Uterine involution in the dairy cow: Comparative study between organic and conventional Dairy Cows

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Brenna Catherine Dobson-Hill

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Abstract

Organic dairying is growing in popularity in New Zealand and the demand for knowledge is ever increasing. Many more farmers are considering organics as an alternative to their current conventional system. However, limited information is available on organic dairying in a New Zealand pastoral situation particularly in relation to its effect on fertility.

The New Zealand dairy industry requires the dairy cow to become pregnant every year at the same time to maximise milk production and utilise the high pasture production in spring. A cow requires 40 to 60 days for uterine involution to occur and for resumption of oestrous cycles. It is critical that the cow becomes pregnant within the six to eight week mating period, however, this can be difficult for late calvers or those cows that had or still have a uterine infection, to undergo uterine involution and be successfully mated. These cows will often not become pregnant and as a consequence be culled for infertility.

During the first few weeks postpartum, the bacterial composition of the uterus fluctuates as a result of spontaneous contamination, elimination and recontamination. Most cows are able to eliminate these bacteria, however, 10 to 17% of cows are unable to do this. In these cows, the bacteria persist, cause infection and inflammation, and delay uterine involution.

The main objective of this present study was to investigate the effect of different management systems (Organic and Conventional) on productivity and reproduction, particularly the process of uterine involution and its relationship with reproductive outcomes. Uterine involution will be studied through the following measurements: cervical diameter as assessed by measurement per rectum, plasma concentrations of glucocorticoids and bacteriology of the uterus. Lactation characteristics: milk yield, cumulative milk yield, milksolids production and somatic cell count will also be investigated.

From the results, it can be concluded that Organic cows have reduced milk yield ($P=0.05$) and milksolids production ($P<0.01$) through there were no major differences at peak lactation or in the rate of decline after. However, overall somatic cell count was not affected by management system though the Conventional cows had high somatic cell counts in early lactation ($P=0.925$). Cervical diameter was affected by the management system with the difference between Organic and Conventional cows almost significant ($P=0.06$). Cortisol concentrations were significantly higher in Organic cows (0.68 ± 0.08 ng/ml) when compared to Conventional cows (0.95 ± 0.06 ng/ml) ($P=0.01$). Cortisol concentrations decreased over time postpartum ($P<0.01$). Additionally Organic cows had a shorter interval from calving to AI ($P=0.017$). However, none of the other reproductive outcomes were affected by management system.

Simple correlations established that reproductive outcomes and bacterial counts, reproductive outcomes and cortisol at Day seven, and cortisol and milk production characteristics, were not independent of each other. Aerobic bacterial counts on Day 28 and the interval from calving to AI were found to be significantly correlated ($r= 0.615$). Stepwise partial regression analysis, plus analysis of variance calculated on the relationships between uterine involution parameters and reproductive outcomes found multiple weak correlations. It found that cervical diameter, and aerobic and anaerobic bacterial counts on Day 28 were significantly correlated with the interval from calving to first AI ($P=0.018$).

The differences in lactation characteristics are related to the lower pasture production on the organic farmlet reducing the maximal potential milk production and the possibly earlier calving dates of the Conventional cows. The differences in bacterial counts and almost significant difference in cervical diameter may be related to the lower cortisol levels and thus suggest that the Organic cows may be less stressed. As stress suppresses the immune system, it may be possible that the higher bacterial counts are indicitative of a less efficient or slightly weakened immune system which takes longer to eliminate bacteria in the uterus; however this is open to conjecture. The shorter interval from calving to AI suggests that Organic cows may be exhibiting oestrus behaviour earlier postpartum or the Conventional cows are having “silent heats” but this difference is possibly related to human factors. The correlation between parameters of uterine involution and interval from calving to AI, and the lower values for uterine involution parameters in Organic cows suggest the possibility of faster uterine involution and this may result in better fertility in the Organic cows. More research needs to be carried out to further investigate the effect of organic dairying on uterine involution and reproduction outcomes but organic dairying at the very least does not negatively impact on uterine involution and fertility.

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1 Literature Review

1.1 Introduction

Organic dairying is growing in popularity in New Zealand and the demand and desire for knowledge on the subject is increasing. People have many different reasons for converting to organic dairying from a desire to be more environmentally friendly and sustainable, to meeting the increasing demand for organic products from overseas markets. Other reasons include frustration at the lack of results within their current conventional systems, a wish for a lower input system and an awareness of the benefits on animal and soil health.

Most of the research in organic dairying has been done in Europe, where cows spend much of the year indoors and are fed concentrates. This is very different to the New Zealand pastoral-based system. There is currently no published research investigating the effect of organic dairying on reproduction in a pastoral system.

The aims of this research are to investigate the effect of the different management systems (organic and conventional) on uterine bacterial contamination, lactational characteristics, cervical diameter and glucocorticoid concentrations. It will also examine the effect of these variables on subsequent fertility outcomes.

This chapter reviews the research into reproduction and fertility in the dairy industry: the process of uterine involution, uterine bacterial contamination and its effects on fertility in both conventional and organic dairy cows.

1.2 Involution of the uterus

In the cow, the third stage of labour, during which the foetal membranes are detached, takes an average of six hours but it can take up to 12 hours. If detachment does not occur within 12 hours then the cow is considered to have retained foetal membranes (RFM) and it can take from three to ten days until they are fully expelled (Noakes *et al.* 2001; Jackson 2004). During the third stage of labour, regular abdominal contractions have largely ceased and myometrial contractions have decreased in amplitude but have become more frequent and irregular. Contractions open up the endometrial crypts causing the foetal villi to shrink due to the loss of turgidity thus leading to the escape of blood from the foetal side as the umbilical cord ruptures (Noakes *et al.* 2001). As a result, the apex of allantochorion becomes inverted and as the sac comes away, the foetal villi are drawn out. When a large piece of the placenta becomes detached it stimulates the reflex contractions of the abdominal muscles.

During the third stage of labour, the endometrium undergoes some changes; namely maturation and collagenisation of the placentomes, the flattening of maternal crypt epithelium, and increased leukocyte infiltration (Slama *et al.* 1991). After the third stage of labour is completed, regular myometrial contractions promote the elimination of lochia, which consists of placental fluids, blood tissue debris and endometrial exudate, and there is an overall reduction of smooth muscle mass (Slama *et al.* 1991).

Uterine involution involves the remodelling of the caruncles, regeneration of endometrial tissue, a reduction in uterine blood flow and endometrial vascularity, as well as a reduction in smooth muscle mass (Guilbault *et al.* 1984, Slama *et al.* 1991). There is also elimination of bacterial contamination. Involution of the genital tract tends to take longer in older cows but often occurs more quickly in cows with high milk yields (Fonseca *et al.* 1983).

Smooth muscle plays an important role in the expulsion of the uterine contents and the reduction in uterine size (Bajcsy *et al.* 2005). The greatest change in the uterus occurs within the first few days postpartum. During the first two weeks postpartum, there are large number and diverse range of bacteria in the uterus (Griffin, 1974a), but they have been eliminated from most animals within the next two weeks (Archbald *et al.* 1998).

Lochial discharge is made up from the remnants of the foetal fluids, blood from the umbilical vessels and sloughed surfaces of the caruncles (Noakes *et al.* 2001). The lochial discharge is usually yellow-brown or red-brown from the blood of the microcotyledons. The greatest flow of fluid is between Days two and three with a dramatic reduction by Day eight. McEntee (1990) found that very little fluid was present after Day 12. Puerperal vulvar discharge (lochia) ceases by the second or third week postpartum (Lewis 1997; Sheldon *et al.* 2006). Okano and Tomizuka (1987) found that lochia is usually present until Day 15 postpartum. Lochia changes from a red-brown fluid to viscous white/yellow material during this period (Sheldon *et al.* 2006).

The diameter of the former gravid horn is halved within five days and the length is halved within 15 days (Gier and Marion 1968). Areas that were not badly damaged during parturition, usually the intercaruncular regions, are quickly regenerated, within eight days, giving the uterus a glistening appearance (McEntee 1990). Complete re-epithelialisation of the caruncle usually takes at least 25 days, but the caruncles continue to decrease in size until Days 40 to 60 (Gier and Marion 1968).

On Day two postpartum, irregular red areas are present on the luminal part of the carnucles with areas of necrosis visible. These lesions are located on the arteries on the superficial part of the vascular stalk of the carnucle. Early vascular lesions consist of the hydropic degeneration of the cells. There is a proliferation of spindle-shaped cells, which narrow the arterial lumen and block the vessels of the fibrous tissue in the carnucle (Hearth *et al.* 2006).

On Day five, a necrotic leukocyte layer covers the endometrium and sloughing of the uterus has begun with the small blood vessels, mainly arterioles, oozing blood. The caruncles are dark, red and soft. Between Days 10 and 15, the sloughing of caruncles is complete, with blood vessels protruding from their endometrial surface (McEntee 1990). The necrotic caruncular material has been sloughed from the vascular stalk, resulting in a surface which is granular with a few remaining haemorrhagic foci that are slowly covered with the growth of the surface epithelium (Grier and Marion 1968; Mc Entee 1990). The surface epithelium in the intercaruncular areas has remained intact throughout the involution process giving it a glistening appearance. The endometrium is a brown colour due to the presence of haemosiderin in macrophages. By Day 30 postpartum, the caruncles appear to be normal (McEntee 1990).

