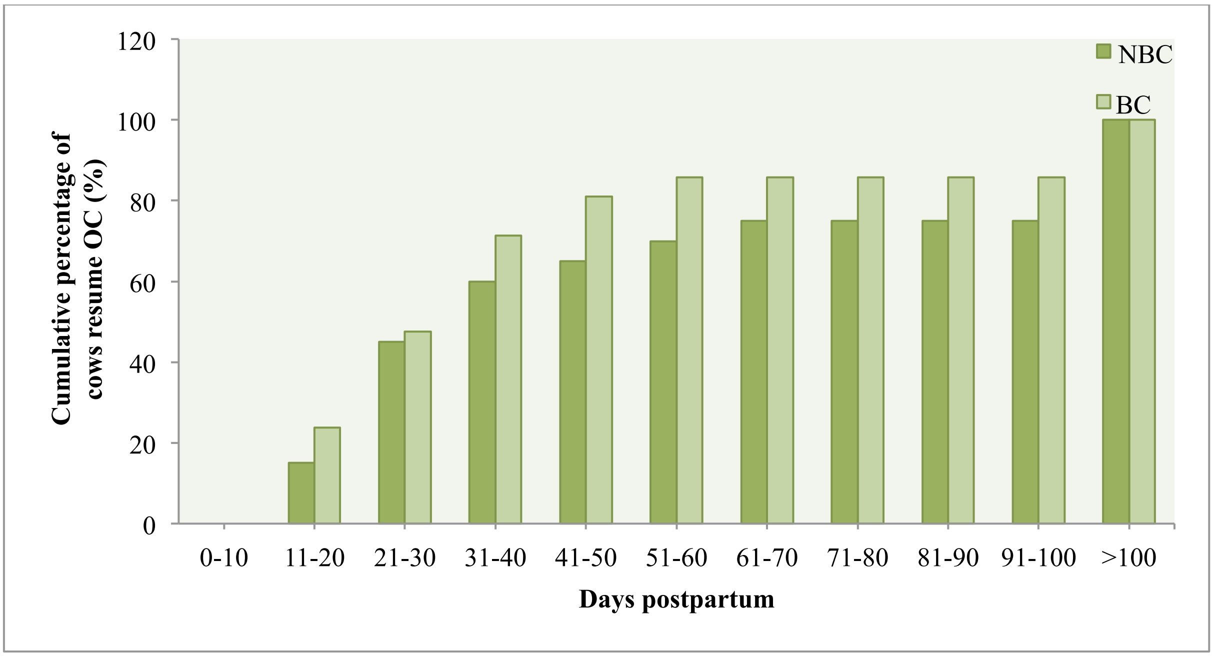


Figure 4.5: The cumulative percentage of all cows from both groups that resumed ovarian cyclicity by different times postpartum as determined by milk progesterone concentration for cows with <100 dpp, and AI records for cows with >100 dpp. (n=41; NBC=20, BC=21)

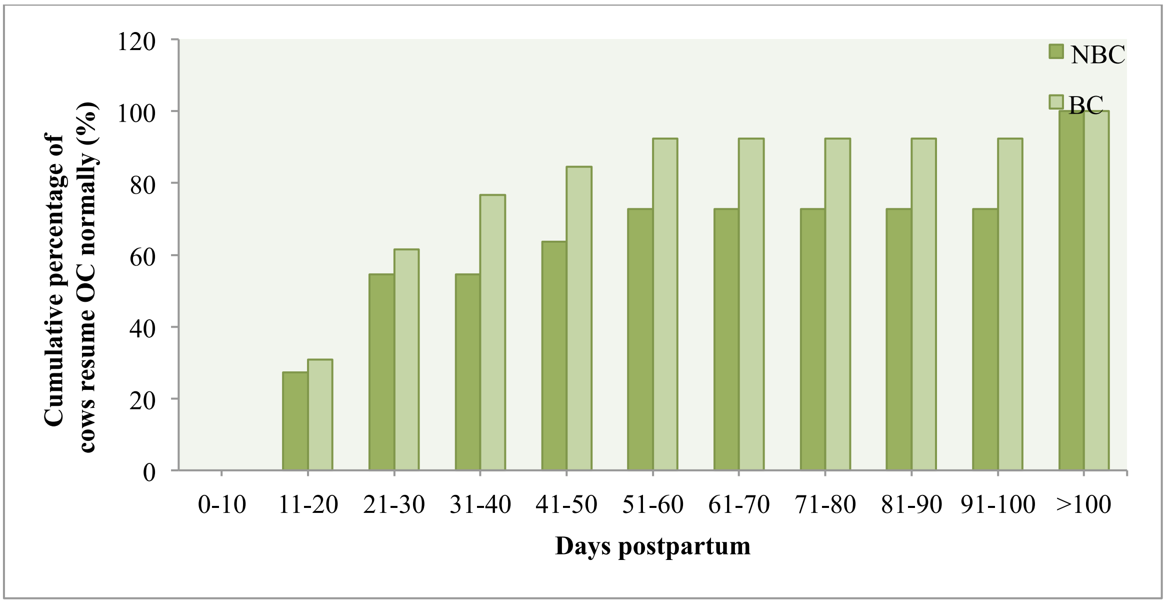


Figure 4.6: The cumulative percentage of cows that resumed ovarian cyclicity naturally without PRID intervention by different times postpartum as determined by milk progesterone concentration for cows with <100 dpp, and AI records for cows with >100 dpp. (n=24; NBC=11, BC=13).

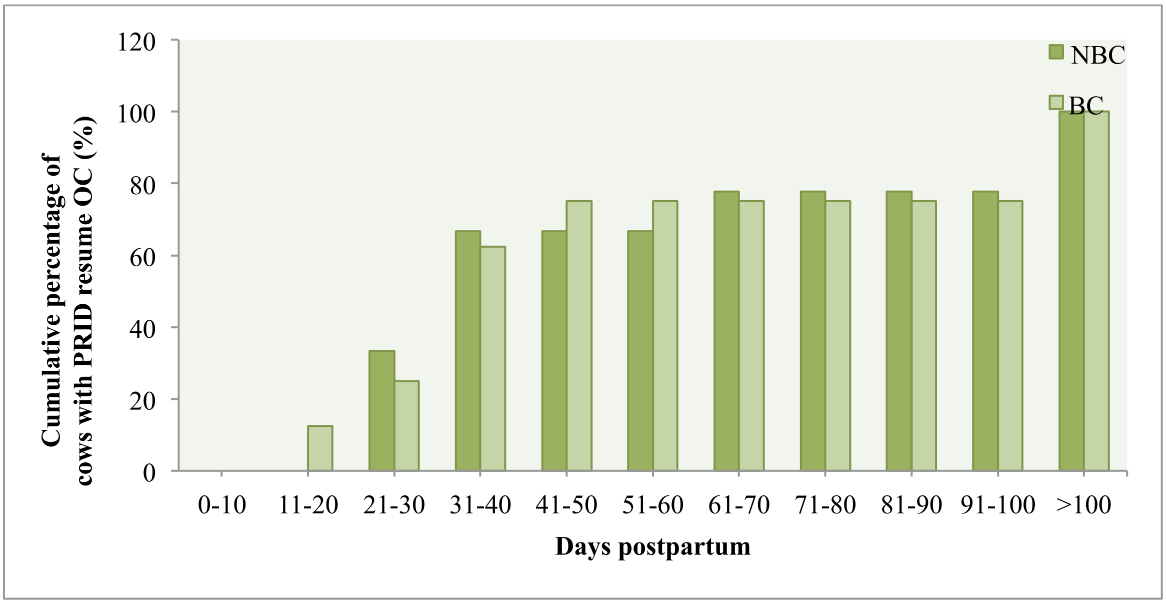


Figure 4.7: The cumulative percentage of cows receiving PRID treatment that resumed ovarian cyclicity by different times postpartum, as determined by milk progesterone concentration for cows with <100 dpp, and AI record for cows with >100 dpp. (n=17; NBC=9, BC=8). Most of the cows in both groups had resumed cyclicity before PRID treatment, only a few cows resumed cyclicity after the PRID.

4.3.5 *Observations of oestrus behavior signs*

The changes in behavior during the inter-luteal interval were observed and every sign of oestrus displayed by each of the cows was recorded and analysed; results are presented in table 4.4. During the oestrus period, not all signs of oestrus were displayed by the cows in this trial and the frequencies of expressing oestrus behaviour varied between cows. Signs of oestrus that were detected throughout the observation period were; stand to be mounted (STBM), mounting other cows, chin resting, vulval discharge and increase in walking activity. However, only 'stand to be mounted' is considered as an actual sign that represents the oestrus period; other than this a cow can be considered as in oestrus if the total points score for oestrus signs detected in one observation period is more than 100 points (Reolofs et al., 2005; see chapter 3 for details of points allocation). There were more cows in BC (n=8) that expressed the behavior of 'stand to be mounted' compared to NBC (n=5). Nevertheless, results show that there was no significant difference in expression of oestrus signs by cows in NBC and BC groups. The distribution of other oestrus signs; mounting other cows, chin resting, vulva discharge displayed by cows from both NBC and BC group were about equivalent. Thus the biostumulation effects did not affect the expression of oestrus signs in BC group.

There were 23 cows from all 41 which were fitted with IceQube pedometers to observe for changes in walking activity during this trial. Results from these 23 cows (NBC=11, BC=12) are used to represent the NBC and BC groups. A change in walking activity during oestrus represents the restless behaviour, as cows spent more time walking than lying down. However, changes in walking activity during oestrus proved to be poor oestrus indicators in this trial as only 4 cows from NBC and 4 cows from BC showed clear changes.

Table 4.4: The percentage of cows observed in NBC and BC groups which displayed various oestrus signs during the observation period.

Oestrus signs	All animals	NBC	BC
Stand to be mounted (STBM)	31.7% (13/41)	25.0% (5/20)	38.1% (8/21)
Mounting other cows	69.4% (28/41)	75.0% (15/20)	61.9% (13/21)
Chin resting	48.8% (20/41)	40.0% (8/20)	57.1% (12/21)
Vulval discharge	61.0% (25/41)	55.0% (11/20)	66.7% (14/21)
Increase in walking activity	34.8% (8/23)	36.4% (4/11)	33.3% (4/12)

The total points scored by cows in NCB and BC for every time they expressed oestrus signs are shown in table 4.5. BC cows tended to score higher points for chin resting and vulva discharge in comparisons with NBC group. However, the total points for stand to be mounted and mounting other cows were numerically higher for cows from NBC, as these behaviour were displayed slightly more frequently in NBC than BC cows. There was no significant difference arising from the effects of biostimulation on the frequencies of oestrus signs displayed by cows in BC group compared to cows in NBC.

Table 4.5: The calculated total points of oestrus behavioural signs displayed by cows in NBC and BC group.

Oestrus signs observed during inter-luteal interval	**Total points**			
	NBC	**BC**	***T-test***	***P* Value**
Stand to be mounted (STBM)	140.0±54.8	137.5±51.8	0.01	0.935
Mounting other cows	109.7±54.3	94.2±46.0	0.65	0.429
Chin resting	24.4±11.2	35.6±17.8	2.29	0.152
Vulva discharge	4.1±1.5	5.4±2.4	2.32	0.142

4.3.6 *Milk progesterone*

The mean milk progesterone concentrations for 21 days after the first rise of progesterone postpartum are shown in Figure 4.8. The mean concentrations for the first oestrous cycle detected was numerically higher for cows that were exposed to the bull, and the progesterone concentrations increased earlier, compared to cows with no exposure to the bull, though there was no statistical difference (P=0.24) in mean progesterone concentrations between groups.

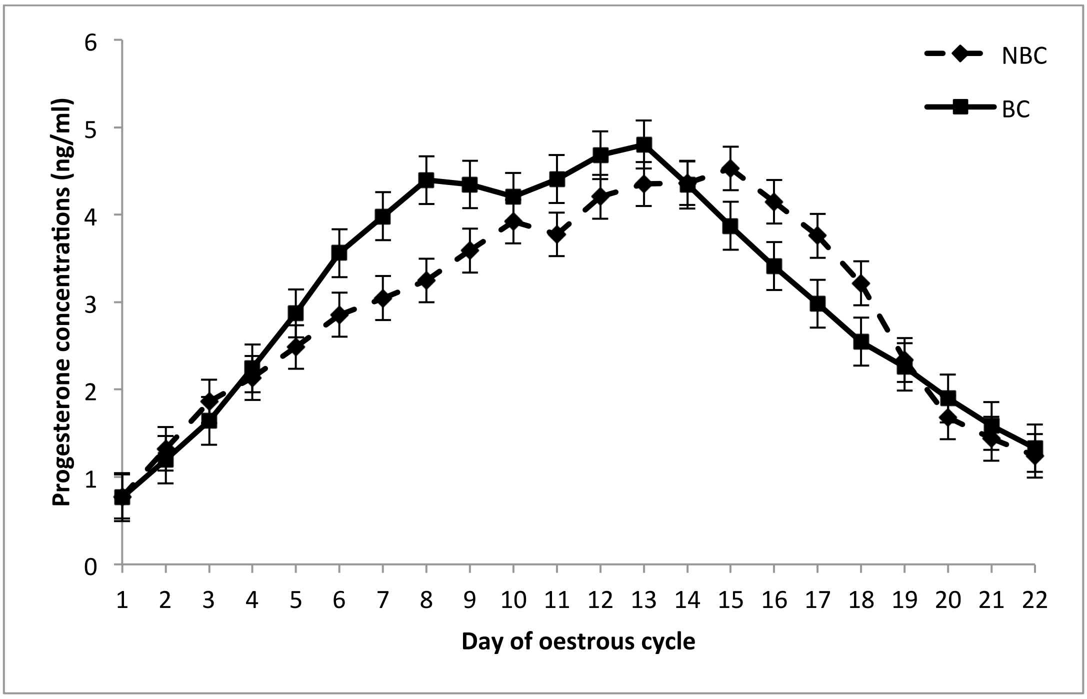


Figure 4.8: The comparison of mean progesterone concentration (±SEM) derived from milk analysis for BC and NBC treatment groups. This extrapolation was based on thrice weekly milk sampling from 31 animals (NBC=14, BC=17) that showed a rise in progesterone for the first time post calving. The values of progesterone concentrations on days that were not sampled were obtained by calculating the average of progesterone concentrations on the previous and the day after the sampling day. The rise in progesterone was not associated with PRID treatment, as all cows resumed cyclicity normally (some of cows received PRIDs but only after the first rise in progesterone).

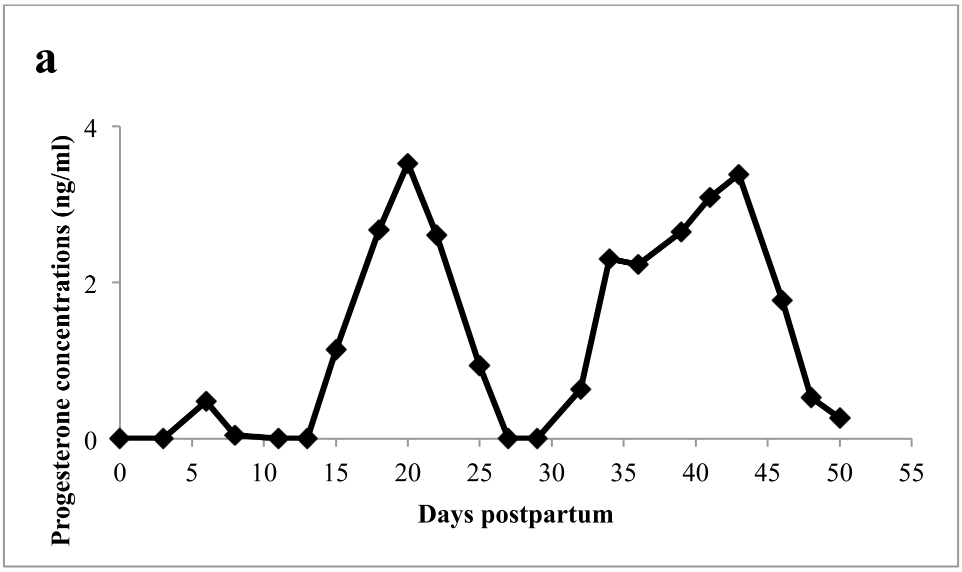
4.3.7 *Comparison of normal or abnormal cycles*

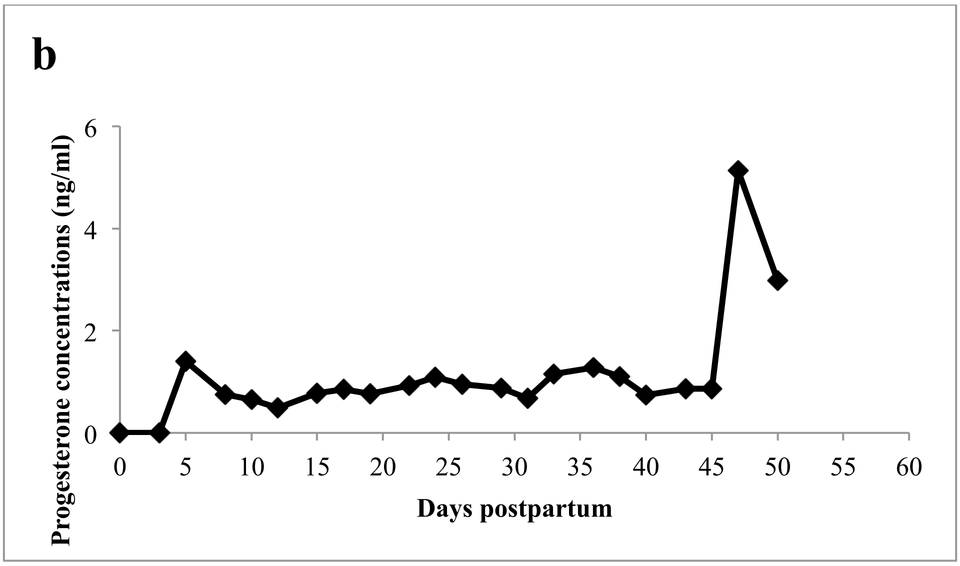
The results from milk progesterone concentration profiles showed that cows from both treatment groups had various types of ovarian cycle after the resumption of ovarian activity post calving, as shown in Table 4.6. A normal ovarian cycle was determined by normal cyclicity of progesterone concentration, which means the progesterone started to increase after ovulation and remained high for about 18 days, then dropped down to allow the next ovulation and started to rise again post ovulation. However, some of the cows experienced abnormal cycles, since the progesterone concentrations appeared to be high for a longer period, or else the concentration of progesterone was too low for a longer period. Progesterone profiles for all types of ovarian cycle are illustrated in figure 4.9.

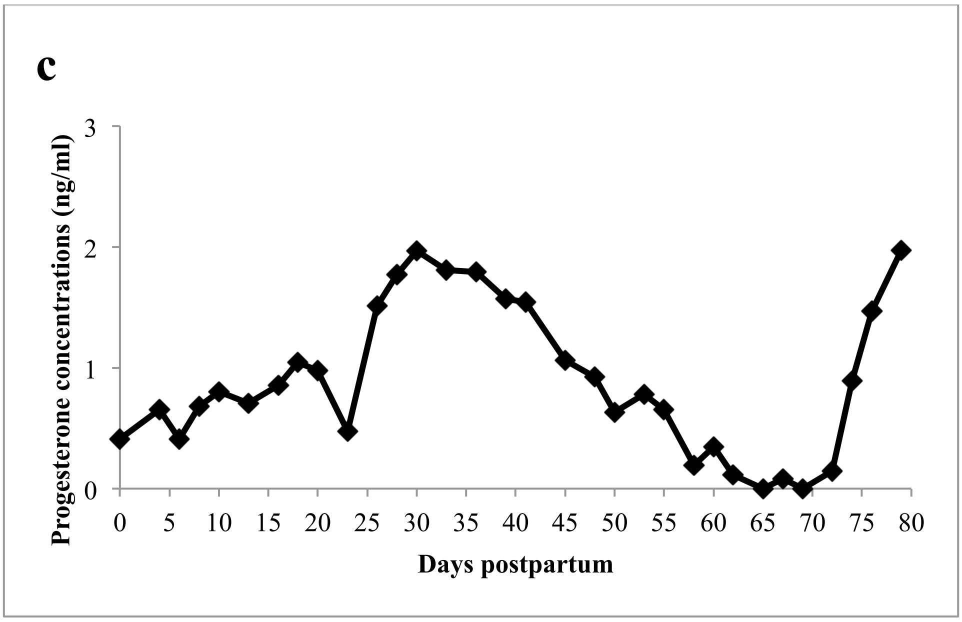
Table 4.6: The proportion of animals, from groups either exposed or not exposed to the bull, showing short, normal, abnormal (delayed ovulation type I and II, persistent CL type I and II based on atypical ovarian hormone pattern) ovarian cyclicity post calving (day 0-80).

	NBC (n=20)	**BC (n=21)**
Normal	50.0% (10)	52.4% (11)
Delayed ovulation type I	40.0% (8)	28.6% (6)
Delayed ovulation type II	0.0%	4.8% (1)
Persistent CL type I	5.0% (1)	4.8% (1)
Persistent CL type II	5.0% (1)	0.0%
Short cycle	0.0%	9.5% (2)

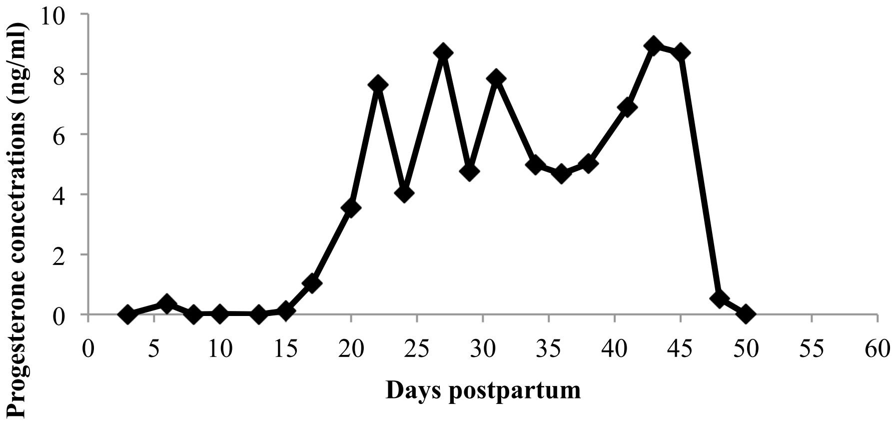
Figure 4.9: Different types of ovarian cycles (normal and abnormal types) in NBC and BC cows and heifers with representative milk progesterone profiles. These profiles were obtained from the point after they had calved. ***a.*** Normal ovarian cycles throughout the study period ***b.*** Abnormal ovarian cycle with delayed ovulation type I, with prolonged low progesterone concentration of 1.5ng/ml or less for 45 days postpartum or more. ***c.*** Abnormal ovarian cycle with delayed ovulation type II, with prolonged interval from first ovulation to second ovulation. ***d.*** Abnormal ovarian cycle with persistent corpus luteum type I, with milk progesterone concentrations of more than 1.5 ng/ml for 18 days or more. ***e.*** Abnormal ovarian cycle with persistent corpus luteum type II, with milk progesterone concentrations of more than 1.5 ng/ml for 17 days or more during the following postpartum oestrous cycles.



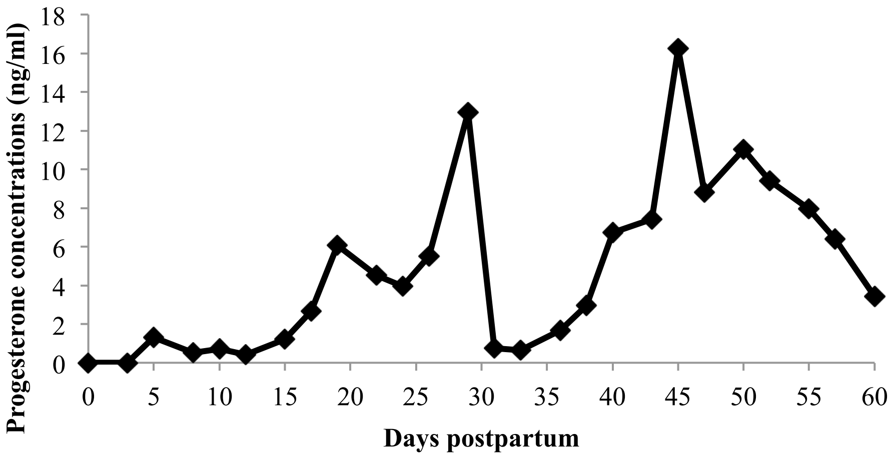

b
Progesterone concentrations (ng/ml)
0
2
4
6
0 5 10 15 20 25 30 35 40 45 50 55 60
Days postpartum


c
Progesterone concentrations (ng/ml)
0
1
2
3
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Days postpartum

d


Progesterone concetrations (ng/ml)
10
8
6
4
2
0
0 5 10 15 20 25 30 35 40 45 50 55 60
Days postpartum

e


Progesterone concentrations (ng/ml)
18
16
14
12
10
8
6
4
2
0
0 5 10 15 20 25 30 35 40 45 50 55 60
Days postpartum

4.3.8 *Interaction with the bull during the inter-luteal interval*

All 21 cows in the BC group had an exposure to the bull every day during this trial on at least two occasions; after they had been milked, cows walked down a passageway and passed the bull pen. The number of cows that had a direct contact with the bull and increased time spent visiting the bull pen was recorded and the average time for five days for this activity is shown in figure 4.10. The longest time cows spent visiting the bull was measured on day -1, which is a day before the day with lowest progesterone concentration (day 0). The number of cows that had voluntary direct contact with the bull was higher (n=8) on day 0 compared to other days, however there was no significant difference in the number of cows that had direct contact, or in the mean time spent visiting the bull, between the five observation days.

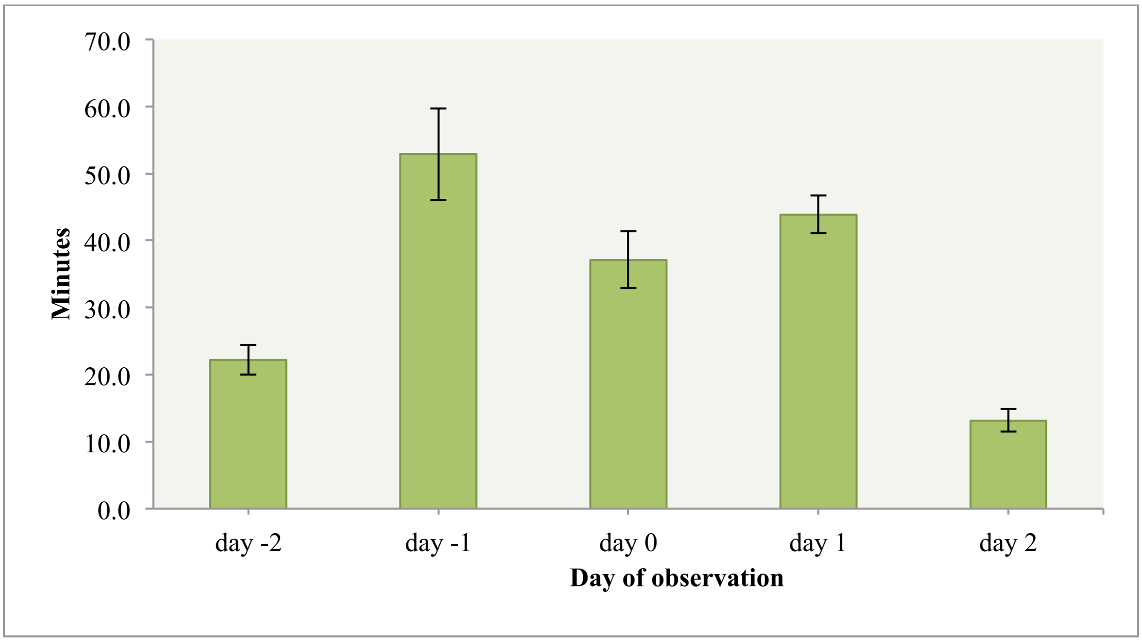


Figure 4.10: Histogram showing the mean (±SEM) time in minutes spent by cows in the BC group which interacted with the bull, measured starting from 2 days (day -1; n=4 and -2; n=7) before the day with lowest progesterone concentrations (day 0; n=8) and 2 days (day1; n=6 and 2; n=6) after that.

4.3.9 *The effects of bull exposure on interval from day of resumption of ovulatory activity (OA) until successful AI*

After the resumption of ovulatory activity in cows was detected, they were served by artificial insemination (AI). For this trial, the number of inseminations required for cows to conceive was over five for several cows. The average number of inseminations per conception was lower in BC cows compared to NBC with 2.0 and 3.4 inseminations, respectively (P=0.010). Table 4.7 shows the mean interval from day of resumption of ovulatory cyclicity (OC) to the first until eighth inseminations. There was no difference between the groups in intervals for all inseminations. The mean interval to first insemination was almost the same for NBC and BC groups. However for the second insemination, the interval for BC group was longer by more than 10 days compared to NBC, and for the third insemination, the difference in interval between groups was only 2 days. Results show that none of the differences in the intervals between groups were significant and thus are unlikely to have contributed to a higher rate of pregnancy for BC cows.

Table 4.7: The mean interval (days) from day of resumption ovulatory activity (OA) to inseminations for both NBC and BC treatment groups for 31 animals that showed a natural first progesterone rise postpartum (these animals were not given PRID treatment before resumption of OC).

Interval from resumption of OA to:	NBC	BC	SEM	*T-test*	*P* value
First insemination	63.8 (n=14)	57.5 (n=17)	18.22	0.33	**0.745**
Second insemination	142.8 (n=12)	155.4 (n=8)	12.93	0.23	**0.640**
Third insemination	226.2 (n=9)	223.7 (n=6)	17.74	0.04	**0.852**
Fourth insemination	241.2 (n=5)	267.3 (n=3)	22.58	0.31	**0.596**
Fifth insemination	292.3 (n=3)	271.0 (n=2)	42.21	0.21	**0.868**
Sixth insemination	367.5 (n=2)	-	-	-	**-**
Seventh insemination	402.0 (n=1)	-	-	-	**-**
Eighth insemination	425.0 (n=1)	-	-	-	**-**

Table 4.8 shows the mean interval from day of calving to the first until eight/fifth inseminations. Similar to results of the interval from resumption of ovulatory activity (OA) to inseminations, the interval from calving to each insemination show that no differences in these intervals between groups. Therefore it is unlike this contributed to the higher rate of pregnancy for BC cows.

Table 4.8: The mean interval (days) from day of calving to first until eight/fifth inseminations for both NBC and BC treatment groups, for all 41 animals.

Interval from calving to:	NBC	BC	SEM	*T-test*	*P* value
First insemination	92.6 (n=20)	90.2 (n=21)	9.80	0.17	**0.868**
Second insemination	163.2 (n=17)	178.4 (n=9)	11.02	0.43	**0.516**
Third insemination	224.3 (n=13)	244.0 (n=7)	14.23	0.44	**0.518**
Fourth insemination	264.5 (n=8)	288.7 (n=3)	20.90	0.27	**0.619**
Fifth insemination	328.0 (n=5)	296.0 (n=2)	34.39	0.32	**0.804**
Sixth insemination	384.5 (n=2)	-	-	-	**-**
Seventh insemination	423.0 (n=1)	-	-	-	**-**
Eighth insemination	446.0 (n=1)	-	-	-	**-**

The interval from day of resumption of ovulatory activity until successful AI for all 33 cows that showed a rise in progesterone concentration, to successful insemination (cows were pregnant after this insemination) differed between treatment groups (Table 4.9). The mean interval was lower for cows in group BC compared to NBC (P=0.009). However, there was no significant difference for 20 of these 33 cows that were not treated with PRID (P=0.148), despite a numerically lower mean value. In the 13 cows treated with PRID, the difference between groups in the interval from the resumption of ovulatory activity to successful insemination also failed to reach statistical significance (P=0.064).

Table 4.9: The mean interval (days) from day of resumption of ovulatory activity (OA) until successful AI for the 33 animals which showed a progesterone (P4) rise.

	NBC	**BC**	**SEM**	***T-test***	***P* value**
All animals	226.0 (n=15)	120.5 (n=18)	18.71	2.81	**0.009**
No PRID	180.0 (n=8)	115.3 (n=12)	20.76	1.53	**0.148**
PRID	278.3 (n=7)	131.0 (n=6)	33.98	2.11	**0.064**

Table 4.10 shows the mean interval from calving to successful insemination for the 33 animals which showed a progesterone (P4) rise while Table 4.11 shows the mean interval from calving to successful insemination for all 41 cows in this trial, which differed significantly between treatment groups. The PRID treatment seemed to be more effective for cows that were exposed to the bull since the interval was reduced in comparison with NBC cows. This suggests that maybe bull exposure has some effect on the efficiency of PRID treatment.

Table 4.10: The mean interval (days) from calving day until successful AI for the 33 animals which showed a progesterone (P4) rise.

	NBC	**BC**	**SEM**	***T-test***	***P* value**
All animals	257.0 (n=15)	149.9 (n=18)	18.63	2.86	**0.008**
No PRID	208.1 (n=8)	142.8 (n=12)	20.71	1.52	**0.151**
PRID	313.6 (n=7)	164.0 (n=6)	33.42	2.19	**0.056**

Table 4.11: The mean interval (days) from calving day until successful AI for all 41 cows.

	NBC	**BC**	**SEM**	***T-test***	***P* value**
All animals	241.0 (n=20)	150.8 (n=21)	15.67	2.87	**0.007**
No PRID	208.3 (n=11)	141.5 (n=13)	17.93	1.84	**0.080**
PRID	291.1 (n=9)	165.9 (n=8)	27.65	2.07	**0.050**

4.3.10 *Pregnancy rate*

The pregnancy rate for animals in this trial is shown in Table 4.12. The highest insemination number required to make cows conceive was five times for cows in BC group, while cows in NBC required more inseminations, up to eight times, to achieve pregnancy. The results show that from all 41 cows, only 15 of them conceived after being served for the first time post calving (NBC=3, BC=12). There was an obvious difference in the number of cows that conceived to the first insemination between groups. A high proportion of cows in the BC group were pregnant to their first insemination compared to the NBC group. However, from all 15 cows that conceived after first insemination, 6 of them were treated with PRID (NBC=1, BC=5).

Table 4.12: The pregnancy rates to first insemination for all animals from NBC and BC group, including cows that were treated with PRID.

	All animals	**NBC**	**BC**	***Fisher's test***	***P* value**
Pregnant to first insemination	36.6% (15/41)	15.0% (3/20)	57.1% (12/21)	7.84	**0.009**
Natural Oestrus	37.5% (9/24)	18.2% (2/11)	53.9% (7/13)	3.23	**0.105**
PRID	25.3% (6/17)	11.1% (1/9)	62.5% (5/8)	4.89	**0.050**

From these results, the pregnancy rate of cows that were cycling naturally (n=24), without treatment of PRID, was higher in BC group compare to NBC; however, this difference failed to reach statistical significance (P=0.105). For cows that were treated with PRID, the pregnancy rate after first insemination was significantly different (P=0.050), with more cows from BC conceiving compared to NBC cows.

Cows that failed to conceive to first insemination were then served again. Results in Table 4.13 show that there was no significant difference in pregnancy rate for all subsequent inseminations between groups. All cows in the BC group had conceived after five inseminations, and only 2 cows from this group required 5 inseminations to get pregnant. In contrast, pregnancy rate for cows in the NBC group was lower than BC; 2 cows from the NBC group required their sixth and eight inseminations to get pregnant. The cumulative percentage of pregnancy to number of inseminations is shown in Figure 4.11.

Table 4.13: The pregnancy rates to second and subsequent inseminations for cows from NBC and BC groups, including 10 cows that were treated with PRID prior to their first insemination.

	All animals	**NBC**	**BC**	***Fisher's test***	***P* value**
Pregnancy to second insemination	23.1% (6/26)	23.5% (4/17)	22.2% (2/9)	0.01	**1.000**
Pregnancy to third insemination	45.0% (9/20)	38.5% (5/13)	57.1% (4/7)	0.42	**0.642**
Pregnancy to fourth insemination	36.4% (4/11)	37.5% (3/8)	33.3% (1/3)	0.02	**1.000**
Pregnancy to fifth insemination	71.4% (5/7)	60.0% (3/5)	100.0% (2/2)	1.12	**1.000**
Pregnancy to six insemination	50% (1/2)	50% (1/2)	-		-
Pregnancy to seventh insemination	-	0/1	-		-
Pregnancy to eight insemination	100.0% (1/1)	100.0% (1/1)	-		-

*From all cows, 10 cows with history of PRID, n=8 from NBC and n=2 from BC group

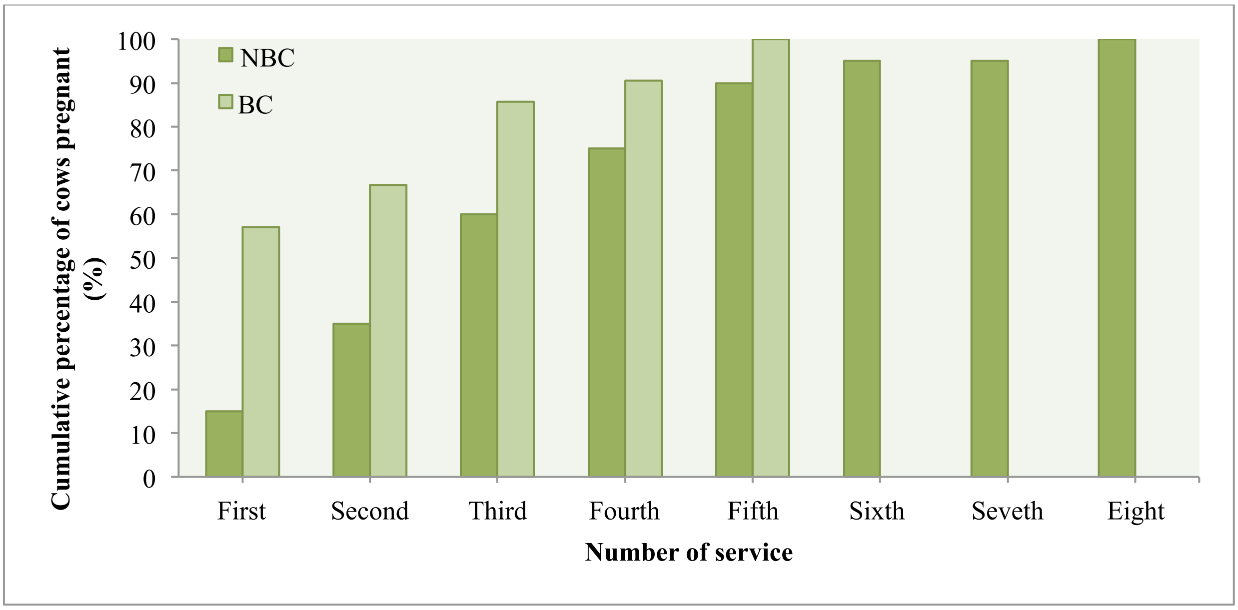


Figure 4.11: The cumulative percentage of all cows for both groups that conceived at different inseminations[1].

[1] The BC cows were generally no longer in contact with the bull after the second insemination as a result of spring turnout to pasture.