1.3 Cervical and uterine involution

Uterine involution is associated with a progressive decrease in vaginal discharge and in uterine and cervical diameters. The cervix decreases from approximately 30 cm immediately after parturition to approximately 2 cm by Day seven postpartum (Wehrend *et al.* 2003). Kasimanickam *et al.* (2004) found that the larger the diameter of the cervix after partition, the longer the time taken for involution to occur (see Table 1.1).

Table 1.1: The decrease in cervical diameter postpartum over time

Cervical Diameter (cm)	20 to 33 days postpartum (%)	34 to 47 days postpartum (%)
Less than 3.5 cm	28.1	57.0
3.5-5	50.5	38.2
Greater than 5	21.5	4.8

Source: Kasimanickam *et al.* 2004.

The process of cervical involution can be divided into four phases. Phase One, occurs the first six hours after parturition, and involves a rapid reduction in the diameter of the cervix. The second phase is the protracted reduction until Day two without the constant re-formation of cervical folds. Phase three occurs between Days two and seven, during which a protracted reduction of size occurs, with constant re-formation of cervical folds. Finally, the fourth stage, from Days seven to ten, involves reopening of the cervical canal and maintaining the structure of the folds (Wehrend, *et al.* 2003). This process means that the cervix allows lochia to drain whilst still protecting the uterus from contamination. The reorganisation of the structures starts at the cranial end of the cervix and continues caudally (Wehrend *et al*. 2003).

The number of days required for the cervix to involute is increased in cows with reproductive abnormalities including dystocia and RFM. These cows are at greater risk of developing a uterine infection because there is more opportunity for bacterial colonisation of the uterus (Sheldon *et al.* 2006). At-risk cows have a longer period with the cervix open than those cows without reproductive problems; this interval has been reported as 16.6 days (Fonscesa *et al.* 1983) and 29.3 days (Coleman *et al.* 1985). This may be of some importance in terms of subsequent fertility, since Le Blanc *et al.* (2002) found that a cervical diameter of less than 7.5 cm between Days 20 to 33 postpartum was associated with a decrease in pregnancy rate. In contrast, Kasmimanickam *et al.* (2004) found that cervical diameter was not associated with the relative pregnancy rate. However, most authors agree that cows with an increased cervical width will have an increased number of days with their cervix open and hence a decreased number of opportunities to become pregnant (Fonscesa *et al.* 1983; Le Blanc *et al.* 2002).

The decrease in the uterine diameter follows a similar pattern to the cervical diameter though the cervix normally involutes more slowly than the uterus (Le Blanc *et al.* 2002). Cervical width is a better predictor of reproductive problems than uterine size as there is less variation in the size and accessibility of the cervix during involution. *Le Blanc et al.* (2002) suggest that optimum time to assess cervical involution is three weeks postpartum and the optimal threshold to assess is between 6 and 7.5 cm. The relationship between cervical width and pregnancy rate is significant particularly between Days 27 and 33 (Le Blanc *et al.* 2002). By Day 40, healthy cows have a diameter of less than 5 cm even though involution may not be complete for another ten days (Kasimanickam *et al.* 2004; Sheldon *et al.* 2006).

1.4 Postpartum ovarian activity

The changes that occur in the ovary postpartum can be divided into three phases:

- The puerperal phase from calving until the pituitary gland becomes responsive to GnRH (gonadotrophin releasing hormone).
- The intermediate phase from when the pituitary gland becomes responsive until first ovulation.
- Post-ovulatory phase from the first ovulation until involution is completed (Olsen *et al.* 1984).

The corpus luteum of pregnancy does not persist following parturition. It begins to degenerate after the onset of parturition, and within a few days of parturition, it is in an advanced stage of degeneration.

Early in the postpartum period, the negative feedback on GnRH is removed, which allows follicle stimulating hormone (FSH) concentrations to increase, over a period of three to five days, leading to the development of new follicles and between Days seven and 14 postpartum (Savio *et al.* 1990). FSH stimulates follicle growth, which becomes minimal by the end of pregnancy. Once a threshold size is reached, follicles produce oestradiol and inhibin, which suppress further FSH secretion. The decreasing FSH concentrations stop further development of any other follicles so only the dominant follicle continues to grow. Whether this dominant follicle ovulates is dependant on the frequency of LH pulses, the size of the dominant follicle and concentration of IGF-I as well as the sensitivity of the hypothalamus to the effects of oestradiol (Savio *et al.* 1990).

The first ovulation is not normally accompanied by oestrus behaviour, as pre-ovulatory exposure to progesterone is necessary for this response. The luteal phase is also likely to be short because of the premature release of $PGF_{2\alpha}$ from the uterus. The first postpartum CL typically regresses on Day eight to ten of the oestrous cycle and the next follicle ovulates on Day nine to 11 (Savio *et al.* 1990). Most cows have ovulated within 30 days of parturition (McEntee 1990).

The post-partum anoestrus period is also affected by breed, age, condition score at calving, nutrition and calving date. The average duration of the anoestrus period is 35 to 40 days in New Zealand dairy cows (McDougall *et al.* 2005).

1.5 Organic Dairying in New Zealand

Organic farming aims to work with natural biological systems. Organic management focuses on creating optimal soil health and management. The soil is regarded as a living system with essential links between healthy soils, plants, animals and people. Organic farming discourages synthetic inputs as well as reducing the overall reliance on inputs from outside sources (Fonterra 2003).

Use of synthetic chemicals including fungicides, herbicides, insecticides, growth regulators and soluble fertilisers is generally prohibited. Organic dairying advocates the use of macronutrient fertilisers such as naturally dried potassium sulphate, reactive phosphate rock, biodegradable microbial plant and/or animal material produced from organic practices such as chicken manure, and soil microorganism enhancers such as compost tea. Very few conventional medicines can be used; Fonterra requires its organic suppliers to conform to United States Department of Agriculture National Organic Programme (USDA NOP) standards (Fonterra 2003). Any use of antibiotics results in the permanent loss of the organic status of the cow (Fonterra 2003; Thatcher 2005).

Key principles of Organic Farming are outlined in Fonterra's Best On-Farm practice for Organic Farmers.

- Encouraging and enhancing biological cycles.
- Maintaining and improving long-term soil structure and fertility.
- Humane management of livestock.
- Maintaining genetic diversity of the system and its surroundings.
- Cycling organic matter and nutrients.
- Integrating management of soil, crops, and the environment for weed, pest and disease management.
- Minimising all forms of pollution.
- Not allowing genetic engineering.

Source: Fonterra, 2003

Herbal leys are often used in organic farming; they contain a mixture of herbs, chicory, plaintain, white and red clover, timothy, cocksfoot and various other grasses (Fonterra 2003). Chicory and timothy species are chosen because they are deep-rooted species and so are more persistent; however, they require careful management in wet conditions (Thatcher 2005; Countrywide 2007). A wide variety of pasture species have advantages for soil fertility, which along with managing stocking rates and rotation lengths, help to maintain pasture quality and ensure good pasture persistence. A variety of pasture species can also have benefits to animal health. For example, chicory and plantain have been found to be copper accumulators and also have fairly high levels of condensed tannins which increase protein uptake by the cow (Thatcher 2005).

Optimal cow health could be achieved through the genetic selection of cows resistant to diseases, parasites and infections, provision of high quality feed and appropriate pasture management, high standards of animal welfare and good husbandry to reduce stress and maintain hygiene standards (Fonterra 2003). All animals should be treated with plant extracts, homeopathic remedies and trace elements (provided they are effective) before conventional medicines are used (Fonterra 2003; Thatcher 2005).

1.5.1 *Differences between Organic and Conventional dairying*

The focus of organic dairying is different to conventional dairying. There is a greater focus on animal welfare, preventative husbandry, maintenance of high hygiene standards and preventing rather than curing disease and illness. In line with this philosophy, organic farmers try to breed for the "ideal" organic cow who should be resistant to parasites and diseases, have a high feed conversion efficiency and have no predispositions to metabolic disorders including hypocalcaemia (Fonterra 2003). Any cows that do not adapt to this system i.e. empty cows and cows with a high somatic cell count tend to be culled (Thatcher 2005).

The lack of artificial nitrogen as a fertiliser means that total pasture production in organic systems is generally less than in conventional systems. Additionally, the seasonal growth curve is altered; early spring growth can be poor but early autumn growth can be increased (Kelly *et al.* 2008). This means that conventional management practices need to be modified, with lower stocking rates, and a later date for the start of calving (Kelly *et al.* 2008). Moreover, since organic dairy farming systems generally produce less grass, lower pasture intakes are more likely to occur than in conventional systems, hence the maximum possible milk production is likely to be lower (Burkitt *et al*. 2007). This is dependant upon the skill of the farmers and the understanding of their individual farming system.

Particular attention is paid to shelter, drainage, races and yards. These factors, particularly well planned and executed races and yards, are important, as they minimise exposure to infectious organisms and stress, and minimise mechanical damage to the feet and limbs (Thatcher 2005).

Data on reproduction from comparisons of organic and conventional farms is scant. However, anecdotal evidence suggests that reproduction may be improved. One farm reported a 7% empty rate and another farm a decrease from 12 to 5 % in the empty rate after conversion to organic farming (Rural delivery 2005; Rural Delivery 2007). The authors suggested that this was due to better nutritional balance. However, it must be remembered that organic farms have a longer calving spread than conventional farms, so this may affect empty rate. There is very little research on the effect of organic dairying on bovine reproduction (Hovi *et al.* 2003; Roesch *et al.* 2005) and there is no published scientific research on reproduction in organic pastoral systems.