4.3.11 *Pregnancy rate associated with type of ovarian cycle*

As described in Table 4.6, animals from both NBC and BC groups showed short, normal, or abnormal ovarian cyclicity post calving (day 0-80). Abnormal ovarian cyclicity was defined as delayed ovulation types I and II, persistent CL type I and II based on an atypical ovarian hormone pattern. Further analysis to measure the association of normal or abnormal ovarian cyclicity with pregnancy rate to first insemination was done. As shown in Table 4.14, from 10 cows with normal ovarian cyclicity in the NBC group, only 1 cow with PRID treatment conceived to the first insemination. In comparison, in BC group of the 11 cows that had normal ovarian cyclicity, 6 of them conceived to the first insemination - 5 cows with no PRID treatment and 1 cow treated with PRID. Results shown in Figure 4.14 explain that there were 2 cows from this group that were inseminated at the wrong time, during the period of high progesterone level; this suggests that if all cows were inseminated at the right time, the proportion of BC cows with a normal cycle which become pregnant to their first insemination could be higher. These comparisons between NBC and BC group show that cows with normal ovarian cyclicity which were exposed to the bull had a better conception rate compared to ones without bull exposure. Other than this, cows with delayed ovulation type I and type II, either treated or not with PRID, had a better conception rate to the first insemination when they were exposed to the bull. Furthermore, 4 of 6 cows with delayed ovulation type I and 1 of 1 cow with delayed ovulation type II were pregnant to first insemination in the BC group, while only 1 of 8 cows with delayed ovulation in the NBC group was pregnant. There were 2 cows with persistent corpus luteum type I which were pregnant to the first insemination, 1 cow from NBC and the other from BC group. However there was no statistical difference for this comparison due to the small number of animals.

Table 4.14: The pregnancy rate to first insemination in cows in relation to the type of ovarian cyclicity post calving, with or without PRID treatment, for both control and bull exposure groups. As shown in table 4.11, 3 NBC cows and 12 BC cows were pregnant to first insemination, this table analyses the effects of normal or abnormal ovarian cycles on conception rates after first insemination (Refer to Figure 4.3 for type of ovarian cyclicity).

Type of ovarian cyclicity	Pregnancy rate to first insemination		
	PRID	**NBC (n=20)**	**BC (n=21)**
Normal	Untreated	-	23.8% (5 of 10)
	Treated	5.0% (1 of 1)	4.8% (1 of 1)
Delayed ovulation type I	Untreated	5.0% (1 of 8)	9.5% (2 of 4)
	Treated	-	9.5% (2 of 2)
Delayed ovulation type II	Untreated	-	-
	Treated	-	4.8% (1 of 1)
Persistent corpus luteum type I	Untreated	5.0% (1 of 1)	-
	Treated	-	4.8% (1 of 1)

4.3.12 *The effects of NBC and BC treatment on calving interval*

Cows in the BC group were exposed to the bull during the anoestrous period, starting from very early post calving (approximately 2 days postpartum). The calving interval was obtained by calculating the days between previous and subsequent calving date. The mean calving interval is shown in Table 4.15. For all animals in the trial, the mean calving interval for cows in BC group was lower compared to NBC group (P=0.007). However there was no significant difference (P=0.088) in calving interval for cows that were cycling naturally (n=24) when compared between groups. Cows that were exposed to the bull and treated with a PRID showed a significant reduction in calving interval (0.050) compared to cows treated with PRID but not exposed to the bull. The pattern of calving interval for both groups is shown in Figure 4.12.

Table 4.15: The mean calving interval (days) for both NBC and BC groups comparing all animals, both with PRID and without PRID treatment.

	NBC	**BC**	**SEM**	***T-test***	***P* value**
All animals	522.3 (n=20)	433.6 (n=21)	16.85	2.85	**0.007**
No PRID	489.3 (n=11)	425.3 (n=13)	18.41	1.80	**0.088**
PRID	562.7 (n=9)	447.1 (n=8)	30.27	2.09	**0.050**

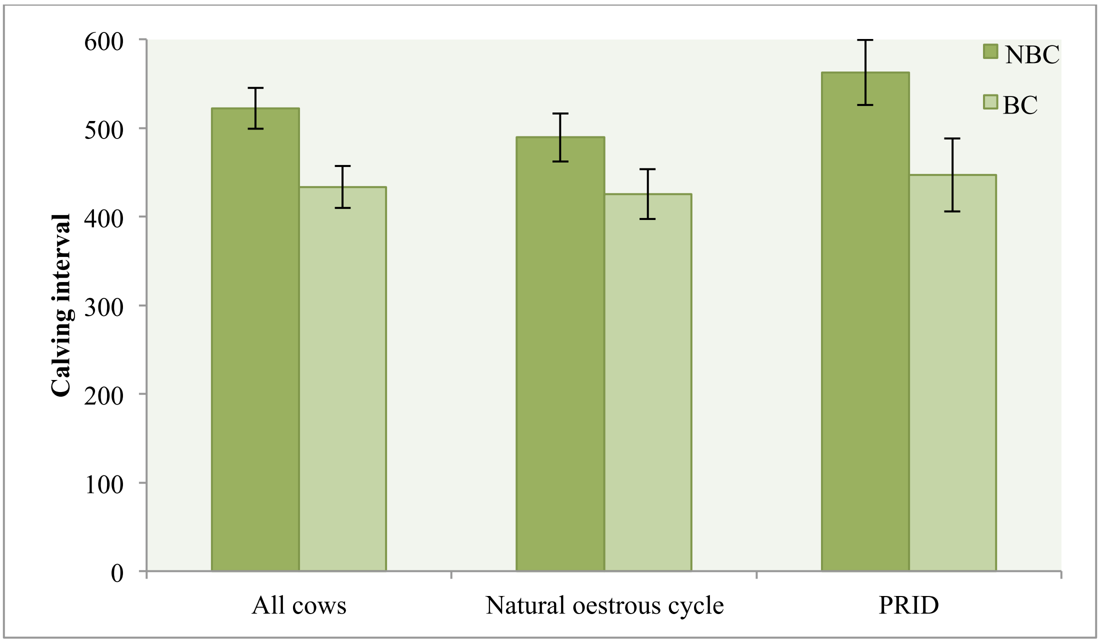


Figure 4.12: The mean (±SEM) calving interval (days), comparing the interval for cows that were treated with bull exposure and without bull exposure for all animals used (NBC=20; BC=21), comparing cows that were not treated with PRID (NBC=11; BC=13) and also comparing the interval for cows that were treated with PRID (NBC=9; BC=8).

4.3.13 *Comparison of time of insemination with milk progesterone concentration*

The time of insemination was compared to the progesterone concentration profile from milk progesterone analysis. Data analysed show that there were 2 cows in NBC and 2 from the BC group which were served at the right time, which is near to the ovulation and, as a result, 3 cows successfully conceived to their first insemination although one cow from NBC failed to conceive. There were 3 cows, 1 from NBC and 2 from BC group, that were served too early before their ovulation; however 1 cow from BC group conceived to this insemination while one cow each from NBC and BC did not conceive. Other than this, 6 cows were served late (4=NBC, 2=BC) and 2 of them did conceived (1=NBC, 1=BC) while ther 4 of them failed to conceive. These observations show that late insemination will cause pregnancy failure, however early insemination may or may not result in pregnancy of the cows. Overall, pregnancy was more likely to occur in cows from BC group compared to NBC at the different timings of insemination. Figures 4.13 and 4.14 show the concentration of the progesterone for cows that were served. The progesterone profiles of one oestrous cycle during insemination show an increase of progesterone for NBC cows with highest progesterone value at 7 ng/ml, and all these animals were confirmed to have conceived to the insemination. However the cycle was not complete since milk sampling was stopped at turnout. For the BC group however, progesterone profiles show a normal complete cycle and the highest progesterone value is 6.8 ng/ml. For cows that failed to conceive to the insemination, their progesterone profiles were normal and not different between groups.

Figures 4.15 and 4.16 show the progesterone concentrations for cows during insemination and the subsequent days. Progesterone concentrations remained above 2.0 ng/ml for all cows that conceived to this insemination, for both NBC and BC group. However progesterone profiles for cows that failed to conceive to this insemination showed a drop below 1.5 ng/ml for several days after insemination. There was no difference in progesterone profiles between groups.

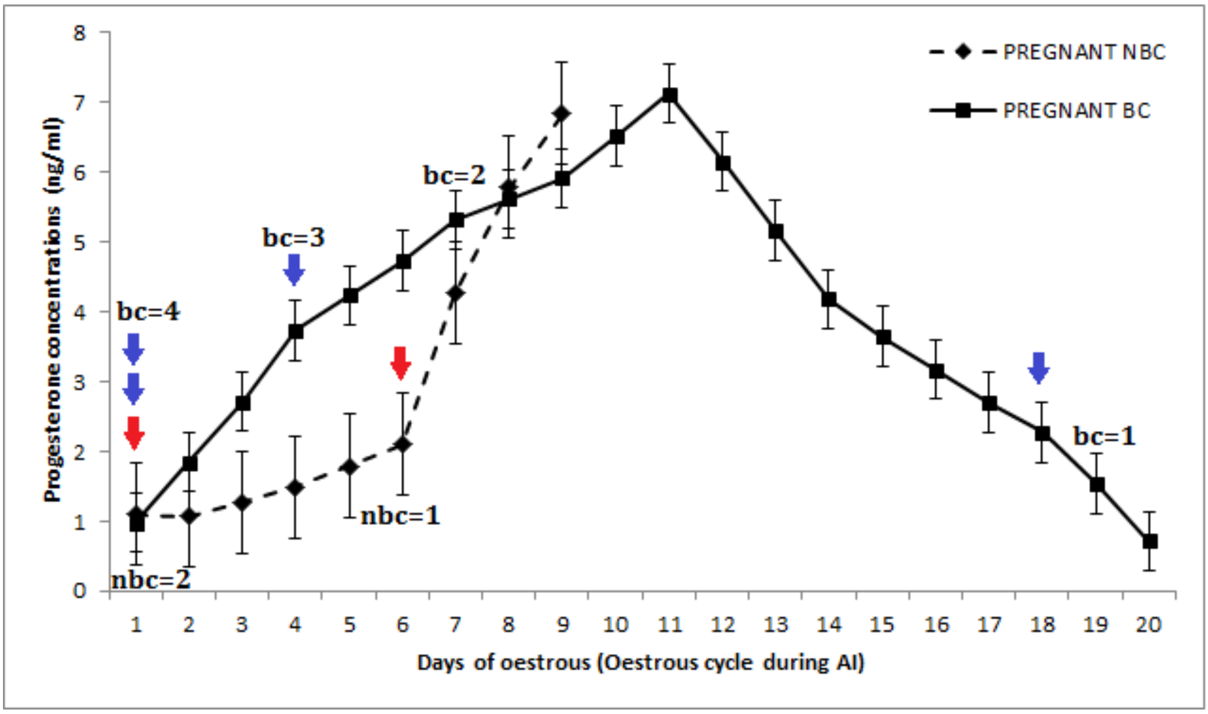


Figure 4.13: Mean progesterone concentrations for animals from both NBC and BC group during the oestrous cycle when they were inseminated and conceived to the insemination. Profiles were obtained from the average concentrations of 2 cows from NBC and 4 cows of BC[2], the number of cows were marked along the profile to show the number of cows calculating to the data at each point. Blue arrows mark the time of AI for cows in BC group and red arrows mark the time of AI for NBC group.

[2] The progesterone concentration curve for BC cows gradually decreases starting from day 11; this is the average of concentrations from two cows, of which one had successful AI and was pregnant while other one was not served until later in the cycle. Thus the progesterone concentrations for this one cow were already decreasing after day 11 but before she was served on day 18.

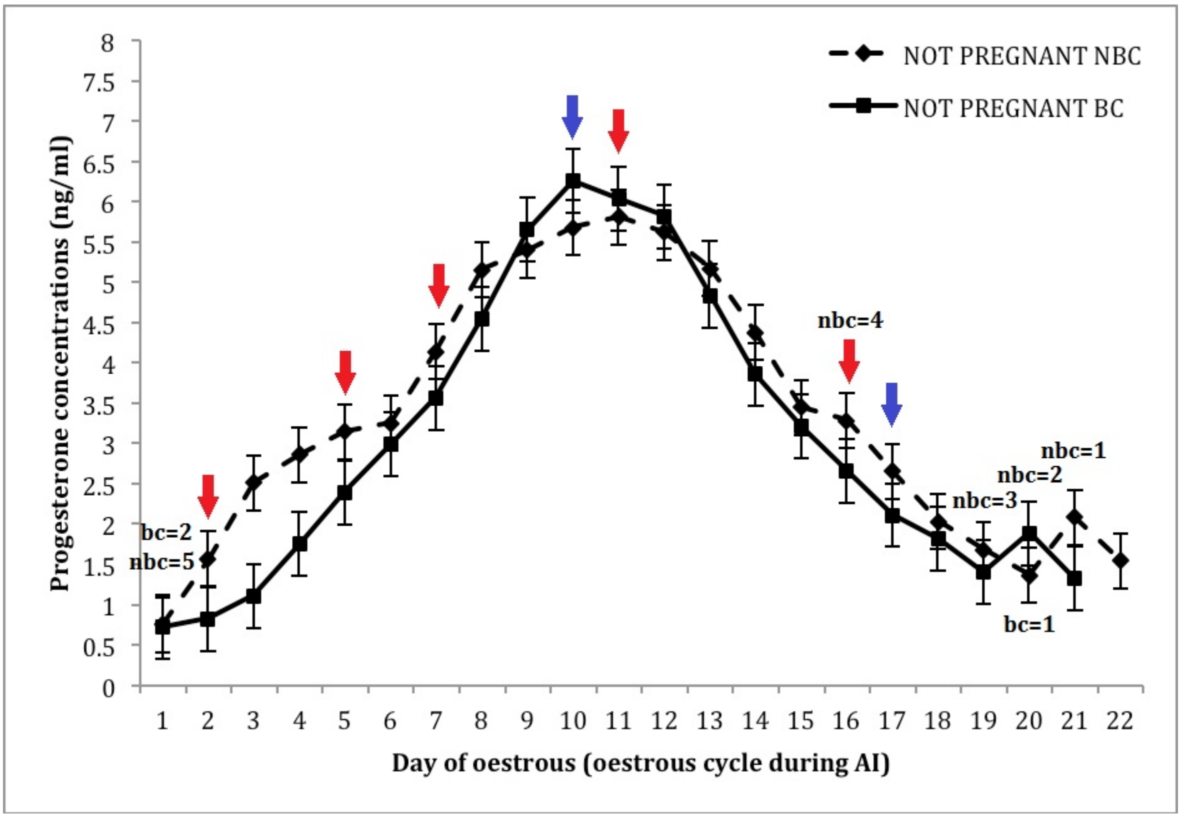


Figure 4.14: The progesterone concentrations for animals from both NBC and BC group on their oestrous cycle when they were served but failed to conceive. Profiles were obtained from the average concentrations of 5 cows from NBC and 2 cows of BC, the numbers of cows are marked along the profile to show average concentration for a number of cows at a certain point. Blue arrows mark the time of AI for cows in the BC group and red arrows mark the time of AI for the NBC group. This shows that most cows that were not pregnant received AI at the wrong time for both treatment groups.

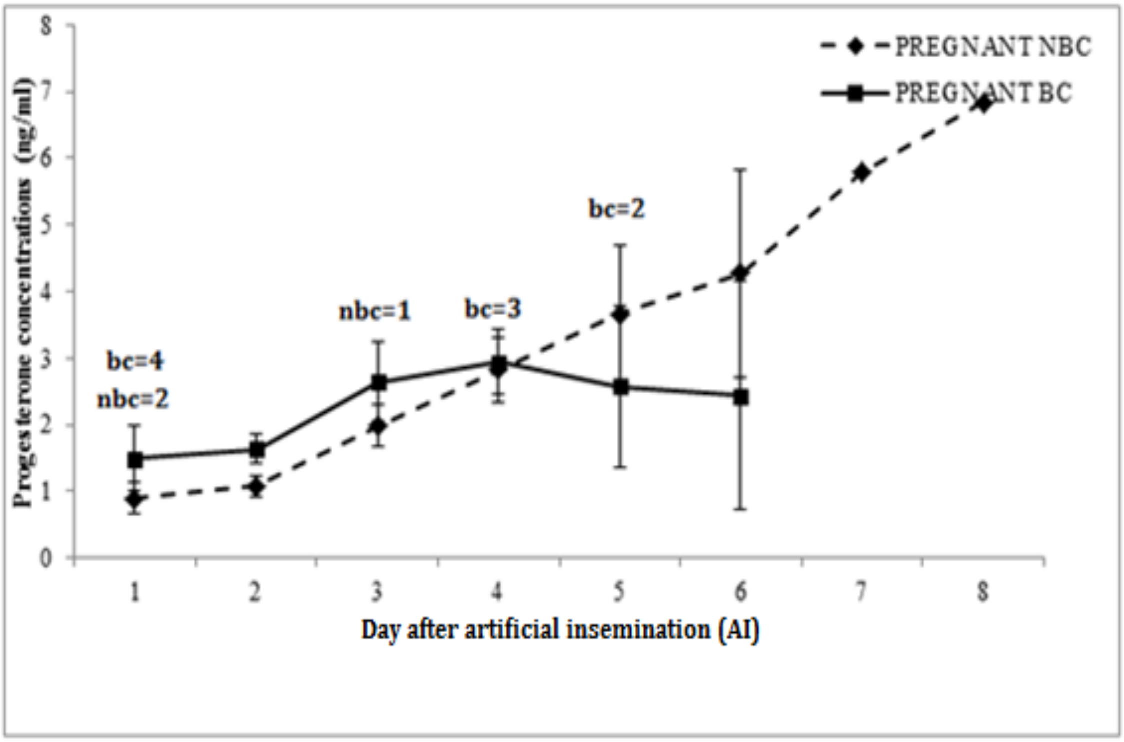


Figure 4.15: The progesterone concentrations for animals from both NBC and BC groups from the day that they were served and conceived at the first insemination.

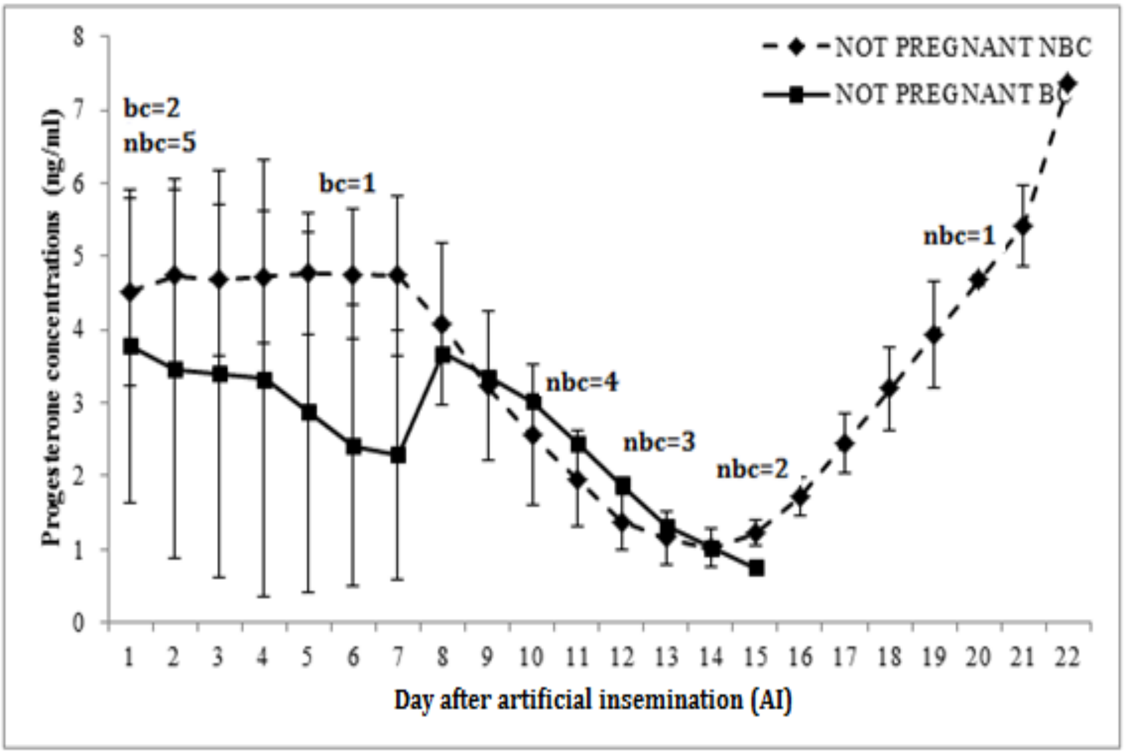


Figure 4.16: The progesterone concentrations for animals from both NBC and BC groups from the day that they were served but failed to conceived to the first insemination.