1.5.2 ***Certification of Organic milk***

Organic milk suppliers in New Zealand must meet the organic production certification standards for both the International Federation of Organic Agricultural Movements (IFOAM), which is a non-government organic standard, and the New Zealand Food Safety Authority (NZFSA) Technical Rules of Organic Production (with its Appendices). The rules are USDA NOP regulations. All processes from on-farm transport and manufacturing are audited annually to ensure that they comply with NZFSA Technical Rules of Organic Production by NZFSA approved Third Party Agencies (TPAs) or Organic Certification Agencies (Fonterra 2003). Currently, there are two organisations that are recognised by NZFSA; namely AsureQuality and Biogro NZ Ltd (Thatcher 2005). NZFSA Technical Rules of Organic Production are recognised by the European Union (EU) and USDA as an equivalent to their own organic standard (Fonterra 2003). It takes two years to become fully certified to EU and IFOAM standards and three years to become fully certified to USDA standards (Fonterra 2003).

The Organic cows on the Dairy Cattle Research Unit at Massey University generally produce about 10 to 20% less milk than conventionally-managed cows (Kelly *et al.* 2008). Currently, the price premium for organic milk is 20% higher than conventional milk and 7% during the conversion to organic status (Reider 2007). This premium and the greater emphasis on animal welfare means the organic farmer does not place as much pressure on their cows for maximum milk production as conventional farmers do. The reduced milk production and associated reduced pressure reduces the total stress on the cow. One of the benefits of organic farming is a likely reduction in metabolic stress and consequentially, of stress hormones (Rosati and Aumaitre 2004).

The less stressful environment of the organic cow may reduce the risk of infection and therefore improve the health of the cow. Organic farming focuses on reducing exposure to infectious agents and reducing stress so that the immune function is not impaired (Hamilton *et al.* 2002). An inverse relationship between milk production and reproductive outcomes in both organic and conventional cows has been found (Reksen *et al.* 1999). However, organically managed cows in this study were fed reduced amounts of concentrates so the low milk yield of organic cows may in part be due to negative energy balance which would also negatively affect reproduction (Roesch *et al.* 2005).

Research done on organic farms in the UK found that the mean calving to first service interval was 80 days (68 - 97) and the mean calving interval was 385 days (370 - 413) (Hovi *et al.* 2002). The mean number of services per conception was 2.3. Organic farms tended to have longer calving periods and have more relaxed attitude to mating (Hovi *et al.* 2002). The organic farms in another study had a mean calving to first service interval (89 days) and mean calving interval slightly lower than conventionally managed herds in the U.K (Blanshard 1999; Hovi *et al.* 2002). However, the number of services was higher than the average number of services in conventional cows (1.7) reported by Blanshard (1999).

Other studies have found no consistent differences in the mean calving interval, calving to first insemination, and inseminations per conception between conventional and organic cows (Reksen *et al.* 1999; Rosati and Aumaitre 2004;

Roesch *et al.* 2005). In one year, the calving interval was shorter in organic cows and in another year, the intervals from calving to first AI were longer in organic cows. However, where mixed model analysis has been used, conventional cows had a shorter calving interval and shorter calving to conception interval. This is possibly related to higher culling rates, particularly in those cows with low fertility in the conventional herds (Reksen *et al.* 1999).

Reksen *et al.* (1999) found that conventionally managed cows had a longer postpartum anoestrus interval than organically managed cows. These authors suggest the cause was that a greater proportion of organic cows were mated naturally. Cows that are mated naturally have fewer days open than cows mated with AI. One of the issues with this study is the small size of the sample herds (5 to 45 cows). Small herds can have less stress through herd interactions and greater individual attention (Rosati and Aumaitre 2004). The lower metabolic stress and lower milk production by the organic cows could account for fewer days open (Rosati and Aumaitre 2004).

1.6 Fertility in New Zealand dairy practice

In New Zealand dairying, it is essential that cows become pregnant every year in order to produce milk. The New Zealand dairy system uses pasture as its main feed source with supplements fed when required. It is therefore necessary that cows calve when the feed supply is best able to meet their requirements and which is why calving occurs in spring. To achieve a 12 month calving interval, a cow must conceive within 85 days of calving (Moller 1970). For this to occur, they must begin cycling a few weeks after calving. Moreover, cows require at least 40 to 50 days for the completion of uterine involution (McEntee 1990). Productivity will not be impaired if the calving to conception interval is between 80 to 85 days, but it will be affected after this period (McMillan *et al.* 1996). However, typically many cows are not detected in oestrus before the planned start of mating.

The New Zealand dairy system has several important differences from overseas systems. In New Zealand, cows are at pasture all year round which is in contrast to many overseas systems where the cows are housed for some, if not all, of the year. Cows in overseas systems have a greater proportion of their diet as concentrates, with some being completely fed on total mixed rations, whereas, in New Zealand, the majority of the feed is pasture with forage-based supplements such as hay and grass silage. The higher energy content of the diet and higher DM intake in overseas systems often means that average milk production is much higher (Roesch *et al.* 2005). Another difference between New Zealand and overseas dairying systems is the average herd size. The average herd in Manawatu and Rangitikei in 2006 was 342 cows (Louise Cook, personal communication) compared to herd sizes of fewer than 100 cows, often less than 50 cows overseas (Busato *et al.* 2000; Bennedsgaard *et al.* 2003; Roesch *et al.* 2005) particularly those herds studied in scientific literature.

1.7 Stress in the New Zealand dairy cow

There are concerns that the growing pressure farmers place on cows for increased milk yields is at the expense of fertility. Stress, in the context of this study, is the changes in the environment that prevent the cow from maximising its reproductive potential. Stress causes an activation of the hypothalamus–pituitary-adrenal gland axis and hypothalamus-pituitary ovarian axis either from a single or caccumulated discrete challenges (Dobson *et al.* 2001). There are many different physiological and social stressors that could be affecting fertility including lameness, mastitis and metabolic diseases. These stressors cause the secretion of cortisol and catecholamines leading to endocrine, immune and neural imbalances that can adversely influence productivity and health (Gwazdauskas 2002). Stress, by itself, can cause cows to become sick and in turn negatively affect milk production and reproduction. A cause of stress postpartum is the fact that the cow is unable to ingest enough feed to meet its nutrient requirements in early lactation. While the effects may be not be evident in the form of reduced milk production, they may be shown by a reduction in fertility. Gwazdauskas (2002) believes that the selection for increased milk production has made cows highly susceptible to stress.

Stressors stimulate the hypothalamus to release corticotrophin-releasing hormone (CRH) which is transported via the hypophyseal portal vessels to the anterior pituitary. The pituitary responds by secreting adrenocorticotrophic hormone (ACTH) which is transported through the blood and binds to the receptors in the adrenal cortex. The binding to receptor causes an increase in steriodgenesis through cAMP leading to cortisol being secreted from the adrenal cortex. Cortisol causes an increase in the rate of gluconeogenesis thus increasing the rate of muscle protein breakdown and enhancing lipolysis, breaking down lipids, with both processes increasing the amount of energy available to the cow (Gwazdauskas 2002).

Glucocorticoid concentration peaked around parturition and decreased back to prepartum levels between 24 hours to nine days postpartum (Smith *et al.* 1973; Torres *et al.* 1997; Preisler *et al.* 2000).

Stressors interfere with the precise timing of the reproductive hormone release in the follicular phase. Stressors disrupt the hypothalamus-pituitary-adrenal axis reducing the pulsatility of the GnRH and LH pulses in the hypothalamus and pituitary; the lack of LH support delays the onset of LH surge (Dobson and Smith 2000; Gwazdauskas 2002). Stressful stimuli affect the neural control of GNRH secretion leading to a reduction in GnRH secretion resulting in slower growing follicles with lower oestradiol production (Dobson and Smith 2000; Dobson *et al.* 2001). Dobson *et al.* (2000) found that stimulation with ACTH caused a disruption to normal follicular activity by interrupting normal LH secretion but it did not affect FSH secretion or its effects as well. It also caused reduced but prolonged secretion of oestradiol. While the ACTH caused a decrease in LH stimulation, the dominant follicle was maintained in all cows which in some cows, resulting in the creation of persistent follicles in which oestradiol secretion stopped prematurely. ACTH also affected the rate of progesterone decrease, this decrease occurred at a much slower rate thus taking an extra four days to decrease compared to the control cows (Dobson *et al.* 2000).

During chronic stress, like severe endometritis, the GnRH frequency will be so slow that while follicular growth occurs, the follicle is unable to be sustained in the later stage when greater pulse frequency is required. These cows will have shorter than normal oestrous cycles. Cows with less acute stress will have sufficient GnRH/LH pulses but will be susceptible to otherwise innocuous stimuli causing disruption to GnRH/LH pulses. These cows may also have impaired integrity of granulosa cells or the oocyte meaning they undergo oestrus and even fertilitisation may occur, but the conceptus is unlikely to develop further into a pregnancy. Another possibility is that the pulse frequency is sufficient for the follicle to develop but not fast enough for the pituitary to be primed by GnRH and/or sufficient oestradiol production. Thus these cows will have cystic follicles as the LH surge produced will not be sufficient to cause ovulation or lutenisation (Dobson and Smith 2000).

Glucocorticoids such as adrenocortoids, metabolic products of nutritional stress including beta-hydroxbutrate (B-OHB) and non-estrified fatty acids (NEFA) are likely to increase the susceptibility of the uterus to infection through their anti-inflammatory actions making them potent inhibitors of the immune system (Kruip et al., 2001). Torres *et al.* (1997) found that postpartum disorders including endometritis affected postpartum cortisol concentrations. The basal concentrations of cortisol in the first week for normal and abnormal cows (including purulent discharges, dystocia, RFM and milk fever) were 3.1 ± 0.9 ng/ml and 6.5 ± 1.9 ng/ml respectively. Furthermore, 42.9% of abnormal cows had elevated basal cortisol concentrations in the first week postpartum and 33.3% in the third week postpartum, compared to only 25% of normal cows with elevated basal cortisol concentrations during these periods (Torres *et al.* 1997).

Previous research into the effect of stress on reproductive outcomes found that there was no difference in first service conception rate, number of services per conception, the interval from calving to first AI or the interval from first AI to conception between cows with high levels of B-OHB (above 1 mM) and cows with normal levels of B-OHB (below 1 mM) (Kessel *et al.* 2008). However, stress disrupts normal follicular function and thus may affect reproductive efficiency in the dairy cow.

1.8 Periparturient diseases: their effects on ovarian activity

The length of the post-partum period is strongly influenced by periparturient disorders including dystocia, endometritis and mastitis (Morton 2000; Kask *et al.* 2000; Le Blanc *et al.* 2002). Cows with abnormal calving and those affected by puerperal disorders, are 3.6 and three times respectively, more at risk of a later postpartum resumption of ovarian activity. Cows with metritis are 11 times more likely to have a delayed resumption of ovarian activity postpartum than cows with normal puerperium (Opsomer *et al.* 2000).

Periparturient disease has been associated with a suppression of LH secretion and the consequent impairment of ovarian follicular growth. Chronic uterine infection and increased lipidpolysaccharide concentrations (LPS) produced by the uterine bacteria are associated with the disruption of LH surge and the failure of ovulation (Sheldon and Dobson 2004). In spontaneous uterine infections which are established within three weeks of parturition and before ovulation, high levels of bacterial contamination are associated with a reduced diameter of the dominant follicle. Suppression of folliculogenesis in the ipsilateral ovary decreases as the postpartum interval advances, the CL regresses, and bacteria are eliminated from the uterus (Sheldon *et al.* 2002).

Although most aspects of ovarian function are largely independent of uterine events, it is possible that uterine bacterial contamination could have a localised effect preventing dominant follicle selection on the ipsilateral ovary. This could be because the bacterial load or the inflammatory response can differ between uterine horns. The difference between horns may be because the inflammatory mediators reaching the ipisilateral ovary may be higher than the concentration reaching the contralateral ovary. Conversely, progesterone can suppress uterine immune defences with the formation of the CL postpartum often preceding the onset of uterine disease (Lewis 1997). There is some evidence that locally high concentration of progesterone in the tip of the uterine horn ipsilateral to the CL may benefit embryo survival in the normal cow (Rowson *et al.* 1970). Such locally high concentrations may also aid the survival of bacteria in the cow with a contaminated uterus (Rowson *et al.* 1970).

1.9 Uterine bacterial contamination

Uterine function is often compromised by bacterial contamination of the uterine lumen after parturition, whilst severe uterine infections can be detrimental to general health and to milk production (Konigsson *et al.* 2001). A wide variety of species of bacteria invade the uterus in the first two to seven weeks postpartum (Griffin *et al.* 1974a,b; Foldi *et al.* 2006). The composition of uterine bacteria fluctuates due to spontaneous contamination, elimination and recontamination (Griffin et al., 1974a,b).

It is important to differentiate between bacterial contamination and bacterial infection. Bacterial contamination is when bacteria are merely present in the uterus and bacterial infection is when the bacteria colonise the uterus. Uterine infection involves the adherence of pathogenic organisms to the mucosa, colonisation or penetration of the epithelium and/or release of bacterial toxins that lead to the establishment of uterine infections (Sheldon *et al.* 2006).

Most cows are able to eliminate bacterial contamination within five weeks, but pathogenic bacteria can persist causing uterine infection (Sheldon and Dobson 2004). Sheldon and Dobson (2004) found uterine contamination decreased from 78% of cows between Days 16 to 30, to 50% between Days 31 and 45. By Days

46 to 60, only 9% of cows still have bacteria present in their uteri. Both pathogenic and opportunistic bacteria cause inflammation and infection and delay uterine involution. The bacteria are able to persist because during parturition, the immune system is suppressed resulting in decreased migration and functionality of neutrophils (Sheldon 2004). The time taken for the elimination of bacterial contamination depends on the progression of uterine involution, the regeneration of endometrium and the effectiveness of uterine defence mechanisms (Sheldon *et al.* 2006).

During parturition and the puerperium, bacteria invade the cervical canal and the uterine cavity from the environment, skin and faeces. During the first ten days postpartum, *Streptococcus, Staphylococcus* and *Bacillus* are the most prevalent species (spp.) isolated (Huszenicza *et al.* 1999). Later on, *E .coli, Arcanobacterium pyogenes, Bacteroides* spp, and *Fusobacterium necrophorum* are commonly found in the uterus of cows, particularly those that have or will develop endometritis (Bekana *et al.* 1996; Dohmen *et al.* 2000). Dohmen *et al.* (1995) found that 65% of uterine swabs taken on Day 14 postpartum contained *A. pyogenes,* 77% contained *Bacteriocides* spp and 61% contained *F. necrophorum*. The prevalence of each bacterial species depends on timing of the sampling, and bacterial growth is generally greatest in the second week postpartum (Bekana *et al.* 1996).

1.9.1 ***Arcanobacterium pyogenes, Bacteroides spp. and Fusobacterium necrophorum***

There is an association between *A. pyogenes* and a higher degree of infiltration by lymphocytes, plasma cells and neutrophils in cows with endometritis (Del Vechio *et al.* 1994). This finding agrees with Dhaliwal *et al.* (2001) that endometritis was principally associated with *A. pyogenes* and Gram-negative anaerobes such as *F. necrophorum and Bacteriodes spp.* Gram-negative bacteria are rarely found alone in the uterus. Where *A. pyogenes* was isolated, *Bacteriodes* was also isolated in 89% of samples and *F. necrophorum* was also isolated in 70% of the samples (Dohmen *et al.* 1995). The presence of *E. coli* and LPS in lochia in the early postpartum favours the development of Gram-negative anaerobes and *A. pyogenes* (Dohmen *et al.* 2000). *Arcanobacterium pyogenes* and Gram-negative anaerobes lack the ability to penetrate intact epithelium to establish infection. However, *E. coli* damages the epithelium allowing the absorption of the endotoxins and the establishment of other bacteria. Stagnating lochia is a good medium for *E. coli* multiplication. *A. pyogene*s and Gram-negative anaerobes can then invade the endometrium, usually down to the level of the submuscosa, and sometimes even into the deeper layers (Paisley *et al.* 1986). *Arcanobacterium pyogenes* produces a growth factor for *F. necrophorum, F. necrophorum* produces a leukotoxin, and *B. melaninogenicus* produces a substance that prevents phagocytosis by bacteria (Paisley, *et al.* 1986). Mild irritation of the endometrium and inoculation of *A. pyogenes* and *B. melaniogenicus* at 12 and 36 hours after calving caused endometritis within 48 hours (El-Azab *et al.* 1988). The amount of cervical discharge and pus present is associated with the prevalence of *F. necrophorum, A. pyogenes and Bacteriodes* spp (Dohmen *et al.* 1995). The presence of *A. pyogenes* after Day 21 was almost always associated with severe endometritis (Griffin *et al.* 1974a; Lewis 1997). Isolation *of A. pyogenes* on Day 26 means that a cow was 2.7 times more likely to become a repeat breeder and isolation on Day 40 means the cow is five times more likely to be a repeat breeder. *A. pyogenes* and *F.necrophorum,* or *A. pyogenes, Bacteriodes* spp. and *F. necrophorum* in combination are almost invariably isolated from cases of pyometra (Farin *et al.* 1989).

The duration of the infection is an important determinant of the severity of its effects (Hartigan *et al.* 1974). Transient infection immediately postpartum does not affect fertility but if infection occurs later postpartum, it can significantly reduce fertility. The presence of *A. pyogenes* in the first three weeks will reduce fertility to first service; however, if infection is eliminated quickly then fertility will not be affected in subsequent services (Hartigan *et al.* 1974). Cows with severe endometritis have a significantly longer period of persistent infection and greater number of Gram-negative anaerobes isolated than cows with mild endometritis (Mateus *et al.* 2003).

Immediately after calving, cows with RFM have a higher concentration of LPS in uterine lochia than healthy cows do and those with uncomplicated dystocia. High LPS concentration is associated with abnormal cervical discharge. Some cows have more efficient detoxification processes, which may help to explain why some cows get sick and others do not (Dohmen *et al.* 2000).

1.9.2 *Endotoxins produced by uterine bacteria and their effects on uterine involution*

Uterine bacteria release endotoxins which are absorbed through the uterine wall. Plasma endotoxins levels peak between Days one and 12 postpartum and remain low until Day 27. Endotoxins are potent inducers of prostaglandins and cytokine secretion which, in turn, enhance the development of endotoxins (Dohmen *et al.* 2000). Endotoxins stimulate the phospholipaseA_2, cyclooxygenase-2 and 5-lipoxygenase enzymes systems which produce prostaglandins (Foldi *et al.* 2006). The prostaglandins then mediate their effects on the reproductive tract including negatively affecting uterine involution and the resumption of ovarian activity.