4.4 Discussion

For this trial, the main purpose was to determine the effects of biostimulation by using fenceline bull exposure from very soon after calving on postpartum anoestrus dairy cows; the average time at introduction was 2 days postpartum. Time taken to resume ovulatory activity, changes in behavior, including oestrus expression and walking activity during the first oestrus, the conception rate and calving interval were all investigated. The results indicate that the use of a bull for biostimulatory exposure during the postpartum period had no effect on the resumption of ovarian cyclicity post calving, which is similar to the result of the first trial. The percentage of cows that resumed cyclicity was the same for cows either exposed or not exposed to the bull stimulation, even though the cows were continuously exposed to fenceline bull contact from a very early day postpartum. The average time to resumption of ovulatory activity was 30±2 dpp, based on progesterone concentration profiles from 33 cows used in this trial. Moreover, there were also cows that only resumed their oestrous cycle after 100 days postpartum, either exposed to the bull or not. These results are in agreement with Custer *et al*. (1990), whose study on beef cows showed that cows on average resumed ovarian cylicity before 45 days postpartum when either exposed or without exposure to the bull. However, the results observed were contradictory to a study by Shipka and Ellis, (1999) where bull exposure during the postpartum period prolonged the anoestrous period and delayed the resumption of ovulatory activity in dairy cows. Result from this study showed that cows without bull exposure had a shorter interval from calving to resumption of ovarian cyclicity; 21±2 dpp compared to cows exposed to the fenceline bull exposure, 2 times a day (30±3 dpp) and cows continuously exposed to the bull (32±4 dpp).

These results were not in agreement with studies on beef cows where bull exposure showed an effect in reducing the length of postpartum anoestrous and accelerated the return of ovarian activity (Fike *et al*., 1996; Zalesky *et al*., 1984). Results from the first trial and current trial in the present study show that whether continuous fenceline bull exposure occurred on average from the middle of the anoestrus period (approximately 30 to 50 dpp) or from the very beginning of the post calving period (approximately 2 to 4 dpp) had no effect on resumption of ovarian activity. This is different to results from Tauck *et al*. (2010), who found a linear relationship between intervals from calving to resumption of ovulatory activity in beef cows, which depended on the daily total duration of bull exposure starting from very early postpartum (D0) compared to cows without bull exposure.

It should be noted that in the present study the cows without bull exposure were housed in the same building as cows with bull exposure, so the NBC group might also receive some pheromones from the bull. Pheromones are airborne chemical substances released from the bull and sensed by the olfactory or respiratory system, but which rapidly diminish. The mechanism of biostimulatory effects of the bull involves pheromonal stimuli (Berardinelli and Joshi, 2005). So, the air in the shed might contains pheromones from the bull and these might be received by NBC cows. For current trial, this could be a reason for no difference in the times taken to resume ovulatory activity between two groups. However, Berardinelli and Tauck (2007) discussed the intensity of bull exposure by comparing results for cows that were exposed to fenceline contact and cows with close physical contact with the bull. They suggest that the response of the cows to biostimulation effects that cows have received from the bull might be affected by the nature and intensity of bull exposure. They conclude that the closer cows are to the bull, the better response will occur. Thus, for this study even though the NBC cows might also receive pheromone effects in the air, this would have been greatly attenuated compared to the treatment group; this may be indicated in other results (the differences in conception rate and calving interval between groups). The cows might not respond, or show very small responses, to the biostimulatory effects of the bull if cows are exposed for an insufficient amount of time or only occasionally exposed to the bull (Berardinelli and Tauck, 2007). In this regard, cows must be in close proximity to the source of the pheromones for a certain period to respond by resumption of ovulatory activity. Fernandez et al. (1996) found that physical intermittent bull exposure of primiparous cows provided by 2 hours of exposure to the bull every three days, for 18 days starting at 33 days postpartum, did not reduce the postpartum anoestrus period and had no effect on hastening the resumption of ovulatory activity.

Similar to results from the first trial, bull exposure had a slight effect on expression of oestrus behaviour. Results from this trial show that a higher proportion of cows that were exposed to the bull expressed detected oestrus behavior, measured during their first inter-luteal period postpartum. However, the intensity of oestrus expression was not much different for cows either with or without bull exposure, and this measurement was only done for the first oestrus after resumption of ovulatory activity. The intensity of expression is mostly dependent on hormone secretion by the ovary and is also influenced by other cows in oestrus at the same time. Thus, changes in hormone secretion resulting from bull exposure may in theory promote more oestrus expression for cows with bull exposure. Roelofs *et al.* (2007) found that acute effects of bull exposure significantly increased LH concentrations and LH pulse frequency, thus improvement in LH might contribute to follicle development. The better quality and size of the largest follicle may increase follicular fluid concentrations of oestradiol (Ginther *et al*., 2001), with consequent effect on higher secretion of oestradiol from the ovary stimulating expression of oestrus behaviour in cows. This is because oestradiol is one of the major hormones that can improve oestrus expression (Allrich, 1994); oestrus will occur once a threshold of oestradiol is achieved, and additional amounts of oestradiol will enhance the oestrous expression (Boer *et al*., 2009). Lyimo et al. (2000) compared the concentrations of oestradiol with the visual symptoms of oestrus and the results showed that there was an association between oestradiol concentration and oestrus expression; they concluded that visual oestrus detection is appropriate to indicate oestrus and to determine the right time for insemination. However, the effects were too small and the mechanism was too complicated to be understood, as this includes the interaction between genes, hormones and the receptors as reviewed by Boer *et al*. (2009). Reolofs *et al*. (2008) showed that there were no significant differences in oestrus behaviour expressed by cows with or without fenceline exposure to the bull, which is similar to the current study.

The results show that, even though the animal numbers were small, fenceline bull exposure increased fertility in cows that received this biostimulation compared to cows that were not exposed to the bull. The result of pregnancy rate shows that, among cows that were exposed to the bull, pregnancy rate was higher at the first insemination compared to cows that were not exposed to the bull. The comparisons between number of inseminations required for cows to conceive shows that the average number of inseminations is lower for the BC group compared to NBC. This suggests that biostimulation might increase the conception rate and reduce number of inseminations required. In agreement to Berardinell *et al*. (2007), there is a possibility that biostimulatory pheromones secreted by the bull may not affect performance during the anoestrous period, thus not accelerate resumption of ovarian activity, but may have an effect on the ovary or reproductive tract physiology to improve the breeding performance. Elevated progesterone during the pre-insemination luteal phase was associated with better conception rates in dairy cows, as reviewed in Royal *et al*. (2000). On the other hand, Shipka and Ellis (1999) found that there was no significant difference in pregnancy rates for cows that were exposed to continuous proximity, fenceline contact or no exposure to the bull. From their results, bull exposure did not improve pregnancy rates; this might be because of the number of cows used in this experiment was too small (n=19) to identify a significant difference between 3 treatment groups.

The comparisons between normal and abnormal oestrous cycles from this study show that there was no difference between cows that were exposed and not exposed to the bull. The percentages for both groups that experienced normal and abnormal oestrus cycles post calving was approximately equal. However, delayed ovulation type I tended to occur more in cows that were not exposed to the bull. Results from Royal *et al*. (2000) show that the percentage of cows with normal ovarian cyclicity decreased in years 1995-1998 compared to years 1975-1982, with only 44.7% compared to 65.7% respectively. In results from the current study, 51.2% of all cows had normal ovarian cylicity, which is usual for a dairy herd, though there was no difference between BC and NBC groups with 52% and 50% respectively. Comparisons of pregnancy rate to first insemination between cows having normal or abnormal cycles, and exposed or not exposed to the bull, show that bull exposure slightly improved pregnancy in cows with normal or abnormal ovarian cycles, treated or not with PRID. It would be expected that there would have been a better conception rate in BC group if all cows were served at the right time, since the wrong time of artificial insemination reduced the chances of pregnancy. In agreement with several studies (Lamming and Darwash, 1998; Nakao *et al*., 1992), the cows that experienced abnormal types of ovarian cylicity, whether delayed ovulation or persistence of corpus luteum resulting from delayed luteolysis, will contribute to the subfertility problem which caused the difficulty to conceive and associated prolonged calving interval in this study. Nevertheless, from the results of this study, the effectiveness of PRID treatment in cows that had delayed ovulation or a persistent corpus luteum was better when cows were exposed to the bull, compared to when cows were isolated from bull exposure. Cows with delayed ovulation, exposed to the bull, started to resume ovarian activity soon after the PRID treatment and conceived to the following insemination.

Even though the effects of bull exposure on reproductive endocrine function in cow are very complicated to understand, results from the current study show that there was an effect of biostimulation on postpartum cows. A better conception rate in the group of cows that was exposed to the bull was seen in the results. There was no effect during the anoestrous period on resumption of ovulatory activity, however the higher conception rate in BC group suggests that exposing post- calving cows to the bull might improve the secretion of reproductive hormones and oocyte quality, which may lead to a better conception rate. According to Diskin and Morris (2008), low progesterone concentrations postpartum might have effects on oocyte maturation and also might reduce the chance of fertilisation; this results in the low pregnancy rates recorded in high producing dairy cows. Lonergan (2011) has listed the effects of progesterone on the oocyte quality, reviewed from a study by Dieleman *et al*. (1983) showing the progesterone dominance in follicular fluids of preovulatory follicles during the interval between LH surge and ovulation is associated with maturation of the oocyte. Besides this, progesterone may affect oocyte quality through its effect on development of the dominant follicle. These suggest the role for progesterone in improvement of reproductive performance. In this study, progesterone secretion in cows that were exposed to the bull during the postpartum period suggested a more rapid rise after day 4 compared to cows without bull exposure; however the difference was not statistically different. According to Spencer and Bazer (2002), higher progesterone concentrations are associated with better conception rates; in the current study, however, the average progesterone concentration was almost the same for both groups during their first oestrus cycle.

In the follicular phase, oestradial secretion and the LH surge will cause ovulation; at this point progesterone concentration will be very low and, if insemination is implemented at the right time, pregnancy will occur. Results on progesterone concentrations during artificial insemination in this study show that low progesterone concentrations, 2ng/ml or less during insemination, will facilitate pregnancy in cows whereas cows failed to conceive when they were served at higher progesterone concentrations. Royal *et al*. (2000) defines that when the progesterone concentrations are 3ng/ml or above, it is the luteal phase and thus the insemination will be unsuccessful when cows are served at this point; some cows in this study failed to conceive as they were incorrectly served during this phase.

Overall, the results suggest that providing bull stimuli to cows post calving may slightly elevate the progesterone rise postpartum, which may consequently improve the oocyte quality, thus increasing conception rates. This could explain the higher proportion of BC cows conceiving to the first insemination. The increase of progesterone in BC cows was not statistically significant for this trial, but still may have contributed to better conception rates. Royal *et al*. (2000) conclude that the lengthening of the average calving interval was associated with the decrease in pregnancy rate, so improving pregnancy rates can reduce calving interval. However, from their study, they found that there were no changes in average interval to first insemination in the comparison of 2 databases from between 1975-1982 and 1995-1998, hence this interval does not appear to be related with pregnancy rate. Even in this study, the average interval to first insemination was not significantly different between groups, but the average calving interval for group NBC was then longer as the pregnancy rate was low compared to BC group. Biostimulation by genital stimulation, pheromone secretion and other external cues from a bull could be an element in improving reproduction performance by increasing pregnancy rates and also reducing calving interval if the treatment is applied in the appropriate circumstances. The results suggest that bull exposure might also affect the efficacy of PRID treatment, thus a further study will be developed to investigate further the influence of bull exposure on this treatment.

In summary, continuous exposure of high yielding dairy cows to a bull during the very early postpartum period increased pregnancy rates and reduced the length of calving interval when compared to cows that were not exposed to the bull. The bull may encourage luteal activity and secretion of adequate amounts of hormones required to improve oocyte quality and prepare a better uterine environment for insemination, however the mechanism leading to improved pregnancy rates is difficult to define. Further research is planned to improve the previous trial and to clarify the effects of bull exposure on PRID treatment, since current results show that bull exposure might improve the efficacy of PRID treatment, increase pregnancy rates, and improve productivity of dairy cows.

Chapter 5

Influence of bull exposure on conception at a synchronised oestrus in postpartum dairy cows

Chapter 5. Influence of bull exposure on conception at a synchronised oestrus in postpartum dairy cows

ABSTRACT

Previous results indicate that bull exposure might improve the efficacy of progesterone releasing intra-vaginal device (PRID) treatment and improve productivity of dairy cows. Therefore, in this study, 28 freshly calved high-yielding Holstein-Friesian dairy cows, were synchronised for oestrus using PRID treatment when cows were 46.3±7.2 days postpartum. They were allocated to two treatment groups; 13 cows for the control group (NBC) and 15 matched cows formed the bull contact group (BC). Data on calving number, date of previous calving, body condition score, milk yield and date seen bulling were recorded. Oestrus signs were continuously observed after PRID removal by visual observation to observe mounting behaviour indicative of oestrus and Icetag pedometers to record changes in activity level. Milk samples were collected every day for 12 days, starting on the day before PRID withdrawal, and continuing thereafter with a 3 times a week sampling routine for 5 weeks. Samples were analysed to determine progesterone concentrations to monitor ovarian cyclicity. After PRID removal, cows were observed for oestrus behaviour and were AIed as a batch 2 days after PRID withdrawal (day 14 after commencement of PRID treatment). The time course of changes in progesterone concentrationafter the PRID removal was not statistically different (P=0.057) between cows with biostimulation from the bull or not. The pregnancy rate to first service of BC cows (6/15) was not significanty different (P=0.221) from NBC cows (2/13). The biostimulatory effects provided by continuous fenceline bull exposure did not improve presentation of oestrus signs in cows with synchronised oestrus and the numerically better conception rate was not significant. However, repeating the experiment with bigger sample groups would be useful to produce a more reliable statistical assessment.

5.1 Introduction

The Progesterone Releasing Intravaginal Device (PRID) and Controlled Internal Drug Release (CIDR) are used in intensive dairy farming to synchronise the oestrous cycle of cows and heifers, either when they are cycling or non-cycling (Butler et al., 2011; Tauck and Berardinelli, 2007; Cavalieri et al., 2003). These devices contain a certain amount of progesterone sufficient to maintain a high concentration of blood progesterone after insertion into the vagina. The system thus mimics the luteal phase of the oestrus cycle, when the progesterone concentrations are high. A sudden drop in concentrations of progesterone occurs when the device is removed from the cow. Low progesterone induces the secretion of gonadotropin releasing hormone (GnRH) generating a pulse of luteinizing hormone (LH) that induces ovulation in treated cows.

Application of oestrus synchronisation protocols allow treated cows to be served at a predicted time in a fixed time AI protocol (Berardinelli et al., 2007). However, prolonged postpartum anoestrus, causing failure to rebreed or later breeding, can cause problems for oestrus synchronisation protocols in cows (Rhodes et al., 2003). In dairy cows, oestrus synchronisation using GnRH seems to be more effective in postpartum cows that have resumed ovarian cyclicity (Geary et al., 2000). Biostimulatory effects by exposure to a bull have the potential to improve the proportion of cows that resume ovarian cyclicity and reduce the interval from calving to resumption of ovulatory activity (Fike et al., 1996). However, findings from both earlier trials in the current study (chapters 3 and 4) were different, with the resumption of ovarian cyclicity being similar for cows either being exposed or not to the bull. In addition, exposing oestrous synchronised beef cows to a bull during the period after device removal significantly increased oestrous expression and pregnancy rates compared to cows undergoing an oestrous synchronisation protocol without bull exposure (Chenoweth, 1983).

The conception rate response in an oestrus synchronisation programme for postpartum cows incorporating AI might be improved by the effects of bull exposure. Results in a previous study showed that primiparous beef cows that were synchronised for oestrus using GnRH and PGF2α have improved conception rates to fixed time AI in response to bull exposure (Berardinelli et al., 2007). Furthermore, the exposure to bulls or bull urine of primiparous beef cows treated with a progestin-based oestrous synchronization protocol improved breeding performance, as more cows had initiated luteal function at the beginning of the oestrous synchronisation protocol and the pregnancy rate was greater (Tauck and Berardinelli, 2007). However, very few studies have investigated the effects of biostimlation by bull exposure in cows with synchronised oestrus, and there is a particular lack of information for dairy cows.

The previous experiment, carried out in winter 2011, showed a significantly better conception rate to first service and calving interval in cows exposed to fenceline contact with a bull between calving and insemination (57.1% v 15.0% and 433.6 days v 522.3 days). This was particularly marked in the cows which had been treated with a PRID prior to service. Patterns of milk progesterone also indicated that failure to conceive was often caused by insemination at a time when cows were not in the optimal stage of oestrus, highlighting the problems of reliable oestrus detection in modern high yielding dairy cows. One way to avoid this problem is to use oestrus synchronisation and fixed time AI. This can give better control of insemination timing, but it is not then known if the influence of the bull will still give improved conception rates.

Therefore, the objective of this experiment was to determine if the biostimulation of daily bull exposure from day 1 post calving has an influence on the ovarian activity and improves the conception rates in dairy cows following oestrous synchronisation and time AI. There were two main objectives to measure the response to bull exposure of the postpartum dairy cows, which were:-

a) To determine the conception rates as measured by the percentage of cows with synchronised oestrus that become pregnant after being inseminated.
b) To measure the number of inseminations subsequently required in obtaining pregnancy in cows following the synchronised oestrus with or without the exposure to the bull.

The null hypotheses were that the conception rates do not differ among cows with synchronised oestrus when exposed to the bull or without bull exposure, and that there is no relationship between the bull exposure and the number of inseminations required achieve pregnancy in dairy cows during the experiment.

5.2 Materials and methods

5.2.1 Experimental protocol

This experiment used similar methodology to that of the second experiment (winter 2011, Chapter 4); cows that were freshly calved were entered into this experiment when they moved to join the main milking herd. They were allocated and remained in one of two treatment groups, which were NBC (no bull contact) and BC (fenceline contact with the bull). All cows were synchronised for oestrus using PRID treatment. These selected cows were PRID treated in batches, according to date of calving, with PRIDs inserted when cows in each batch were 40 to 55 days postpartum. Cows were not PRID treated before 40 days postpartum, as ovarian activity is volatile before this point, and no later than 55 days postpartum as this will unduly increase the next calving interval. All cows were inseminated using artificial insemination after PRID removal and PGF2α injection. These cows then continued to be maintained under NBC or BC treatment conditions. The flow diagram of this trial is explained in figure 5.1.

Cows in the BC group were continuously exposed to a sexually mature Friesian-Holstein bull, age 15 months and sexually active, to provide the appropriate stimuli. They had fenceline access throughout the 24h period, and were diverted to walk past the bull pen following each milking. Cows in the no bull contact (NBC) treatment were housed as much distance as possible away from bull, and with no direct exposure or possibility of interaction with the bull.

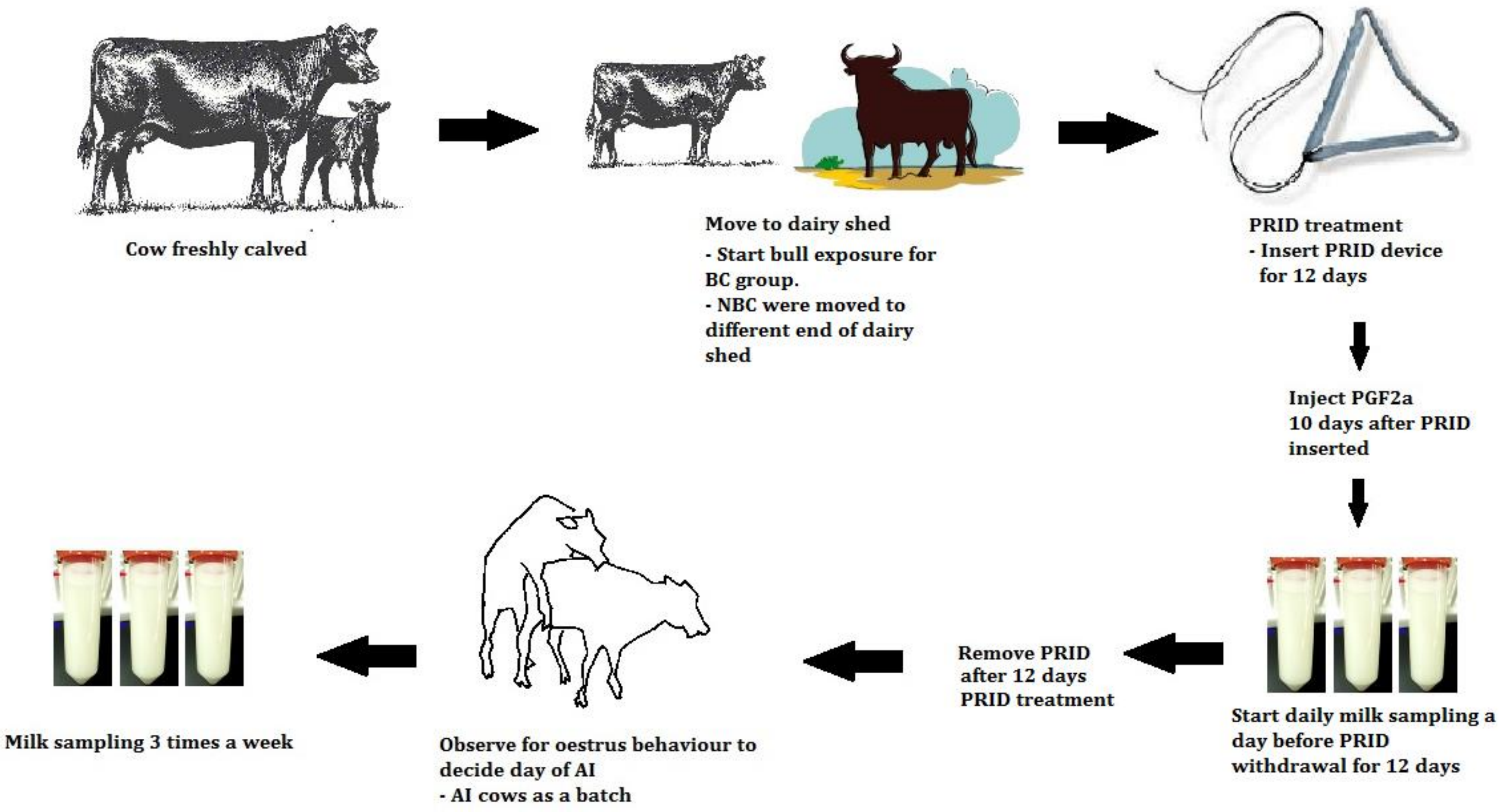


Figure 5.1: The illustration of the experimental protocol.

5.2.2 Animals and routines

Cows housed in the cubicle shed at Cockle Park Research Farm were allocated between two balanced groups of 28 cows, depending on their previous (for recently calved cows) or expected calving date. This was to ensure that each group would have a similar level of oestrus expression over time, so that social effects would not bias the treatment comparison. The details of the cow such as calving number, date of previous calving, body condition score and milk yield were recorded. Body condition score (BCS) of cows was assessed and recorded during the selection process and every two weeks during the study until the end of experiment. No cows were inseminated before the PRID treatments were implemented.

The cows were fed ad libitum on a total mixed ration (TMR) diet formulated to meet the nutrients requirements according to their production level: for either freshly calved cows, high or low milk production. This was provided by the stockperson every day. They were milked two times a day in a Fullwood low-line 16:16 herringbone milking parlour.

5.2.3 Oestrus synchronisation using Progesterone-releasing Intravaginal Device (PRID)

Oestrus synchronisation was applied to cows using a progesterone-releasing intravaginal device (PRID® Delta, Ceva Animal Health Ltd, Amersham, UK). Cows were administered PRIDs by batch according to calving date, and every batch received PRIDs on a Friday. This device contains 1.55g of natural progesterone. Throughout the treatment period, circulating progesterone is maintained at higher levels, avoiding formation of persistent dominant follicles. Synchronisation of oestrus in cows by the PRID was used in combination with a prostaglandin ($PGF2\alpha$) injection to ensure luteolysis; a prostaglandin analogue (Estrumate, Intervet UK Ltd, Walton, UK) was given on day 10 of PRID treatment. The PRID device then was withdrawn from the cow on day 12 (Figure 5.2); the removal process was scheduled to be on Tuesday for all cows to allow milk sample collection daily for 12 days, starting from day 11 of PRID treatment to day 11 following PRID withdrawal. The sudden drop of progesterone level after PRID withdrawal caused a synchronisation of oestrus. Cows that were displaying observed oestrus were used as the indicator for AI time; they were then inseminated as a batch, with the insemination time decided for the whole batch when any cow showed oestrus expression, approximately 12 hours after showing oestrus (Figure 5.2) on day 14 of PRID treatment, either in the morning or afternoon. Data on serving date and pregnancy outcome were recorded to determine any changes of pregnancy rate relating to treatment.

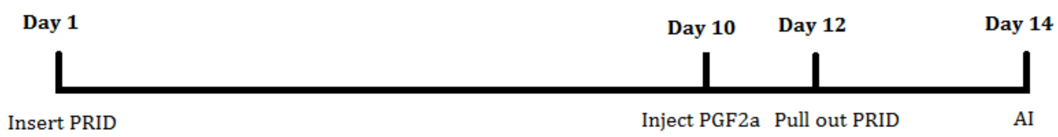


Figure 5.2: The diagram of PRID protocol.

5.2.4 Activity measurements and observation of oestrous behaviour

The same pedometer system used in the previous trial (IceQube, Ice Robotics Ltd, Roslin, UK) was also used to detect the changes in walking activity that defines one behavioural indicator of oestrus. As previously, this pedometer was assigned to record daily activity of cows, and report details of walking activity summarised in 15 minute blocks. The pedometer was attached to one of the rear legs of the cow, above the fetlock, during milking in the parlour on the first day they entered the main herd in the dairy shed. It then continuously monitored their stepping behaviour until the day when it was removed from the cow. Pedometer readings were taken at the end of the study by downloading data using a pedometer reader (The IceReader, Ice Robotics Ltd, Roslin, UK) that communicates by wireless. Data were then analysed with the dedicated software installed on a computer in Newcastle University. The period of oestrus was predicted from milk progesterone profiles following the PRID treatment, and the results were used to measures number of steps over that particular period. An increase in the number of steps, as measured by the pedometer, was calculated based on the median number of steps (Roelofs et al., 2005). The number of steps taken in each 4 hour time period starting at 06.00 on the day of oestrus, as predicted from the milk progesterone profiles, were compared with the number of steps taken in the same time period during the 10 days before and 10 days after. The ratio was calculated by dividing the number of steps in the 4 hour time period with the median number of steps of 10 days before PRID withdrawal and, if this ratio exceeded a threshold of 5.0, this was defined as an actual increase in number of steps and designated as an oestrus alert based on pedometer readings.

Secondly, observations of oestrous behaviour were carried out for two sessions, morning and afternoon for 60 min every session, each day for 3 days after PRID removal to determine the appropriate time to inseminate the cows. The characteristic behaviours of oestrus were evaluated to determine if the cows were really in oestrus. An assigned number of points were given for every single sign of oestrus recorded (see chapter 3 for details). Besides this, a video camera was placed to view the area of fenceline with the bull to measure the frequencies that cows in oestrus had contact with the bull. Similar to other trials, milk production was recorded based on data on the farm computer system. A difference in amount of milk production was notified by the system; if any cows had drop in their milk production, they were probably will coming into oestrus on that particular day. Data were used to measure number of cows that had changes in their milk production when they were in oestrus.

5.2.5 Milk sampling to measure progesterone concentrations

Milk samples were collected daily, starting one day before PRID removal until 11 days after the removal (12 days). After this, milk samples were collected 3 times a week, every Monday, Wednesday and Friday. All samples were collected during the afternoon milking session at 2 pm. Milk samples of at least 5ml were collected into clean tubes, securely capped and stored in a freezer at -20^{o}C until the end of the trial. They were then subject as a batch to progesterone assay in the laboratory of the School of Agriculture, Food and Rural Development, Newcastle University. The milk progesterone test was used to measure the progesterone changes during the normal oestrous cycle, to identify periods of oestrus and if ovulation had occurred.

Progesterone concentrations were determined using the commercial milk progesterone test kit (Ridgeway Milk progesterone enzyme immunoassay, Ridgeway Science, Gloucestershire, UK) and the procedures were the same as for the previous experiments.

The frozen milk samples were thawed, and then milk samples from 28 cows from both treatment groups were tested. The assay included the milk progesterone standards: 0ng/ml, 1ng/ml, 2ng/ml, 5ng/ml, 10 ng/ml, 20 ng/ml and 50ng/ml in duplicate and two milk samples were used as a quality control in each assay. The mean inter-assay coefficient of variation was 9.8% and intra-assay was 8.7%. The limit of detection of the assay was 0.2 ng/ml.

5.2.6 Data analysis

All the data were statistically analysed at the end of the trial. Data sets on progesterone concentrations and changes in walking activity were tested using the normality test (Minitab 16) and found to be normally distributed. Milk progesterone concentrations and changes in number of steps were compared by a repeated measures ANOVA, using the general linear model routine in the Minitab 16. The conception rates after insemination following the PRID treatment and oestrus expression were compared using a chi-square test to identify any significant difference between groups in the percentage of cows which conceived to first service, or exhibited particular signs of oestrus. The significance level was set at $P \leq 0.05$ for all statistical tests.

5.3 Results

5.3.1 Overall comparisons between groups at allocation

The 28 freshly calved cows assigned to this trial were divided into two groups; a no bull contact group (NBC) (n=13), without any contact or exposure with the bull, and a bull contact group (BC) (n=15), which had fenceline contact for 24 hours every day, starting from the day they entered the dairy shed (2 days postpartum) and throughout the trial period. The distribution of animal characteristics in the two treatment groups at allocation was compared to check for balance on body condition score (BCS) at selection, average daily milk production for the previous lactation period, parity, previous calving interval, and also subsequent interval from calving to PRID treatment. The results, shown in Table 5.1, demonstrate that there were no significant differences in these characteristics between groups.

Table 5.1: The comparisons of body condition score (BCS), parity number, previous milk production, previous calving interval and interval from calving to PRID treatment between cows allocated to the two treatment groups.

	NBC (N=13)	**BC (N=15)**	**SEM**	***T-test***	***P* value**
BCS	1.98±0.28	2.01±0.37	0.06	0.04	**0.838**
Parity	2.7±1.18	2.9±1.44	0.25	0.22	**0.647**
Milk production (litres/day)	27.8±7.00	30.1±5.64	1.34	0.72	**0.405**
Previous calving interval (days)	452±86.6	442±80.8	17.43	0.07	**0.791**
Interval to PRID treatment (dpp)	45.9±6.5	46.6±7.8	1.37	0.08	**0.786**

The average changes of body condition score (BCS) and average daily milk production from calving to time of AI was compared to measure if the conception rate was affected by these factors. BCS at the time of AI for NBC cows was 2.02±0.28 and for BC was 1.99±0.26. The results of all BCS assessments show that BCS did not significantly affect the conception rate BCS measured from the start of trial until they were inseminated for cows that were pregnant was 2.00±0.03 and 2.10±0.24 for NBC and BC cows respectively, while BCS for non-pregnant cows was 2.02±0.27 and 1.93±0.22 for NBC and BC cows respectively.

The average milk yield from calving to AI time was also measured to compare milk production between groups. There was no significant difference in average milk yield from calving to day of AI for NBC and BC groups, with 31.15±2.50 l/day and 32.32±3.92 l/day, respectively.

Table 5.2: The comparisons of body condition score (BCS) and average milk production from calving to time of insemination between two treatment groups.

		NBC	BC	SEM	*T-test*	*P* value
BCS	**All**	2.02±0.28 (n=13)	1.99±0.26 (n=15)	0.069	0.01	**0.992**
	Pregnant	2.00±0.03 (n=2)	2.10±0.24 (n=6)	0.053	1.03	**0.349**
	Non-pregnant	2.02±0.27 (n=11)	1.93±0.22 (n=9)	0.156	0.76	**0.460**
Milk production (litres/day)	**All**	31.2±2.50 (n=13)	32.3±3.92 (n=15)	0.801	0.73	**0.478**
	Pregnant	30.7±3.20 (n=2)	33.9±4.35 (n=6)	1.427	1.04	**0.409**
	Non-pregnant	31.4±2.60 (n=11)	30.8±3.16 (n=9)	0.960	0.29	**0.785**

5.3.2 Conception rate

All cows were inseminated 2 days after PRID removal, approximately on day 60 of postpartum. The conception rate for animals in this trial showed that a higher proportion of cows in the BC group (n=6) were pregnant to the insemination following the PRID treatment compared the NBC group (N=2) (table 5.2). However this difference was not statistically significant (Fisher test=2.068, P=0.221), due to the low number of cows conceiving from both groups. Cows that were not pregnant were inseminated again, and only 1 cow from NBC conceived to the second service.

Table 5.3: Conception rate to services following PRID withdrawal

	All animals	**NBC**	**BC**	**Fisher's test**	**P Value**
Pregnancy rate for first AI	28.6% (8/28)	15.4% (2/13)	40.0% (6/15)	2.068	**0.221**
Pregnancy rate for second AI	5.0% (1/20)	9.0% (1/11)	0% (0/9)	-	-

5.3.3 Observations of changes in behaviour after PRID treatment

Technically, after the PRID device is withdrawn from the vagina, cows should come into oestrus. Thus oestrous behaviours for all treated cows were observed, starting from the day of PRID removal until they were inseminated (two days after removal). From observations, 12 cows expressed 'stand to be mounted' behaviour, 4 cows from NBC group and 8 cows from the BC group, approximately one day after PRID removal. Other oestrus signs were also observed including mounting other cows, chin resting and vulva discharge. 17 cows were observed mounting other cows, chin resting was observed in 8 cows and vulva discharge detected in 13 cows (Table 5.3) and total scores for oestrus signs expressed were calculated (Table 5.4). Results found that the frequencies of oestrus signs displayed by cows were not significantly different between groups. Only one cow from NBC that had expressed standing oestrous behaviour was pregnant to the first service after PRID treatment, while for the BC group 5 of the cows showing standing oestrus were pregnant (1 of 4 and 5 of 8 cows for the NBC and BC group respectively). Only 1 cow in each group that did not showing standing oestrous behaviour became pregnant. Overall, however, there was no significant difference in oestrous behaviour score detected from both groups.

From all 28 cows, 17 were fitted with Icecube pedometers to observe for changes in walking activity. However not all cows increased their walking activity during oestrus; for some of them, the average count of walking steps showed the same average throughout the trial period. Results show that only 5 cows increased their walking activity during the oestrus period (NBC=2, BC=3), with the increase in walking detected approximately one day after PRID withdrawal. For the cows which showed a change, the mean count of walking steps on a normal day was 1099.4 for NBC cows (n=2) and 1053.3 for BC cows (n=3), and there was no significant difference (F=1.86; P=0.094) between groups. During the oestrus period, the day after PRID withdrawal (when the lowest milk progesterone value was observed) the average count of walking steps increased to 1831.6 steps for NBC cows (n=2) and 1952.0 steps for BC cows (n=3); there was again no significant difference (F=0.09; P=0.778) between groups. Figure 5.3 shows the average count of walking steps for NBC and BC cows for 21 days, with day 0 defined as one day after PRID withdrawal when the highest count was detected. From the 2 NBC cows that increased their walking activity during oestrus, only one of them was pregnant to the first service after PRID treatment, while for BC cows, 2 from 3 cows were pregnant.

In addition, a decrease in milk production on the day of oestrus, based on the automated oestrus detection alert from the farm computer system, was detected in 9 cows (NBC=6, BC=3). There was a significant difference between normal days and oestrus day (F=8.13; P=0.017) in average milk production. The mean production for a normal day was 32.94±2.50 l/day and 33.59±3.60 l/day respectively, while on the day of oestrus the average milk production was 23.79±4.00 l/day for NBC and 26.47±8.20 l/day for BC cows. However, there was no significant difference in the extent of reduction in milk production during oestrus between NBC and BC group.

Table 5.4: Signs of oestrus observed after PRID withdrawal

	All animals	**NBC**	**BC**	***Chi-square***	***P Value***
Stand to be mounted	48.9% (12/28)	30.8% (4/13)	53.3% (8/15)	1.448	**0.229**
Mounting	60.7% (17/28)	53.8% (7/13)	66.7% (10/15)	0.480	**0.488**
Chin resting	28.6% (8/28)	38.5% (5/13)	20.0% (3/15)	1.163	**0.410**
Vulva discharge	46.4% (13/28)	46.2% (6/13)	46.7% (7/15)	-	**-**
Increase in walking	29.4% (5/17)	25.0% (2/8)	33.3% (3/9)	0.142	**1.000**
Reduction in milk production	32.1% (9/28)	46.2% (6/13)	20.0% (3/15)	2.184	**0.228**

Table 5.5: The total points of signs of oestrus recorded for cows in the NBC and BC group.

Oestrus signs observed during inter-luteal interval	**Total points**			
	NBC	**BC**	***T-test***	***P* Value**
Stand to be mounted (STBM)	150.0±57.7	137.0±51.8	0.14	**0.711**
Mounting other cows	90.0±44.5	73.5±34.8	0.74	**0.404**
Chin resting	18.0±6.7	15.0±0	0.56	**0.482**
Vulva discharge	3.0±0	3.0±0	-	-

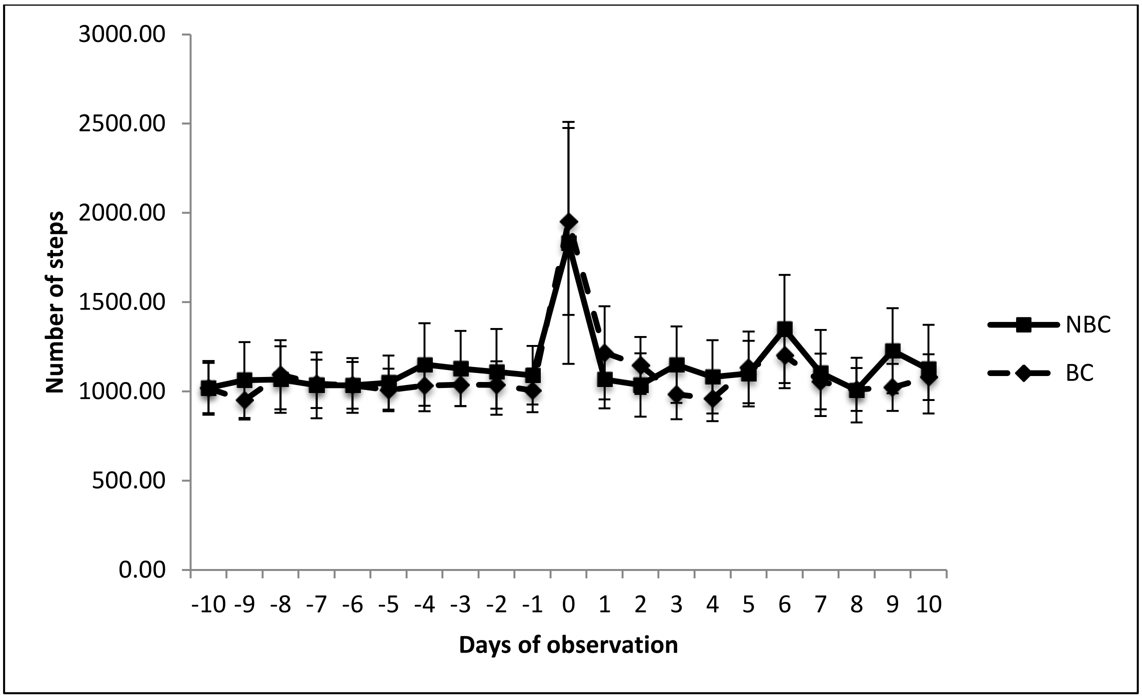


Figure 5.3: Mean (± SEM) number of steps shown by cows during the PRID treatment and following the oestrous synchronisation protocol. Day 0 indicates a day after PRID withdrawal, results shows that there is no significant difference in the increase of steps during oestrus NBC and BC cows showing this indicator (NBC=2, BC=3).