Endotoxin concentrations are proportional to bacterial numbers. Cows with RFM and dystocia have high concentrations of endotoxins (Dohmen *et al.* 2000). There is a linked relationship between uterine infection, endotoxin production and resumption of ovarian activity (Mateus *et al.* 2003). Endotoxins induce proinflammatory cytokines including tumour necrosis factors and interleukins (Foldi *et al.* 2006). Endotoxins also provide a positive chemotatic signal to leukocytes, activating them and enhancing their migration from the blood into the uterus. This signal is particularly strong in cows with severe endometritis (Mateus *et al.* 2003).

The actual process of how the endotoxins are absorbed and enter the blood stream is poorly understood. Dohmen *et al.* (2000) could not detect endotoxin in the plasma of cows (normal puerperium, RFM and dystocia) but found significantly increased IGF anti-LPS concentrations suggesting that either the endotoxins were not being absorbed or had already been detoxified. In contrast, Mateus *et al.* (2003) detected endotoxins in some cows with mild endometritis and in all cows with severe endometritis. The concentration of endotoxins was much higher in the uterine fluid than in the corresponding plasma, which may account for the difference between the two findings.

Direct cytotoxic effects favour the establishment of bacterial infections (Dohmen *et al.* 2000). On Day seven, 75% of cows had high numbers of uterine pathogens, with the majority being *E. coli.* In these cows, there is also a reduced ovarian follicle growth with the resulting dominant follicle taking longer to mature and producing less oestradiol. The resultant CL was much smaller and produced less progesterone (Williams *et al.* 2007). The likely effects on fertility are a reduced incidence of oestrous behaviour which may be missed by the farmer and an 'unhealthy' CL which does not produce enough progesterone to sustain pregnancy.

One of the ways *E coli* mediates its effects is through the secretion of an endotoxin, lypopolysaccharide (LPS). Peter *et al.* (1989) found the administration of intravenous LPS to the uterus disrupted the pre-ovulatory surge of LH. It has also been observed that it inhibits peripheral oestradiol concentrations in response to LH episodes (Xiao *et al.* 1998).

1.10 Repeat breeder syndrome

On a New Zealand dairy farm, repeat breeders are cows that require more than three inseminations to become pregnant (Ayalon 1974). Failure of the cow to conceive within the 12 to 14 week mating period means that either the cow is culled or is carried over to the next mating period (McDougall 2006). This leads to increased costs, due to an increased number of services, an increased calving to conception interval and higher veterinary costs. It also means less days in milk per cow, increased culling, and a reduced number of possible replacement heifers which reduces the potential genetic gain of the herd (Bartlett *et al.* 1986; Bosberry and Dobson 1989; Dohmen *et al.* 1995; Archbald *et al.* 1998; Le Blanc *et al.* 2002). Repeat breeders are likely to have been so in the previous lactation therefore it is probable that these cows are inherently sub-fertile (Gustafsson and Emanuelson 2002). Many repeat breeders have sub-clinical endometritis but no overt infection (Moss *et al.* 2002).

1.11 Metritis, Pyometra and Endometritis and their immune responses

The immune response to bacterial infection is characterised by an influx of neutrophils into the uterus and increased acute phase proteins (Hearth *et al.* 2006). An increased number of polimorphonuclear neutrophils (PMNs) are present in the uterine lumen (Kasimanickam *et al.* 2004). Uterine neutrophils also increase over the last 15 days of pregnancy with the number of cells therefore decreasing at calving and then increasing in the first 14 days postpartum (Saad 1989; Lewis 1997). A decrease in phagocytising –PMNs in the first week postpartum is likely to be associated with an increased susceptibility to postpartum uterine infection (Mateus *et al*. 2002).

Metritis is an acute systemic illness that usually occurs within ten days after parturition. Metritis involves inflammation of all layers of the uterus with evidence of odema, infiltration by leukocytes and myometrial degeneration (Sheldon *et al.* 2006). Cows with metritis generally have a fetid red-brown watery uterine discharge, an abnormally large and tonic uterus and a systemic illness due to endotoxaemia and have a markedly reduced milk yield (Sheldon *et al.* 2006). Vaginal discharge becomes fetid at about three to four days after parturition in severe cases, and in milder cases between six to ten days (Foldi *et al.* 2006). The degenerative and infiltrative processes lead to excessive damage to luminal and glandular epithelium. Metritis is associated with dystocia, retained foetal membranes and stillbirths at the preceding calving, and will substantially impair fertility thereafter (Morton 2000).

In cows with metritis, the prepartum increase in neutrophil numbers and function is reduced and the postpartum increase was attenuated (Lewis 1997). In cows with endometritis, inflammatory cells infiltrate the endometrium and the superficial epithelium can become desquamated and necrotic. The endometrium becomes congested with a large infiltration of neutrophils, plasma cells and lymphocytes. The uterine glands can become cystic, sometimes with scar tissue replacing the endometrium (Lewis 1997). Increased inflammation of the stratum compactum and other areas of the epithelium, and reduced numbers of lymphocytic foci are associated with better reproductive performance. In contrast, cows with increased areas of intact non-sloughed epithelium are at greater risk of cows developing Repeat Breeder syndrome (Bonnett *et al.*1993).

Pyometra is an accumulation of purulent and mucopurulent material within the uterine lumen, and a closed cervix. There is always an active CL. The wall of the uterus is thick and doughy (Sheldon *et al.* 2006). Pyometra usually starts to develop about ten days after the first ovulation postpartum (Farin *et al.* 1989). Dohmen *et al.* (2000) suggest that *A. pyogenes* and Gram-negative bacteria work synergistically to generate pyometra during the luteal phase of the oestrous cycle. However, pyometra is rarely seen in New Zealand.

Cows with pyometra have high numbers of bacteria, neutrophils, and an ulcerated endometrial surface coated with mucus. Lymphocytes, plasma cells and neutrophils are scattered and aggregated in deep lamina. On Day 13, the epithelial cells are large and surrounded by neutrophils (Dohmen *et al.* 2000). The endometrial glands are decreased, and the stroma is necrotic and filled with lymphocytes, plasma cells and neutophils, and inflammatory exudates accumulate. In 70 to 75% of cows, the exudate can spread to the uterine tubes (Lewis 1997).

Endometritis only involves the inflammation of the endometrium with inflammation no deeper than the stratum spongiosum (Sheldon *et al.* 2006). Clinical endometritis is characterised by a purulent (more than 50% pus) discharge after 21 days or mucopurulent discharge after 26 days, but is not associated with systemic illness (Le Blanc *et al.* 2002; Sheldon *et al.* 2006). It is characterised by degenerative changes to the surface epithelium, vascular congestion with stromal oedema, migration of neutrophils, granulocytes and other inflammatory cells to the endometrium (Foldi *et al.* 2006). The purulent discharge is derived from perished neutrophils, phagocytised bacteria and tissue debris (Foldi *et al.* 2006). It is often associated with a cervical diameter larger than 7.5 cm at 21 days postpartum (Le Blanc *et al.* 2002).

Subclinical endometritis is a major cause of infertility in the dairy cow. It is characterised by cervical discharges that are not overtly purulent, but contain significant numbers of neutrophils (Sheldon *et al.* 2006). The presence of intra-uterine fluid is another characteristic of subclinical endometritis (Archbald *et al.* 1998). Subclinical endometritis usually occurs as a result of an abnormal postpartum event such as RFM (Bosberry and Dobson 1989). Cows with greater than 18% neutrophils between Days 21 and 33, or greater than 10% neutrophils between Days 24 and 37 postpartum in cervical swabs are considered to have subclinical endometritis (Sheldon *et al.* 2006).

The age of the cow affects the incidence of endometritis. Le Blanc *et al.* (2002) found that cows have a 12 and 13% incidence of endometritis in their first and second lactations while cows in their third lactation or later have a 21% incidence of endometritis. These figures are likely to be higher than in New Zealand cows for a number of reasons. Firstly, the cows in that study were raised inside in barns whereas New Zealand cows are raised in pastoral conditions and will likely have fewer opportunities for infection to occur. The herd size in that study (30 to 230 cows) was much smaller than the average herd size (342 cows) in Manawatu, New Zealand (Louise Cook, personal communication. Also the genetics of the Canadian cows (that study) and New Zealand cows are quite different and may perhaps affect their susceptibility to uterine infection (McDougall 2006). Dohmen *et al.* (1995) found that 20% of cows had discharge with flecks of pus, 29% had purulent discharge, 45% had mucopurulent discharge and 7% had a fetid discharge. The amount of cervical discharge and pus is associated with the prevalence of *F. necrophorum* and *A. pyogenes (*Dohmen *et al.* 1995*)*.

Cows with a longer duration of infection or inflammation have a higher risk of impaired reproductive performance. Uterine infections increase the days to first oestrus by seven days and cows require 0. 31 extra services (Coleman *et al.* 1985). High levels of PMNs or fluid in the uterus were associated with reduced fertility. Uterine fluid volume remains high with severe puerperal infection compared to mildly infected and control cows (Mateus *et al.* 2002; Kasimanickam *et al.* 2004). Cows with a normal puerperium and cows with endometritis have similar uterine body and intrauterine fluid volumes by the sixth week postpartum (Mateus *et al.* 2002). Cows with peripartum reproductive events such as dystocia and RFM were 3.15 times more likely to have high PMN or fluid in the uterus on Days 20 to 35 and 3.18 times more likely between Days 34 to 47 postpartum (Kasimanickam *et al.* 2004).

Cows with purulent or fetid discharges between Days 20 and 26 postpartum had a 17% decrease in pregnancy rate compared to cows with no discharge or clear mucus. The presence of a mildly purulent uterine discharge in the first month postpartum is likely to indicate a successful immune response by the cow to bacterial contamination (Kasimanickam *et al.* 2004).