5.3.4 Interaction with the bull

From 15 cows in the BC group, only 6 cows had a direct voluntary contact with the bull and showed an increase in time spent visiting and interacting with the bull through the fence. Other cows in this group passed the bull two times a day after milking, however they did not show any interest in contacting the bull voluntarily. Interactions with the bull of these 6 cows were then measured, starting from one day before PRID withdrawal (on day -1) for 6 days, including the day of insemination (day 2). The highest increase in time spent with the bull was measured on day 1 after PRID withdrawal and there was a significant difference between days ($F=12.20$; $P<0.001$) in time spent interacting with the bull by cows. From these 6 cows that did interact with the bull during oestrus, only 3 of them were pregnant to the first service after PRID treatment.

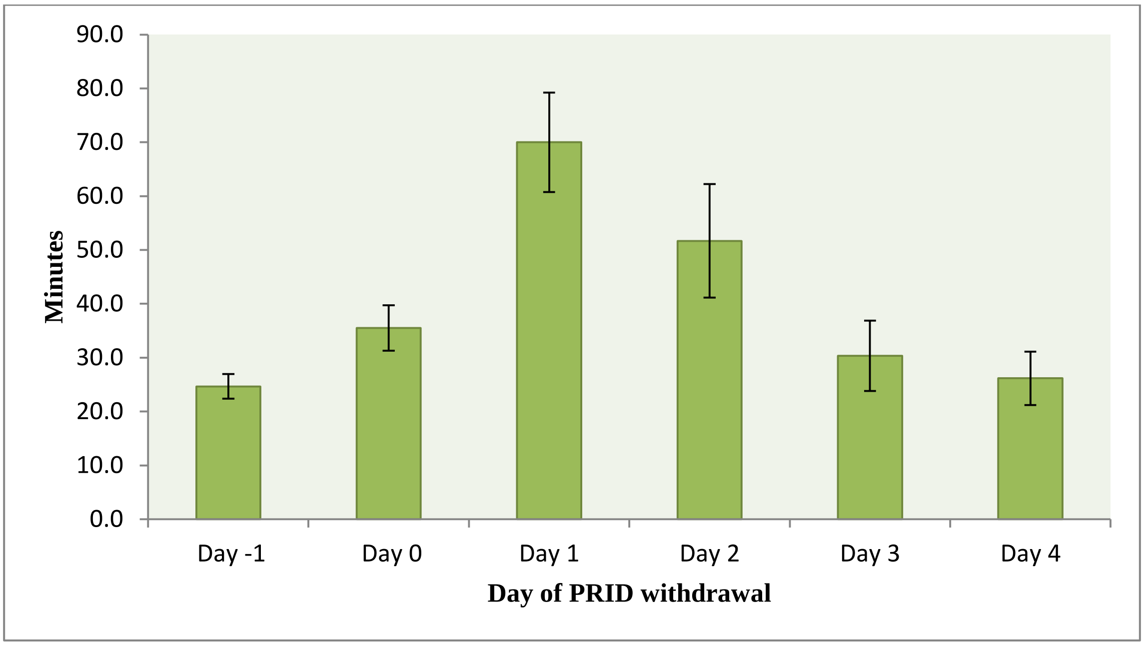


Figure 5.4: The mean (±SEM) time in minutes spent by cows in the BC group that showed increased interaction with the bull (n=6), measured from 24 hours before PRIDs were removed from cows (on day -1) and continuing during the predicted period[1] of oestrus until after cows received AI (day 2).

[1] Observations starts from a day before PRID withdrawal, day 0 indicates the day of PRID withdrawal and day 2 indicates the day of artificial insemination for treated cows.

5.3.5 Milk Progesterone

The daily milk progesterone concentrations from a day before PRID devices were removed from cows were screened by milk progesterone analysis. Milk samples were initially collected daily for 12 days then continued with 3 days a week sampling. Progesterone concentration profiles for 12 days during daily sampling from all cows in the NBC and BC group are shown in Figure 5.5. There was no significant difference (F=4.56; P=0.06) in concentrations of progesterone between groups during this period, despite a tendency for higher progesterone in NBC cows with mean concentrations of 1.39±0.78 and 1.09±0.55 for NBC and BC cows respectively.

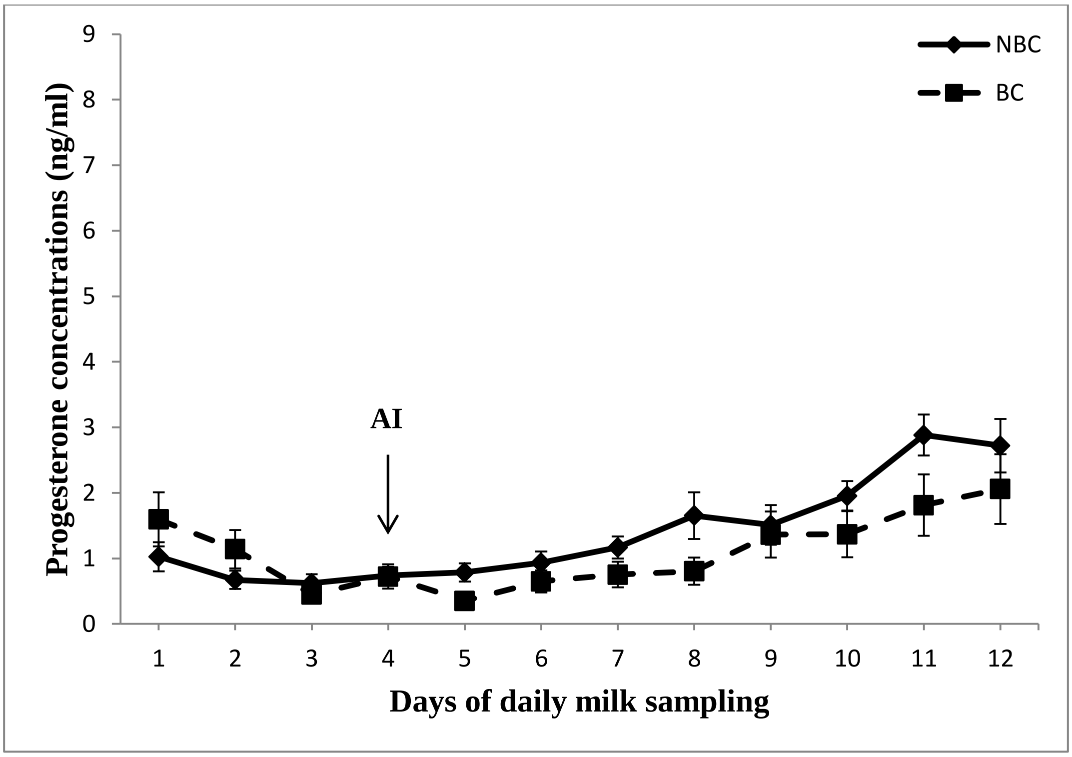


Figure 5.5: Mean (±SEM) of progesterone concentration from daily milk samples for all animals either pregnant or non-pregnant for a 12 day period starting from the day before PRID withdrawal until 11 days after PRID treatment in both the NBC and BC group (NBC=13, BC=15).

Figure 5.6 shows the progesterone concentrations profiles for all cows in the NBC and BC group throughout the trial period; there was no significant difference between groups (F=4.00; P=0.057).

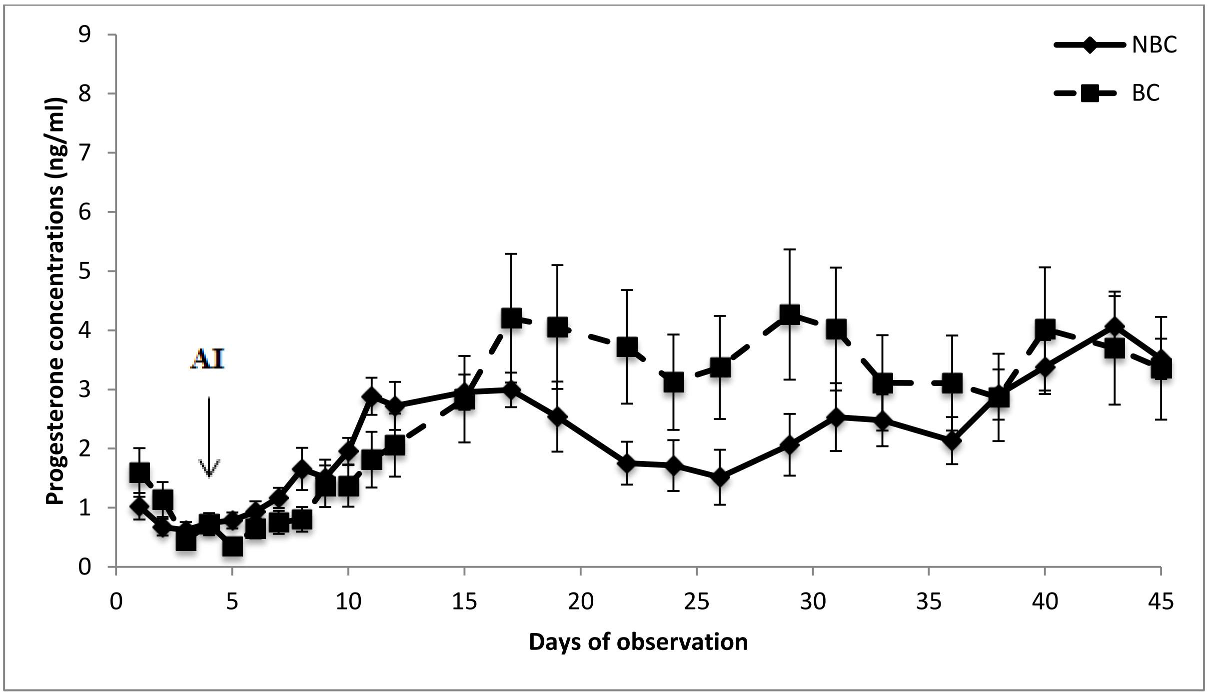


Figure 5.6: The mean (±SEM[2]) of milk progesterone concentration for all animals, either pregnant or non-pregnant, from one day before PRID removal (starting day; Day 0) until 45 days[3] in the treatment groups (NBC=13, BC=15).

The progesterone concentrations measured for cows that were pregnant, detected by constant high progesterone concentrations (≥1.5 ng/ml), shows that there was a significant difference in concentrations (F=11.48; P=0.002) between groups, with BC cows showing a faster rise and higher mean value (Figure 5.7). Cows that were not pregnant (as detected by a decrease in progesterone concentrations after an initial rise) showed no significant group difference (F=0.53; P=0.475), with the mean progesterone concentrations shown in Figure 5.8.

[2] Standard error of mean (SEM) marked is calculated for every day rather than a pooled SEM from the repeated measures variance test.
[3] Progesterone concentrations were measured daily for 12 days then sampling was continued 3 times per week.

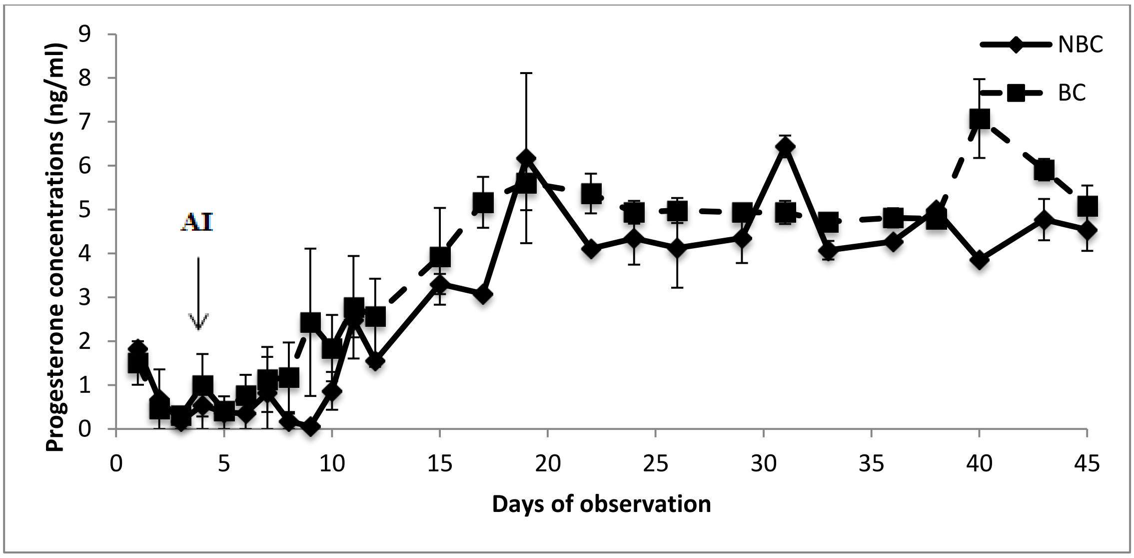


Figure 5.7: The mean (±SEM[4]) progesterone concentrations for cows that were pregnant, detected by sustained high progesterone concentrations[5] for more than 30 days after insemination, in the treatment groups (NBC=2, BC=6). Day 1 indicates the day before PRID withdrawal.

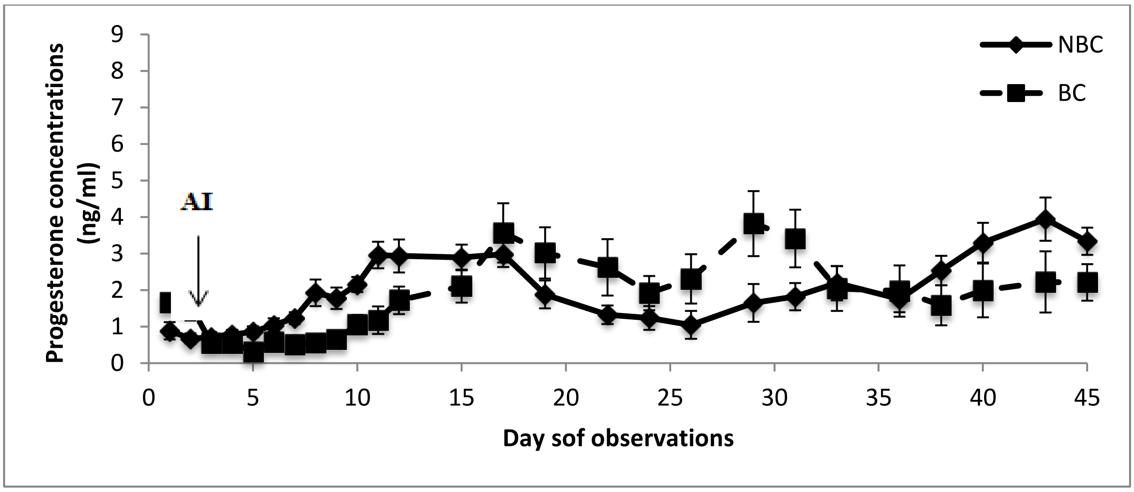


Figure 5.8: The mean (±SEM) progesterone concentrations for cows which were not pregnant, determined by a rise in progesterone concentrations followed by a decrease to below 1.5ng/ml, for the treatment groups (NBC=11, BC=9). Day 1 indicates the day before PRID withdrawal.

[4] Standard error of mean (SEM) marked is calculated for every day rather than a pooled SEM from the repeated measures variance test.
[5] Progesterone concentrations were measured daily for 12 days then sampling was continued 3 times per week.

5.4 Discussion

Chenoweth (1983) stated that exposing oestrous synchronised beef cows to a bull during the period after device removal significantly increased oestrous expression and pregnancy rates compared to cows undergoing an oestrous synchronisation protocol without bull exposure. Results on conception rates from the second trial (Chapter 4) are in agreement with this previous study. Although results of this trial did not demonstrate a significant benefit, the percentage conception rate to the first service after PRID removal in cows with fenceline bull exposure was numerically much higher than for cows without bull exposure and the difference was comparable to that seen in the previous study (chapter 4). By using a power test (Minitab 16), the estimation for sample size necessary to demonstrate significance for the difference in conception rate between treatment groups seen here is at least 17 cows per group. Conception rates were poor in both control (NBC) and treatment (BC) groups in this trial compared to the previous trial (Chapter 4). According to previous study (Dare et al., 2010) the pregnancy rate for dairy cows that were synchronised for oestrus was around 60% to 70%. However in the current trial the pregnancy rates for NBC and BC groups were only 15.4% and 40.0% respectively. This may be related to other reasons, since all cows were synchronised for oestrus using a standard method, which might include effects of metabolic state or body condition of the cows during the trial, the handling of the AI process, the relatively early stage of the postpartum period when the AI took place and stress as these animals related to management changes.

In this study, the reduced response to biostimulation from the presence of a bull, the limited changes detected in behaviour during oestrus and the lower conception rate in this trial, even though all cows were synchronised for oestrus, may be due to their body condition. The average body condition score (BCS) at the beginning of this trial was similar for both groups, however the BCS was slightly lower for selected cows in this trial compared to both previous trials (trial 3: NBC=1.98±0.08, BC=2.01±0.08; trial 2: NBC=2.09±0.10, BC=2.16±0.12; trial 1: NBC=2.40±0.80, BC=2.10±0.60). An appropriate body condition is important for successful insemination. Monje et al. (1992) reported that the percentage of cows responding to the biostimulation from bull contact is positively related to the nutrition. However, in another study (Stumpf et al., 1992), a greater response to the biostimulation of bull exposure was found in postpartum cows with modest body condition rather than cows with higher body condition. Furthermore, primiparous cows with low body condition at calving have a prolonged anoestrous period; McDougall et al. (1995) reported that most of the early postpartum dominant follicles failed to ovulate in cows under severe nutritional stress. This is probably because of the lower LH pulse frequency, which was inadequate to stimulate sufficient estradiol secretion by the dominant follicles to induce the preovulatory LH surge. In heifers, a positive relationship was observed on ovulatory response to male exposure with heavier body weight (Quadros and Labato, 2004), thus suggesting the presence of an optimum body weight is necessary to respond to biostimulation effects of male exposure. In a study by Fiol et al. (2010), exposure of peripuberal beef heifers to androgenized steers caused hastening of puberty in heifers with heavier body weight; the response was directly related to the initial body weight of the heifers. Pheromones affect reproductive activity via the hypothalamus, which produces pulses of gonadotropin-releasing hormone (Rekwot et al., 2001). However the responsiveness of reproductive performance in females to pheromonal cues probably depends on their body weight (Izard and Vandenbergh, 1982). Moreover, progesterone was not effective for the induction of an LH surge, ovulation and oestrus in anoestrous cows with a lower body condition score (Nation et al 2000), suggesting that poor body condition could reduce the effects of PRID treatment in postpartum cows. In the present study, the initial body condition of the postpartum cows could be one of the critical factors affecting the reproductive response to the PRID treatment as results show a low conception rate following the PRID in both cows that were exposed to the bull or not exposed to the bull.