Le Blanc *et al.* (2002) found that apparently normal cows performed better than untreated controls indicating that many cows that were given intrauterine antibiotics during the postpartum period are likely to have an undiagnosed uterine infection. These infections may be occurring in New Zealand cows causing a number of cows to be subfertile (McDougall and Murray, 2000).

1.12 The effect of postpartum disorders on the development of uterine infection

Dystocia, twins and RFM predispose the uterus to postpartum disorders due to uterine inertia and the impairment of neutrophil function. Mellado and Reyes (1994) found that most peripartum reproductive disorders increase the interval from parturition to first service by between 4.4 to 9.2 days. Other authors have made similar findings (Fonscesa *et al.* 1983; Coleman *et al.* 1985).

In cows without uterine infections, the average number of days from calving to first ovulation was 20.4 days; 55 to 65% of cows will conceive to their first service, 75% will be pregnant within six weeks and about 90% by the end of the breeding season (Fonseca *et al.* 1983). Cows with dystocia during the preceding calving had a pregnancy rate of 82.5% Cows with RFM take 25 days longer to conceive, whilst those cows that develop endometritis take 51 days longer (Coleman *et al.* 1985; Kim and Kang 2003). Cows with RFM increase the number of days between parturition to conception from 14.3 to 21.4 days. Cows with RFM are 4.7 times more likely to develop metritis than cows with uncomplicated calvings (Mellado and Reyes 1994). Cows with RFM needed an extra 1.2 services per conception, whilst those cows with dystocia needed an extra 1.8 services per conception (Kim and Kang 2003). Coleman *et al.* (1985) found that difficult calvings require an extra 2.33 services per conception.

The pregnancy rate 28 days after the planned start of mating and the final pregnancy rate were reduced by RFM. McDougall (2001) found that 1.5% of the herd have RFMs. The pregnancy rate for RFM is 70.9% compared to 93.9% in cows that experienced normal third stage labour. Kim and Kang (2003) found that the average interval from calving to first service and calving to conception is delayed by 23 days and 36 days respectively by endometritis. Le Blanc *et al.* (2002) found that cows with endometritis required 10% more inseminations than cows without endometritis. Cows with endometritis are 1.3 to 1.7 times more likely to be culled for reproductive failure (Lewis 1997; Le Blanc *et al.* 2002). This means the farm is likely to be incurring unnecessary costs. On the other hand, if infection is detected early, these cows can be treated and conceive during the normal mating period.

Cows with delayed involution are often those that have had a short-term uterine infection or some other factor which the animal is quickly able to eliminate. Archbald *et al.* (1998) found that there was no difference between cows with normal uterine involution and delayed uterine involution in services per conception and days per conception. However, the final pregnancy rate was higher for those cows with normal uterine involution suggesting that the delayed uterine involution (likely to be cows with transient infections which have been resolved) increases pregnancy loss. The final pregnancy rate was 47.6% for cows that suffered from endometritris compared to 63.6% in control cows (Morton 2000; Xu and Burton, 2000).

Cows that calve late have a pregnancy rate of 54.1%, 28 days after the planned start of mating compared to 68.1% in the rest of the herd (McDougall 2001). The problem with calving later is that cows have a much shorter period of time in which to undergo involution before the start of mating, resulting in cows being mated while they are still undergoing involution. These cows are not infrequently culled for failure to conceive because their uterus was not at the stage where it was able to carry a pregnancy at the start of mating. This means that they either do not become pregnant or they suffer an early embryonic loss of pregnancy (Stevens 2000). In New Zealand, mating subfertile cows is a major cause of unnecessary culling and is also a waste of both time and resources.

Cows with previous calving difficulties are five times more likely to have calving difficulties at subsequent parturitions. Cows with retained foetal membranes are 5.5 times more likely to retain their membranes at subsequent parturitions; cows with uterine infections are 1.8 times more likely to have another uterine infection after subsequent parturitions (Coleman *et al.* 1985). However, other authors found

RFMs do not predispose cows to future occurrences of RFMs at their next calving (Roberts 1986)

The consequences of sub-clinical endometritis lead to management decisions that cause culling and reproductive failure (Kasimanickam *et al.* 2004). Subclinical endometritis occurs often in cows that are assumed to be clinically normal. Subclinical endometritis could currently be having a major effect on the dairy industry in New Zealand (Xu and Burton 2000).

1.13 The effect of milk production on fertility

There is strong evidence that high milk production negatively affects fertility of the dairy cow (Lucy 2001a). However, the evidence from research on the effect of milk production on fertility is conflicting. Some researchers have found a correlation between high milk production and low fertility; but others have found no such evidence (Harrison *et al.* 1990; McMillian *et al.* 1996; Grosshans *et al.* 1997). However, the effect of milk production on fertility is less clear in pastoral systems (McMillian *et al.* 1996; Xu and Burton 2000, McDougall 2006). The association between high milk production and lower reproductive outcomes are compounded by the effects of management and herd size (McMillian *et al.* 1996).

Throughout the world, there has been a decline in reproductive efficiency as shown by the increase in the intervals of calving to first AI, calving to first ovulation and conception rate to first insemination over time, while over the same period milk production has greatly increased (McMillan *et al.* 1996; Roche *et al.* 2000).The interval from calving to first ovulation has increased over time, in the 1970s, it was between 14 and 21 days postpartum (Marion and Grier 1968; Morrow *et al.* 1966). It is interesting to note that deVries and Veerkamp (2000) found that the mean interval to first ovulation was 29.7 but the mode was 16 which was similar to the interval in the 1970s (Grier and Marion 1968).

Higher milk production has been linked to the later onset of oestrus behaviour (Harrison *et al.* 1989; Royal *et al.* 2000; Verkerk *et al.* 2000). High producing dairy cows particularly those with a high percentage of overseas genetics (high breeding value for milk production) can ovulate without exhibiting oestrus behaviour (McMillian *et al.* 1996). The number of days from visual oestrus (66 vs 43 days) and the number of ovulations (1.6 vs 0.7) before the first visual oestrus were higher for cows that had high milk production than for those cows that did not (Royal *et al.* 2000). By Day 75, only 50% of the cows with high milk production had showed a visual oestrus but all the cows with average milk production had shown a visual oestrus. Optimal uterine involution is associated with early and frequent occurrences of oestrus behaviour leading to increased reproductive performance. Harrison *et al.* (1989) found that many of the metabolic differences occurring between cows with different levels of milk production occur before oestrus is visible.

However, Harrison *et al.* (1990) found no association between uterine involution and milk production. There were no significant differences between cows with high or average milk production in the interval from parturition to uterine involution, and days to first ovulation between cows with high or average milk production (Harrison *et al.*1990). This supports the theory that milk production negatively impacts on the expression of oestrous behaviour but not on ovarian functions.

While there is an antagonistic relationship between milk production and fertility, many authors believe it is minor compared to effects of other factors such as postpartum diseases including endometritis and postpartum nutrition (Lucy 2001a; McDougall and Murray, 2001). The degree of negative energy balance helps determine to what extent high milk production negatively impacts on fertility (Lucy, 2001a,b). The severity of negative energy balance and the delay in resumption of normal postpartum cycling is associated with reduction in body weight and body condition loss. Butler and Smith (1982) found a negative correlation between average energy balance during the first 20 days postpartum and days to first ovulation. The increase in the average interval to first ovulation in modern dairy cows may be caused by a subpopulation of sub-fertile cows. It is possible that these cows are in a greater negative energy balance perhaps because of their high percentage of overseas genetics and their high milk production. While, deVries and Veerkamp (2000) found that only 3 or 4% of the variation in the interval could be explained by total energy deficit; this is in conflict with the research conducted (Lucy 2001a; Wathes *et al.* 2007).

Wathes *et al.* (2007) found clear evidence that a period of severe negative energy balance adversely affects the future fertility of the cow. It may be that it is in fact the degree of negative energy balance rather than high milk production itself that is the reason for lower fertility. Often, high producing cows are the cows that are in severe negative energy balance which is why high milk production is often associated with poor fertility. Most studies only found the negative effect of milk production on fertility at high production levels. However, high producing cows are necessarily those with the greatest negative energy balance or the lowest body condition. Higher producing herds can actually have healthier cows than lower producing herds, because their feeding and reproductive management is superior.

1.14 Conclusions

The process of involution involves the restoration of normal follicular activity, the shrinking of the genital tract back to pre-pregnancy size and the elimination of bacterial contamination. Cervical and uterine involution is usually completed by Days 40 to 60 postpartum. The immune system plays an important role in the progression of involution. Cervical involution is a good indicator for the progression of involution. Cows with a large cervical diameter on Day 28 (greater than 6 cm) commonly have a longer anoestrous period than with cows with a cervical diameter less than 6 cm.

Endometitis, metritis and pyometra are all forms of uterine infection. Uterine infection prolongs the interval from calving to first ovulation, the interval from calving to conception and increases the number of services per cow. Repeat breeders are cows that require three or more services. These cows often have undiagnosed subclinical endometritis and cause unnecessary expenditure on extra services, veterinary costs, lost income from milk and culling due to infertility. Periparturient disorders including dystocia, RFM and twins can often prolong the anoestrus period and predispose these cows to uterine infection.

Bacterial contamination of the uterus occurs during parturition and until the third week postpartum with most bacteria eliminated by Day 28. In the uterus, there is a continuous cycle of the elimination of bacteria and spontaneous recontamination particularly in the 2nd week postpartum. The main bacteria found during this period are *A. pyogenes, Bacteroides* and *F. necrophorum*. These bacteria and their endotoxins work syngestically to provide an environment conducive with uterine infection. Subclinical endometritis is likely to be important cause of subfertility.

Organic dairying focuses on prevention of infection and maintaining high hygiene standards rather than treatment of diseases and illness. Organic dairy cows cannot be treated with conventional medicines such as antibiotics and their pastures cannot be treated with commercial fertilisers such as superphosphate. Pasture growth can be less than on conventional farms as nitrogen cannot be applied therefore milk production may be reduced. The emphasis on high quality shelter, races and yards reduces stress. Stress has a negative effect on the immune system therefore unstressed cows are less prone to illness and disease. It is therefore proposed that Organic dairying does not adversely affect the key parameters of production particularly lactation and reproduction

This research will examine issues relating to uterine bacterial contamination, cervical diameter, cortisol concentrations and their relationship on subsequent fertility, in both conventional and organic dairy cows.

2 Methods

2.1 Study Design

This study was a cohort perspective observational study of the relationship of postpartum uterine bacteria, cortisol concentrations and cervical diameter on subsequent reproductive outcomes. This study also investigated the effect of management systems on postpartum uterine bacteria, cervical diameter and cortisol concentrations. Finally this study compared milk yield, cumulative milk yield, milk solids and somatic cell counts between the management systems. The study was conducted during the 2006/2007 milking season (3 July 2006 to 1 June 2006). Uterine bacteriology and blood sampling were undertaken between 3 August and 13 October 2006.

2.2 Farm and Herd Characteristics

The two herds were located on the Dairy Cattle Research Unit, Massey University (Palmerston North). In August 2001, the farm was divided into two farmlets, with one farmlet beginning the conversion to organic status, whilst the other farmlet remained under conventional management. The cows were divided evenly into two herds, based on breeding worth, production worth, somatic cell count, age and liveweight. The Organic farmlet achieved its certification from AgriQuality in August 2003. Each farmlet was managed independently according to "best practice" for its particular management system and environmental conditions.

The Organic farm was 20.4 ha and the Conventional farm 21.3 ha (including the quarantine area). The two herds were kept completely separately on different areas of the farm with the Organic cows using the milking shed first. At the time of sampling, there were 41 Organic and 45 Conventional cows.

During the 2006/2007 milking season, the organic herd produced 347 kg MS/cow and 792 kg MS/ha and the conventional herd 379 kg MS/cow and 942 kg MS/ha. The average number of days in milk was 250 for the organic herd and 269 days for the conventional herd. The Organic pasture production was 8.8 t DM/ha, compared to 9.5 t DM/ha on the conventional farmlet. The average stocking rate was 2.2 cows/ha for the organic farmlet and 2.4 cows/ha for the Conventional farmlet.

2.3 Selection criteria

A total of 37 cows managed in the organic farming system ('Organic cows') and 38 conventionally-managed cows ('Conventional cows') were selected for this study. These groups contained all of the cows that calved before 15 September.

2.4 General Reproductive Management

Both herds of cows were spring-calving, with a planned start of calving of 27 July 2006. The mean calving date of the organic herd was 21 August and the mean calving date of the conventional herd was 19 August. The AI mating period of six weeks began on 26 of October. The bulls were run with cows from 6 December 2006 until 12 February 2007. All insemination dates and heats after the start of planned start of mating were recorded. All cows were pregnancy tested by palpation *per rectum* in February 2007.

The Dairy Cattle Research Unit did not induce any cows, nor were any hormones used to treat anoestrus cows.

2.5 Observation and Sampling Regimen

Previous studies have shown that similar cytology and histological results from cervical and uterine swabs (Ahmadi *et al.* 2005; Yavari *et al.* 2009) therefore in this study, cervical swabs are considered to be representative of the cytology of the uterus postpartum Cervical swabs were collected for bacteriology on Days seven, ten and 28 after calving (Dauod, 2008). On each occasion, the tail was tied to the side and the perineal area was cleaned. It was first wiped with disposable paper towels, and then was thoroughly cleaned with disinfectant and warm water. The perineal area was dried with another disposable paper towel. The vulval lips were parted by an assistant and a 40 cm disposable guarded swab (Continental Plastics

Corp, Delavan, Wisconsin, U.S.A) inserted. The disposable guarded swab is composed of sterile cotton tip that is attached to a rod which is enclosed by an inner sheath and finally an outer protective sheath. A hand was inserted into the rectum to guide the rod into the cervix. The guarded swab was inserted into the vagina and guided to the cervix per rectum. Once inside the external os of the cervix, the rod was pushed out of its protective guard and rotated to ensure that the swab was well-covered in cervical secretions. The swab was then retracted into the outer protective sheath and withdrawn from the cervix and vagina. The cervical swab was cut using sterile scissors into transport media (Thyoglocate broth, Fort Richards, Auckland, New Zealand) and transported back to the laboratory for microbiological culture.

The diameter of the cervix was assessed by palpation *per rectum*. The width of the cervix was estimated by hand and previously correlated to a ruler previously as a means of calibrating the estimates that were made.

Blood samples (lithium heparin anticoagulant) were taken by caudal venepuncture for the measurement of plasma cortisol concentrations. The plasma was separated by centrifugation at (1000 g) for ten minutes. The plasma was aliquoted into separate containers and stored at -20℃ until assayed.

Body condition score was assessed by an experienced technician as in MacDonald and Roche (2004) in Week one and Week four postpartum. Milk data was collected using Westfalia Surge megatrons and the data was then entered into DairyPlan database. The somatic cell counts were taken during the LIC monthly herd tests and the data was entered into DairyPlan. The data was retrieved from DairyPlan at a later date.

2.6 Microbiology

All swabs were inoculated onto microbiological culture plates within two hours of collection. After vortex sampling, a 100 µl aliquot of the transport media (thyoglocolate broth) was pipetted into a sterile jar. A second aliquot (10 µl) was removed from the transport media and placed in a further sterile jar, after which 900 µl of peptone water was added and the aliquot vortexed. Validation of this method was performed using serial dilutions and comparable results were found.

A spiral plater (WASP2, Don Whitley Spiral Scientific Limited, Shipley, U.K) was used to plate 100 µl of aliquot onto an agar plate. For each aliquot, a Fastidious Anaerobe agar plate (Fort Richards Laboratories, New Zealand) was inoculated for anaerobic incubation and a sheep blood agar plate (Fort Richards Laboratories, New Zealand) was inoculated for aerobic incubation. Anaerobic plates were placed in an anaerobic jar with an anaerobic envelope (AnaeroGen, Oxoid Limited, U.K). Both aerobic and anaerobic plates were then incubated for five days at 37˚C. Counting of the bacterial colonies was undertaken on the incubated plates. The number of colonies was calculated in Colony Forming Units for 1 ml sample (CFU/ml). The colonies were counted using a colony counter (Whitley Spiral aCOLtye Supercount, Don Whitley Spiral Scientific Limited, Yorkshire, England). The species of bacteria were not identified.

2.7 Radioimmunology of Cortisol

Samples were assayed in duplicate against a standard curve made up in charcoal-stripped serum. Samples (24µl) were incubated with 1000 µl of iodinated cortisol (20,000 cpm) in anti-cortisol coated tubes (^{125}I-cortisol and tubes, ImmunChem™ Coated Tube Cortisol ^{125}I RIA kit, MP Biomedicals, USA) for 45 minutes at 37°C, then the supernatant was aspirated off. The pellets were counted on a PerkinElmer Wallac 1470 Automatic gamma counter for 5 minutes each.

The crossreactivity of the cortisol antibody with other steroids was tested by MP Biomedicals. Cross-reactions are as follows: prednisolone (45.6%), 11-desoxycortisol (12.3%), corticosterone (5.5%), prednisone (2.7%), cortisone (2.1%), 17α-hydroxyprogesterone (1.0%), progesterone (0.25%) and dexamethasone, dihydrotestosterone and testosterone (<0.10%).

A serial dilution of plasma in charcoal stripped serum was parallel to the cortisol standard curve. The quantitative recovery of cortisol was measured by adding different amounts of standard cortisol to three plasma samples. The recoveries of added cortisol were 96.2 ± 3.4%, 97.4 ± 7.4% and 96.0 ± 5.3%.

2.7.1 ***Assay Sensitivity***

The sensitivity of the cortisol assay was the minimum hormone level that could be consistently distinguished from zero. It was determined as the hormone concentration at the mean two standard deviations from zero hormone point on standards on the standard curves. The assay sensitivity, expressed as µg steroid/dl plasma, was 0.42 µg/dl.

2.7.2 ***Intra- and inter-assay variation***

Sheep plasma samples that gave approximately 70%, 50% and 30% binding on the standard curve were used as low, medium and high quality controls in four assays. The mean concentrations of cortisol in these solutions were 5.37± 1.15, 13.4± 2.0 and 30.6± 4.9 µg/dl respectively (n=4). The intra-assay coefficient of variation for each solution was determined by a formula using a sum of squares of beginning and end of assay quality controls. The intra-assay coefficients of variation for cortisol were 23.5%, 14.5% and 12.1% for low, medium and high solutions respectively (n=4).

2.8 Statistical Analysis of Data

From the records of reproductive events, the following indices were calculated:

- Interval from calving to first observed oestrus
- Interval from calving to first recorded insemination
- Interval from the start of mating to the first recorded insemination
- Interval from calving to conception. This was calculated as the interval between calving and the last recorded date of insemination or natural service, where this was subsequently confirmed by manual diagnosis of pregnancy per rectum.
- Interval from the start of mating to conception. This was calculated similarly to the previous index.
- Number of services per conception. All recorded insemination and natural service matings were included. If the last recorded mating date did not agree with the gestational age of the cow at pregnancy diagnosis, an additional mating date was generated and added to the cow's record.