In addition, the condition of animal itself in relation to different stressors contributes to the success of the insemination. Unfortunately, during this trial the herd was managed by three different stockpersons from October 2012 until April 2013. This situation could contribute to increased stress levels in the cows. Furthermore, the success of the AI process may have been influenced by their current stress levels, as cows in earlier batches in this trial, from late October 2012 until February 2013, were handled by a relief stockperson and had a very low pregnancy rate of only 7.1% from all animals, compared to 21.4% conception rate in animals from later batches that were handled by a different stockperson.

The existence of progesterone in cow's milk has been well proven in many previous studies and widely used as a less invasive option to measure progesterone concentrations than using a blood sample (Nakao et al., 1982, Gillis et al., 2002, Rioux and Rajotte, 2004). In this study, milk progesterone profiles indicated that average progesterone concentrations were slightly above 1ng/ml on the day before PRID removal after effects from PGF2a injection (NBC=1.03±0.22, BC=1.60±0.41), and then then dropped to near 0 ng/ml on the day of PRID removal. All cows were inseminated artificially 2 days after the PRID had been removed, with this interval validated by behavioural observations on the cows. After this, the progesterone concentrations gradually increased up to above 2.5 ng/ml after 10 days from insemination (NBC=2.95±0.30, BC=2.83±0.73). The concentrations remained high for more than 30 days, above 1.5ng/ml, for cows that were pregnant. However, for cows that failed to conceive, progesterone concentrations dropped again after several days, and the new luteal phase was detected as progesterone concentrations increased again after the drop. However the timing of the drop in progesterone was different among cows, as the length of cycle for every cow varied. Overall, there was no significant difference in average progesterone profiles between NBC and BC cows, providing no evidence of better luteinisation attributable to better LH activity in biostimulated cows.

Observations on the interaction between cows and the bull showed that some cows would visit and interact with a bull more often during the oestrus period than when they were not in oestrus. Similarly, in sheep, ewes in oestrus may display active ram seeking behaviour; when placed in a large area they tend to approach and mate with rams (Lindsay and Robinson, 1961; Lindsay and Fletcher, 1972). Strong oestrous behaviour is very useful in detecting the oestrous period and to predict the right time for insemination. In this study, oestrous behaviour such as standing to be mounted, mounting other cows and chin resting was observed. However, standing to be mounted is the most reliable visible sign of the correct time of oestrus compared to mounting and chin resting. The conception rates of primiparous and multiparous cows were similar when they were artificially inseminated immediately after exhibiting several oestrus signs such as mounting and standing to be mounted (Nebel et al., 1995). In addition, lower conception rates were found in cows being inseminated without showing standing oestrous behaviours (Gwazdauskas et al., 1986). In this trial, behaviours such as mounting and chin resting were not necessarily shown on the same day as standing to be mounted behaviour, but expressed either earlier or later. Nevertheless, it is very critical to detect oestrus behaviour in cows, especially during the early postpartum period with high milk production. High milk yield production is associated with lower signs of oestrus (Van Vliet and van Eerdenburg, 1996).

The mounting behaviour has a direct positive relationship to an increase in pedometer reading (Van Vlient and van Eerdenburg, 1996), where the highest walking activity and increased mounting behaviours occur approximately 16 hours before oestrus (Arney, et al., 1994). Thus, the activity monitoring devices such as pedometers have a potential application in research and practice to monitor behaviours continuously and automatically without need for human intervention (McGowan et al., 2007). The current results show that several cows which showed ‘stand to be mounted’ behaviour during oestrus also increased their walking activity as recorded by pedometer, but a few cows with mounting behaviour did not have changes in their walking activity. In cows that were detected with increased walking behaviour, this was confirmed to occur on day of oestrus by comparing to the results from progesterone profiles; changes was detected on the same day as the drop in milk progesterone. However, the high milk production and the fact that all these cows were in the very early days of the postpartum period with poor body condition could explain why a lot of cows in this study, either exposed or not to the bull, had poor oestrus signs. Lower concentrations of serum oestradiol in lactating cows is a consequence of higher metabolism for milk synthesis and alters their reproductive physiology (Sartori *et al*., 2004) and may be associated with difficulty in expression of oestrus signs in lactating cows (Dransfield *et al*., 1998). Holstein genetics have been associated with an increased rate of metabolic problems, including lameness, mastitis and infertility, with lameness recognised as the third most important health problem of dairy cows (Biefeldt *et al*. 2005). Lame cows usually have difficulty in expressing oestrus due to unstable body movements. The results in the current study may have been affected by the genetic problems of this breed, although lameness testing has not been systematically done in this study.

There was no difference in expression of oestrous behaviour between groups different cowsin this trial, which is an observation similar to both previous trials. This observation agrees with the results found in a study by Shipka and Ellis (1998), where there were no differences observed in oestrous behaviour expressed by dairy cows either not exposed to the bull, exposed twice daily with fenceline bull contact or continuously exposed to the fenceline bull contact. Van Eerdenburg *et al*. (2002) explained that fewer than 50% of cows displayed oestrus behaviour during oestrus. Although there was no difference in oestrus behaviour detected in cows either with fenceline bull exposure or not, Roelofs et al. (2008) claimed that fenceline bull exposure could be a useful aid for visual detection of oestrus as cows in oestrus increase the frequency of visiting the bull. However, in this study the changes in visiting the bull by cows during oestrus were low and do not support this as an appropriate method to detect oestrus. A decrease in milk production could be used to detect oestrus; results show that 32.1% of cows in this trial had a reduction in milk production during oestrus, and there was a significant difference between milk production during a normal day and the oestrus period for these cows. There is usually a drop in milk yield of about 12 to 16% on the day before oestrus that could be designated as an oestrus sign (Firk *et al*., 2002). Eradus *et al*. (1992) explained that lower milk production during oestrus is associated with increased restlessness, increased activity and decreased feed intake. For this study, the reduced milk production was detected in either one milking session in the day or at both morning and afternoon milking. However, no difference was observed in reduction in milk production in oestrus cows in relation to whether they were exposed to the bull or not.

It can be concluded that, under the conditions of this current experiment, where PRID treated postpartum dairy cows exposed to a mature bull during the very early postpartum period had no effects on changes in expression of oestrus behaviour, changes in walking activity, and conception rate after first insemination following PRID removal. Poor oestrus behaviour was recorded from cows either when exposed to the bull or not. Some cows did show interest in interacting with the bull more on the day of oestrus, though the mechanism behind this particular behaviour is not well understood. Nevertheless, cows in better body condition or slightly better after calving may have had a more favourable response to biosimulation from bull exposure, since all of the reproductive functions are strongly related to the number of days postpartum, nutrition and body condition. Besides, percentage conception rate to first service following PRID treatment was higher in BC group compared to NBC group, although the difference was not statistically different. Therefore, to determine the true significance of results, repetition of this experiment with bigger sample groups will be useful for more reliable statistical assessment.

Chapter 6

General discussion and conclusion

Chapter 6. General discussion and conclusion

Fenceline bull exposure during the postpartum period has been shown in this thesis, presented by results in chapter 3 and 4,to be an ineffective method for hastening the resumption of ovulatory activity or enhancing oestrus expression in high producing dairy cows. However, in chapter 4 a significant effect of fenceline bull exposure on conception rate to the first service and reduction of calving interval in cows was demonstrated. Whilst a similar numerical trend was shown in chapter 5, the difference was not statistically significant against a background of generally poor conception. The factors that contribute to different responses of postpartum dairy cows tofenceline bull exposure will be discussed to understand how cows might be affected. In addition, the cost-benefit of keeping a bull as a stimulus animal in the herd as an approach to reduce reproductive problems among postpartum dairy cows will be discussed.

6.1 The resumption of ovarian cyclicity in postpartum dairy cows

In cattle, anoestrus is one of the physiological states after parturition, during which no oestrus and ovulation occurs. This is because, during pregnancy, the concentration of progesterone remains high until parturition and this causes a negative feedback suppressing activity of the hypothalamus-pituitary axis (Bulman and Wood, 1980; Kyle et al., 1992). However, a prolonged anoestrous period significantly affects the fertility through prolonging the calving interval of the animals.Dairy cows with high milk production or low body condition score (BCS) tend to experience a longer anoestrous period after parturition. Extended periods of postpartum anoestrus and poor conception when served, result in cows failing to conceive or conceiving later. Therefore, the resumption of ovulatory activity is very critical in postpartum dairy cows.

Interaction with a bull may encourage oestrus expression and hasten the onset of oestrus after calving (Berardinelli et al., 2005; Fike et al., 1996). However, this response to bull exposure during the postpartum period is determined by many other interacting factors, as shown in figure 6.1.

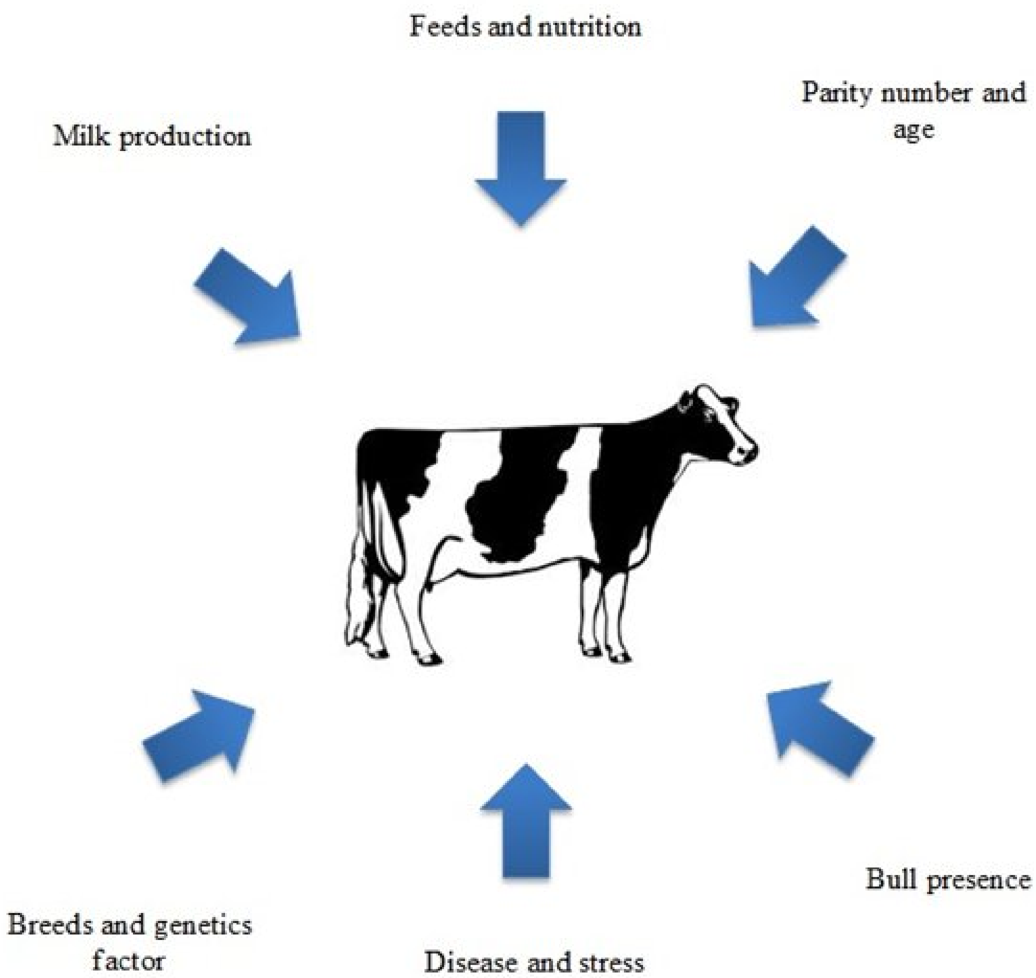


Figure 6.1: Diagrammatic illustration of the factors that influence the interval to resumption of ovarian cyclicity from calving in postpartum dairy cows.

In the current study, the average resumption of ovarian cyclicity was approximately 47 and 30 days postpartum in trial 1 and 2, respectively, while in trial 3 the interval to natural resumption ovarian cyclicity was not measured as cows received PRID treatment in the early postpartum period. Dairy cows that are not nutritionally stressed normally ovulate their first postpartum dominant follicle by approximately 15 days post postpartum with a mean 3 days to first ovulation (Crowe, 2008). This suggests that cows that were used in the current studies may have had a nutritional stress,it may have increased the intervals for resumption of ovarian cyclicity in these animals. Dunn and Kaltenbach (1980) stated that the interval from calving to resumption of ovarian cyclicity is strongly dependent on nutrition. Usually cows have a problem with energy balance in the early postpartum period caused by increased of energy out put in milk production (Robinson *et al.* 2006). Besides, body condition score at calving and during the postpartum period (Rutter and Randel, 1984), the conditions during calving (Bellows et al., 1982) and post calving conditions such as suckling for beef breeds (Short et al., 1976) or lactation in dairy breeds (Inbar et al., 2001) also contribute to the interval to resumption ovarian cyclicity. This is because lactating cows have lower concentrations of serum oestradiol compared to the non lactating cows or heifers,and this affects the onset of oestrus as well as the duration. A lower concentration of serum oestradiol in lactating cows arises from higher metabolic demands for milk synthesis that alter the reproductive physiology and hormone synthesis (Sartori*et al*., 2004). Thus, this could be counted as a factor that had affected the interval to resumption of ovarian cyclicity in cows used in current study. Crowe (2008) outlined that one key for optimising the resumption of ovulatory cyclicity was the optimal body condition at calving, suggestingbetween 2.75 to 3 BCS units,reducing by less than 0.5 BCS during post calving period. The average BCS for all cows used throughout the present study was less than 2.25 during the postpartum period. This explains that cows may have been nutritionally stressed during the trial periods, causing a prolonged anoestrus period.

In some previous studies, bull exposure was found to be an effective treatment in reducing the interval to resumption of ovarian cyclicity in mature postpartum beef cows (Zalesky et al., 1984; Alberio et al., 1987; Naasz and Miller, 1987). Gokuldas el al. (2010) also found that the postpartum interval to resumption of ovarian cyclicity (47.4 ± 2.58 days vs. 56.0 ± 2.37 days, $p < 0.05$) was significantly shorter for buffaloes that were exposed to a bull compared to buffaloes with no bull exposure. These findings are supported by other previous studies in which resumption of ovarian cyclicity was earlier inbeef cows(Burns and Spritzer, 1992) and Zebu cows, used either for dairy or beef production (Rekwot et al., 2000), that were exposed to the bull and indicate a rapid response of ovarian activity in females to stimulation by a male animal. Studies in sheep have demonstrated that biostimulation by a ram influenced the hypothalamic pituitary axis and consequently increased pulse frequency of LH in anoestrus ewes (Martin et al. 1980). Similarly, in some studies, the biostimulation effect of bull exposure to beef or dairy cows during the early postpartum period has increased the LH secretion and hastened the resumption of ovulatory activity (Baruah and Kanchev, 1993; Fernandez et al., 1996).

In spite of these previous findings, contrary results were obtained in all experiments in the present study; high producing dairy cows exposed to a mature bull throughout the postpartum period had no difference in interval from calving to resumption of ovarian cyclicity in comparison to cows without bull exposure as shown in table 6.1. In this study cows were exposed to the bull either in the mid postpartum period,from between 1 to 45 days postpartum (Trial 1),or in the early postpartum period,from 2 days postpartum (Trial 2), yet the findings were similar in both trials. The reason for longer interval in trial 1 with approximately 47 days postpartum compared to 30 postpartum in trial 2, is because cows that were resume ovarian cyclicity earlier during the allocation of trial 1 was excluded from this experiment as only anoestrous cows were used in this trial. The current results are in accord with one study of suckling beef cows, in which there was no significant difference observed in the interval from calving to resumption of ovarian cyclivity and oestrus between cows exposed to the bull in the early postpartum period (10 days postpartum) and without bull exposure (Alberio et al., 1987).

Table 6.1: The comparisons of average interval (±SEM) from calving to the resumption of ovulatory activity in NBC and BC groups from trial 1 and 2.

	Interval of resumption of OC (dpp)	
	NBC	**BC**
Trial 1 (Chapter 3)	46.5±17.3 (n=8)	48.6±20.5 (n=12)
Trial 2 (Chapter 4)	31.3±14.5 (n=15)	29.4±12.4 (n=18)

The timing of the bull exposure to the cows could have an effect on resumption ovulatory activity in cows (Berardinelli and Tauck, 2007).Tauck (2005) stated that exposing a bull to the cows at the very early period after parturition, results in cows not responding to the pheromones emitted by the bull. Alberio et al. (1987) explain that start time of bull exposure to the postpartum cows could be a critical factor for the effect of biostumulation, as the neuroendocrine restoration of cyclicitydevelops gradually after 20 to 30 days postpartum. However, the trial presented in chapter 3 showed no different in speed of response to the biostimulatory effect in cows that were exposed to the bull in a later postpartum period.

One reason for a lack of effect on time to resumption of cyclicity in the experiments in the current study might be the limited degree of bull exposure. The extent of response to the bull exposure on reproductive performance and behaviours in postpartum cows is strongly dependent on the degree of exposure; close physical contact may cause better results compared to fencelineor intermittent bull exposure. Whilst present throughout the whole period, the bull was in an adjacent pen behind the cubicle area, which cows had to make a voluntary effort to visit except when walked past twice daily after milking. Results from the video observations indicated that many cows did not spend very much time in the vicinity of the bull pen; the percentage of cows making a visit were only 50.0%, 38.1% and 40% in trial 1, 2 and 3 respectively. In a previous study, heifers exposed to the close physical proximity of a hormone treated steer reached puberty earlier than heifer that were placed far away from the steer (Fiol et al., 2010). Ababneh et al. (2012) suggest that limited exposure to the bull is insufficient to produce a convincing effect on cows, and Berardinelli and Tauck (2007) stated that fenceline bull exposure was not very effective in comparison to close physical contact between cows and bull. This may explain the reason for insignificant differences in interval to resumption of ovarian cyclicity in cows in the current study.

6.1.1 *Environmental conditionand lameness affecting anoestrous in postpartum cows*

In intensive dairy farming, modern dairy breed cattle tend have serious problems with leg conditions. There are large numbers of cows recorded as having suffered with lameness due to limited space in the yard, slurry on the floor, metabolic stress linked to dietary intakes and weak legs. Leg problems have a direct effect on the fertility of cows (Melendez *et al*., 2003).Holstein genetics have been associated with an increased rate of metabolic problems including lameness, mastitis and infertility (Biefeldt*et al*. 2005). The current study was carried out in an intensive dairy farm; the cows were housed for 24 hours a day during the experiment.Under this condition, the cowssituation was associated with all these environmental and breed factors. Throughout this study, foot treatment was done on several occasions as cows in this herd suffer from foot problems. Hence, this might have contributed for poor reproductive performance, whether they were exposed to the bull or not. However, no systematic locomotion scoring was done for this study. This intensive housing situation was similar to study by Shipka and Ellis (1998), who found no effects of bull exposure in cows kept in a tie-stall barn. In contrast, earlier studies that were done with cows raised in open pasture seem to achieve a more positive response to bull exposure in reproduction performance (Zalesky et al., 1984; Burns and Spritzer, 1992).

6.1.2 *Influence of nutrition on cows during postpartum period*

In general, nutritional factors may influence the outcome by promoting or inhibiting cyclic ovarian function in cows (Allrich, 1993), either with or without bull exposure. In the current study, no differences were found on the effects of bull exposure during the postpartum period in cows that had a body condition score between 2 to 3. This range of scores represents the targeted body condition for lactating cows to make sure they have not suffered from excessive negative energy balance or are likely to have affected fertility level due to excessive fatness (Hulsen, 2005). After parturition, cows that suffer from insufficient nutrition may preferentially divert nutrients away from reproduction (Leroy et al., 2008); as a consequence, this causes a prolonged anoestrus period by limiting the number of ovarian follicles, reducing growth and maximum size of the dominant follicle, delaying the first ovulation, interfering with the process of ovulation, hindering the expression of estrus, and lowering plasma progesterone concentrations. In addition to energy deficits, increased feed intake will also suppresses reproduction by promoting steroid hormone metabolism (Peter et al., 2009).Thus, effects of biostimulationmay not work very well in cows in a negative energy balancecondition. This is related to the findings in this study, as in trial 2 (Chapter 4) there were a few cows with BCS less than 2 at the beginning of trial in the NBC and BC groups, that had a prolonged anoestrus period. Moreover, cows that had low BCS and resumed ovarian cyclicity had difficulty in getting pregnant, thus they were treated with a PRID. In contrast, intrial 3 (Chapter 5), many cows were at poor condition during the beginning of the trial; the average BCS for NBC was1.98±0.28 and for BC was 2.01±0.37. This situation was associated with unfavourable results after PRID treatment, with low conception rate in these animals.

6.2 Progesterone concentrations

During the postpartum period, the concentration of progesterone remains low, until the normal ovarian activity resumes.The resumption of ovarian cyclicity after parturition can be detected by measuring progesterone concentrations during this period. Progesterone concentrations show a sudden decrease during the oestrus period in cows, thus milk progesterone analysis is commonly used to decide if the cow is in oestrus (Bajema et al., 1994). In this study, there was no difference in any of the experiments (Chapter 3,4 and 5)in the proportions of cows exposed to the bull and cows without bull exposure that had a pre-oestrus progesterone rise.

In chapter 4, the average progesterone concentrations elevated above 1.5 ng/ml on day two after insemination and continued to rise in pregnant cows throughout the sampling period. In contrast, the concentrations declined below 1.5 ng/ml on day 13 in the non-pregnant cows for both groups either with or without bull exposure. However the progesterone profiles were different in results in chapter 5, in which all cows were synchronised for oestrus using PRID treatment. The progesterone concentrations were above 1 ng/ml before PRID withdrawal then started to decline after PRID removal and the concentrations during AI were below 1 ng/ml for all cows in BC and NBC group. It was interesting to note that progesterone concentrations during the first week after insemination were significantly lower in pregnant cows without bull exposure. The rise of progesterone after AI was more gradual in NBC cows and some of them had a concentration below 1 ng/ml until day 6 after insemination, starting to rise only on day 7, howeverthis observation obtainedfrom a very small number of cows. In contrast, in BC cows the concentrations were initially at a lower level and started to elevate above 1 ng/ml after day 5 from insemination. The concentrations then remained high throughout the sampling period for cows from both NBC and BC group. Conversely for cows that were not pregnant, the progesterone concentrations declined below 2 ng/ml on day 21 after insemination for cows in both groups. Results on progesterone concentration profiles in this study are in agreement to progesterone profiles presented in previous studies in cattle (Ababneh et al., 2012; Knickerbocker et al. 1986). However the decline and elevation in progesterone concentrations seems different inthe present study compared to the results from these two trials,as the decline and elevation of progesterone was slower in current study. This reflects the fact that the profiles were obtained from the average concentrations of all cows in both treatment groups, whereas in reality the profile for eachcow wasdifferent as the length of oestrous cycle varied in each cow.

From these results, there were no significant effects on the progesterone concentrations at the time of AI for cows that were exposed to the bull compared to cows without bull exposure, similar to the observation in a study on dairy cows by Ababneh et al. (2012). Besides, Shipka and Ellis (1999) described that cows that were not exposed to a bull during the postpartum period had a higher peak (9.1 ng/ml) of progesterone concentrations in comparisons to cows with bull exposure (6.6 ng/ml) during the sampling period. However, a slight increase in progesterone concentrations after exposure to a bull in the early postpartum period has also been reported (Landaeta-Hernandez et al., 2004), but the difference was not statistically significant. Conversely, some previous studies (Burns and Spirtzer, 1992; Bolanos et al., 1998) report contrasting findings, as the concentrations of progesterone were increased in beef cows and zebu cows that were exposed to the bull during the early postpartum period. Hence, the contradiction in the changes in progesterone concentrations in cows that were exposed to the bull highlight that the mechanisms by which bull exposure affects cows during the anoestrus period is not well understood.

6.3 Effects of biostimulationon pregnancy rate

Results from this study report that bull exposure during the mid of the postpartum period (Trial 1) did not improve the pregnancy rates in these cows. However, results in trial2 and 3difference with results in trial 1, as cows that were exposed to the bull during the early postpartum period, with some then synchronised for oestrus using PRID (Trial 3), had higher pregnancy rates compared to the cows without bull exposure as shown in table 6.2. It seems that the improvement of pregnancy rates with bull exposure does not depend on the synchronisation protocol used. Berardinelli et al. (2007) suggestedin their study, that biostimulationaffects cows by hastening the resumption of ovarian activity so the cows responded better in an oestrussyncronisation program. They found that primiparous beef cows that were synchronised for oestrus by using GnRH and PGF_2, then exposed to a mature bull or bull execretory products for more than 60 days, had higher conception rates compared to cows without exposure (57.6% and 35.6% respectively).

The differencein conception rate was significant for cows with bull exposure in trial 2, but not statistically significant in trial 3. This is maybe due to the lower sample size in trial 3 compared to the previous trial, with only 28 cows (Chapter 5) in comparison to 41 cows (Chapter 4) used in trial 2. In previous studies in cows and pubertal heifers, biostimulation has also affected the conception rate, ascows and heifersthat were exposed to the bull had higher conception rate compared to control animals in some trials (Small et al., 2000; Berardinelli et al., 2007). However, in other study in beef cows, exposure to a bull or androgenized steer with fixed time AI did not improve the conception rates of cows (Ungerfeld, 2010).

Table 6.2: The comparisons of conception rate following first service between NBC and BC group for trial 1, 2 and 3.

	NBC	**BC**
Trial 1 (Chapter 3)	25% (2/8)	16.7% (2/12)
Trial 2 (Chapter 4)	15.0% (3/20)	57.1% (12/21)
Trial 3 (Chapter 5)	15.4% (2/13)	40.0% (6/15)
Total	17.1% (7/41)	41.6% (20/48)

The pregnancy rate for the first insemination postpartum in cows that were exposed to the bull in the current study was 57.1% and 40.0% in trial 2 and 3, respectively, while for cows with no exposure to the bull was 15.0% and 15.4%.This indicates that the overall pregnancy rate was higher in cows with bull exposure. The pregnancy rate in cows with bull exposure can be considered as within the range of current UK first service pregnancy rates which averagebetween 40-42% (Dairy Co., 2012). In addition the percentages were also in the range reported in previous studies with fixed time AI in cows that were syncronised for oestrus (Nebel and Jobst, 1998; Ababned et al., 2012). In contrast, thepregnancy rate for cows without bull exposure in the present study was below the average rate from the literature and below current UK national range. However in trial 1, the percentage was very low in both cows with bull exposure or not with only 16.7% and 25.0% respectively, which is below the national target percentage of conception rate to the first service. This is may due to the fact that more cows with longer anoestrus being selected into this trial, shown by the higher average of interval to resumption ovarian cyclicity with 46.5±17.3 days postpartum for NCB and 48.6±20.5 days postpartum for BC cows in comparison to the average of interval to resumption ovarian cyclicity in trial 2 with 31.3±14.5 and 29.4±12.0days postpartum for NBC and BC cows respectively. This was because cows showing early cyclicity did not meet the criterion of being anoestrus for recruitment into the trial. Besides this, length of bull exposure could be another reason for lower conception rate in cows in the first trial, as BC cows were exposed to the fenceline bull exposure for only approximately 40 days, while in trial 2 and 3 the length of exposure is much longer at approximately 80 days.Ababned et al. (2012) suggest that longer interaction between cows and bull before AI could be more important than post AI, and short exposure of the bull may not improve pregnancy rates. Furthermore, Rodriguez and Rivera (1999) claimed that exposing a bull to the cows during the oestrus detection period has increased pregnancy rate, but this was not the case for cows that were only exposed to the bull during AI.This suggest that maybe exposing the bull to cows before AI could increase pregnancy rate through changes in hormones secretions induced by both pheromonal and behavioural influences of the bull, such as sniffing and licking the cows. Thus, this situation may promote better oestrus detection and the conception rates could be increased by insemination of cows at the right time (Heres*et al*., 2000).

6.4 The influence of bull exposure onbehavioural responses of the postpartum cows

Previous studies concluded that stand to be mounted, mounting other cows, chin resting, vulva discharge, flehmen and some other behaviours are the best visual indicator of oestrus in cows (Van Vliet and van Eerdenburg, 1996, López-Gatius et al., 2005, Yàniz et al., 2006). However, McGowan et al. (2007) explained that several false positive alerts from visual observation occur in detecting oestrus behaviourally when compared tousing measurements of progesterone levels.In the current study, the signs of oestrus displayed were not significantly different between cows with bull exposure or not (Table 6.3); this was explained in results fromchapter 3,4 and 5.This indicates that bull exposure does not increase expression of oestrus signs among cows. All the cows used in this study were in the early postpartum period and were in an anoestrus state at the beginning of the experiment; the oestrus signs were observed at the first inter luteal phase post calving, as shown in Figure 6.2. Normally, the first ovulation post calving is a silent oestrus, which means no oestrus signs can be observed (Crowe, 2008). A study by Isobel et al. (2004) found that 83% of silent oestrus occurred during the first ovulation in the first oestrous cycle post calving, and the percentage decreased as the number of cycles increased with 46%, 13% and 0% in second, third and fourth ovulation respectively. So, this can be one reason for the poor oestrus detection and poor response ofoestrus expression to bull exposure in the current study.

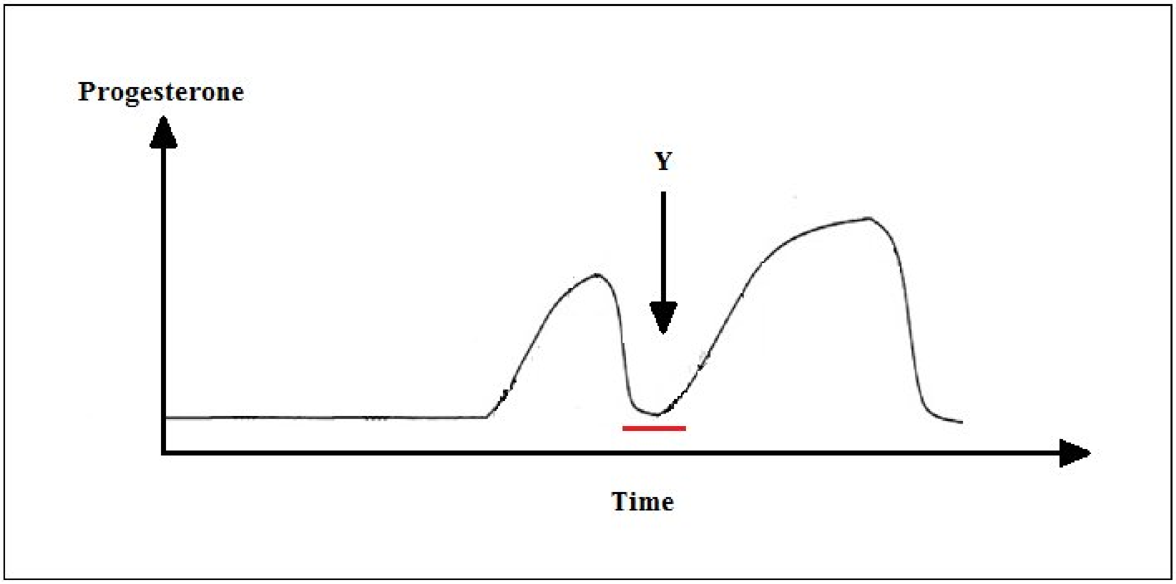


Figure 6.2: Diagrammatic representation of the progesterone concentrations in cows post caving. The first rise of progesterone occurs after the first ovulation, which normally silent oestrus. The interval during low progesterone concentrations is the first inter luteal phase (marked as Y), oestrus signs were observed during this period in this study.

Table 6.3: The percentages of oestrus signs observed in trial 1 (Table 3.4), 2 (Table 4.3) and 3 (Table 5.4).

Oestrus signs		**Trial 1 (Chapter 3)**	**Trial 2 (Chapter 4)**	**Trial 3 (Chapter 5)**
STBM	**NBC**	37.5% (3/8)	25.0% (5/20)	30.8% (4/13)
	BC	58.3% (7/12)	38.1% (8/21)	53.3% (8/15)
Mounting other cows	**NBC**	50.0% (4/8)	75.0% (15/20)	53.8% (7/13)
	BC	66.7% (8/12)	61.9% (13/21)	66.7% (10/15)
Chin resting	**NBC**	50.0% (4/8)	40.0% (8/20)	38.5% (5/13)
	BC	41.7% (5/12)	57.1% (12/21)	20.0% (3/15)
Vulva discharge	**NBC**	37.5% (3/8)	55.0% (11/20)	46.2% (6/13)
	BC	16.7% (2/12)	66.7% (14/21)	46.7% (7/15)
Increase in walking	**NBC**	25.0% (2/8)	20.0% (4/11)	25.0% (2/8)
	BC	16.7% (2/12)	19.0% (4/12)	33.3% (3/9)

In a previous study, the proportion of cows in oestrus, detected by visual observation twice a day,was significantly higher for cows with bull exposure compared to cows without bull exposure (67.9% and 32.7% respectively) (Alberio et al., 1987). The findings in a study of buffaloes similarlyfound that the interval to detect first oestrus signs after parturition was significantly shorter for buffaloes that were exposed to the male compared to without male exposure (57.7 ± 3.61 days vs. 71.3 ± 5.13 days, respectively) (Gokuldas et al., 2010).The different findings between the present study and previous studies can be explained by the different stimulation methods, asa vasectomised bull was used as their stimulus animal, and by the fact that the studies used different species or breeds. Moreover, Orihuela (2000) stated that the findings on oestrus detection could be attributed to the differences in oestrus detection techniques, types of housing and frequency of handling. Besides this, the season of the year, humidity and genetic factors also affect the oestrus period and intensity in animals (Hernandez et al., 2002). In this study, a scoring system modified from a previous study (Van Eerdenburg et al., 1996; Roelofs et al., 2008) was used in oestrus detection to measure the frequency and intensity of oestrus signs expressed by the cows. Strong intensity and high frequency of oestrus displayed by animals will increase the chance of detecting oestrus in cows (Van Eerdenburg et al., 1996). However the total point scores in this study did not differ significantly in cows either exposed to the bull or not.

Detection of oestrus by measuring the oestrus signs is strongly related to the number of cycles after parturition (Peter and Bosu, 1986). They measured changes in walking activity using pedometers and theproportion of cows that had increased walking activity during oestrus was increased as the number of oestrous cycle increased. Therefore increases in walking activity were not necessarily obvious for the first ovulation and first oestrus cycle post calving. In the current study, walking activity was measured in cows at their early postpartum period, that is, the first inter luteal period after resumption of ovarian cyclicity. Results showed that the change in walking activity during oestrus was not significantly different in cows either exposed to the bull or not. Yàniz and López-Gatius (2003) indicated that walking activity is related to the fertility;an increasing walking activity during oestrus means the fertility level is good and may increase conception rates. However, for this current study, the changes in walking activity were not observed after the firstinter luteal period and thus no comparisons in walking activity during oestrus between different post partum cycle numbers can be made.

6.5 The cost-benefit of keeping a bull in the herd

The cost of keeping a bull is dependent on the system applied in any particular farm. For example, Cockle Park farm uses a rolling system in keeping the bull. Bulls were kept until suitable for sell for beef, and until that time they were used as a stimulus animal to provide biostumulation effects. Some other farms keep a mature bull for breeding purposes, thus this is also suitable to be used as a stimulus animal with some extra cost of fencing to house the bull near the cows. The net margin for selling a beef bull at an age of 12 to 14 months is approximately £196, as reported in UK industry literature (Farmers Weekly, 2013). If the farm keeps the bull to use for biostimulation, rather than sell for beef, they will lose £196 profit from selling it and £1.92 per dayfeeding cost for the medium size of bull (Eblex, 2008). In contrast, for an average performing 100 cow herd, poor fertility costs over £25,000 per year, which includes cost of conception failure (Dairy Co., 2012). The cost of conception failure for each cow of a 21 day cycle is £65.02 (Blowey, 2011); this amount is a total from all related costs from lost production, labour, additional AI and extra veterinary treatment. From the results of conception rate to the first service in this study for normal cows without PRID treatment, the average improvement for cows that were exposed to the bull was approximately 36% compared to cows without bull exposure. Thus, for example in one herd with 100 cows, if an extra 36% of the herd were pregnant to the first service, the total cost saved would be at least £2,300 while the cost for keeping a bull for a year is approximately £896.

So, for this situation, the better conception of cows treated with biostimulation will greatly outweigh the cost of not selling the bull. In addition, the cost of using a bull can be reduced by using a young bull as a stimulus animal, which can be sold while still suitable for market and so incur little depreciation cost, rather that a mature one (age 3 years above), since the effects of bull exposure were not significantly different between young or mature bulls (Cupp et al., 1993).Thus this calculation proves that keeping a bull could be an organic, economical, clean, green and ethical approach in improving the postpartum reproduction problem in dairy herds.

Conclusions

Under the conditions of this study, continuous fenceline exposure of high yielding dairy cows to a bull during the very early postpartum period increased pregnancy rates and reduced the length of calving interval when compared to non exposed cows. However, there was no significant effect on cows that were exposed to the bull in the later postpartum period and there was no absolute effect on the time to resumption of ovarian cyclicity in this study. The biostimulation effects of bull exposure may stimulate ovarian activity and secretions of hormones required to improve oocyte quality and prepare a better environment for insemination. Furthermore, although intensity of typical oestrus behaviours was not changed, some cows exposed to the bull did interact more with the bull when in oestrus, giving a clue about the timing of oestrus. The biostimulatory effects provided by close physical contact with a bull through continuous fenceline exposure during the early postpartum therefore improved the breeding performance of high yielding dairy cows. Furthermore, bull exposure might also improve the efficacy of PRID treatment to reduce prolonged anoestrus, and improve productivity of dairy cows.

However, the best exposure method needs further exploration to obtain optimal results frombiostimulation, as effects of biostimulation on reproductive performance in cows during the postpartum period have been shown to be linearly related to the manner of bull exposure (Tauck et al., 2010). In addition, a critical part in the investigation of the effects of biostimulation by bull exposure is to understand the underlying mechanism of this stimulation. The cues frombull exposure, whether pheromonal or visual, received by the postpartum cows are poorly understood. Furthermore, the efficiency of response to the stimulation may be influenced by the effects of other surrounding factors, such as stress, milk production, body condition and environment. Further studies with larger numbers of cows are needed to confirm the important indications from findings in this project; it is important to improve reproductive performance in dairy cows in any manner possible, as this will critically influence the efficiency and profitability of dairy production.

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APPENDIX 1

Total mixed ration (TMR)

Total mixed ration of home grown wheat and bought in straights such as soya and distillers grains. Here is the list of ingredients used to produce TMR for dairy herd in Cockle Park.

1. Grass silage
2. Straw
3. Processed feed: Northern Gold is a high, digestible fibre energy and protein, moist complementary feed that is denser than many other moist feeds of similar dry matter. It is produced by mixing wheat distillers' syrup and sugar beet.
4. Premix, produced from ingredients below:
   - Rolled Barley 20%
   - Rolled Wheat 20%
   - Sugar Beet Pellets 10%
   - Hipro soya 13%
   - Maize distiller 13%
   - Regumaize molasses 22%
   - Hi Phos minerals 2%

The current TMR formulated for dairy herd is as follows:

Grass Silage 44kg /head
Straw 1kg/head
Northern Gold 8kg/head
Premix 5.5kg/head

This ration formed to meet the nutrition requirement for cows that freshly calved to drive the cow towards high milk production and for high groups to maintain high milk production. Feed intake is important in order to maintain adequate nutrient for high milk production and to prevent any metabolic disorder.

THE END