Data from cows that failed to conceive was excluded from this index and cows that were culled were assumed to have conceived to their last service.

Statistical analyses were carried out using Genstat 5 (Lawes Agricultural Trust, Rothamsted, UK).

Data were subject to an analysis of variance with respect to time (days after calving) and management system, in a repeated measures model, in which individual animals were nested within treatment. Data that were not normally distributed were normalized by $\log_e$ transformation prior to analysis. Simple regression, stepwise regression and principal component anaylsis were also carried out between lactational and reproductive parameters, cortisol concentrations, cervical width and bacterial contamination .

One cow was euthanised and two cows were culled in the middle of the sampling period. Their data was used for analysis of uterine bacterial contamination and cortisol concentration but was excluded from the reproductive data analysis

2.9 Ethics Approval

All procedures using animals were approved by Massey University Animal Ethics Committee.

3 Results

3.1 Age Structure

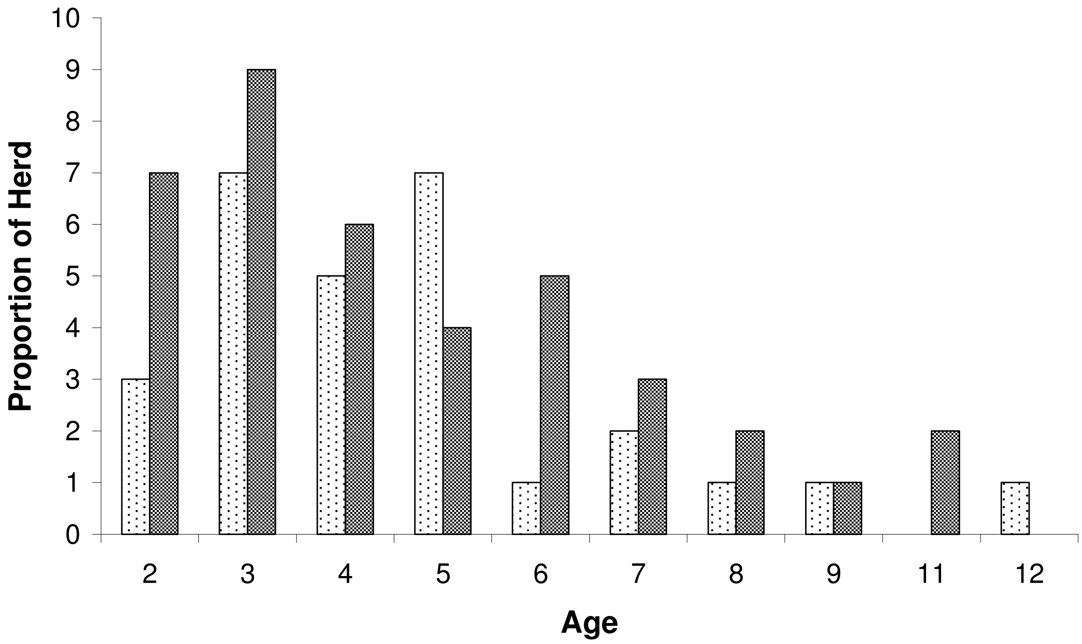

Figure 3.1: Age structure of Organic and Conventional herds

There was a tendency for the Organic herd to have younger cows, 34% of Organic herd were two year olds opposed to 19% of Conventional herd and for the Conventional herd to have a higher proportion of middle aged cows (6, 7 and 8 year olds (Figure 3.1).

3.2 Production Data

3.2.1 *Condition Score*

There was no significant difference in the body condition score of Conventional and Organic cows, in either Week one (Day seven and Day ten; (p=0.604) or Week four (Day 28; p=0.449) (Figure 3.2). The mean body condition score (1-10 scale) in

Week one was 4.10 (± 0.04) and 4.14 (± 0.05) for the Organic and Conventional cows respectively; in Week four, the mean body condition score was 3.9 (± 0.06) in the Organic herd and 4.0 (± 0.06) in the Conventional herd.

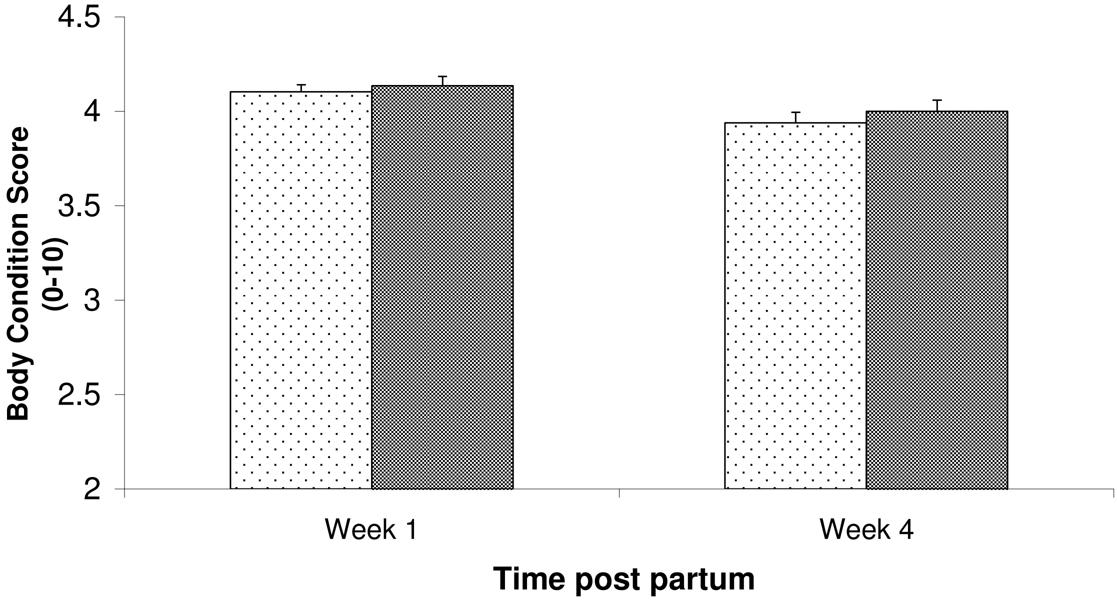

Figure 3.2: Mean (+ s.e.m) body condition scores for Week one and Week four postpartum for cows managed in Organic and Conventional farming systems. □ Organic ▩ Conventional

3.2.2 ***Milk yield***

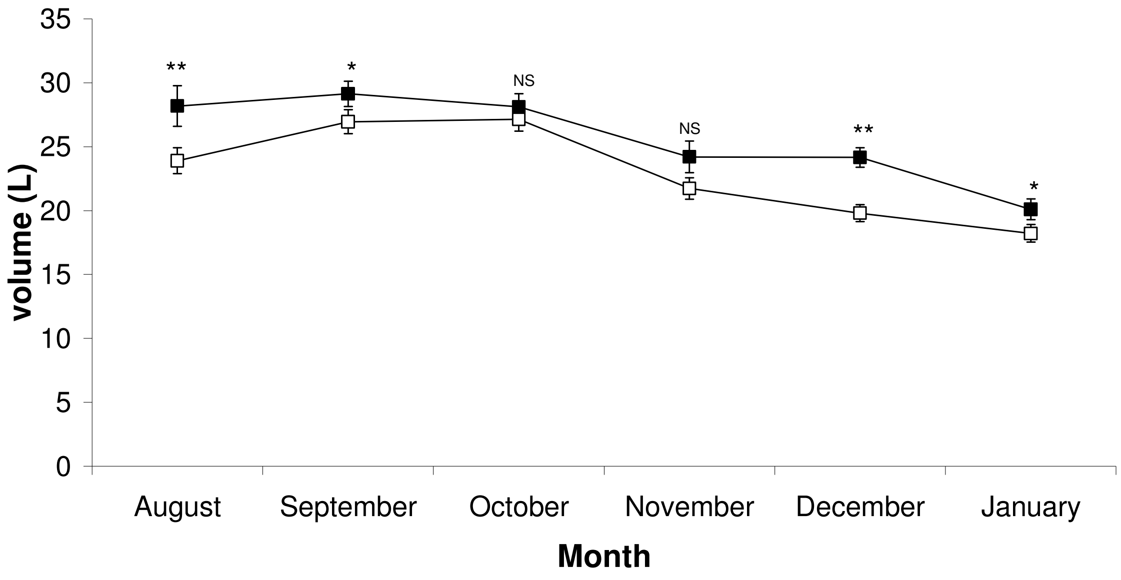

Figure 3.3: Mean (+ s.e.m) individual milk yield (L) for cows managed in Organic and Conventional farming systems between August 2006 and January 2007. Differences between means within months are significant where indicated (*P<0.05, ** P<0.01). conventional organic

Organic cows produced significantly less milk than Conventional cows (P=0.005; Figure 3.3). Days in milk had a significant effect on milk yield (P<0.001). After correction for this effect, there was an even greater difference in milk yield over time between Organic and Conventional cows (P<0.001). The peak milk yield occurred in September (26.95 + 0.95 L; 29.13 + 1L) with a decrease in milk yield after this month, the Conventional cows showed a slower decrease in milk yield, with values remaining above those of Organic cows during Dec (P<0.01) and Jan (P<0.05).

3.2.3 ***Cumulative Milk Yield***

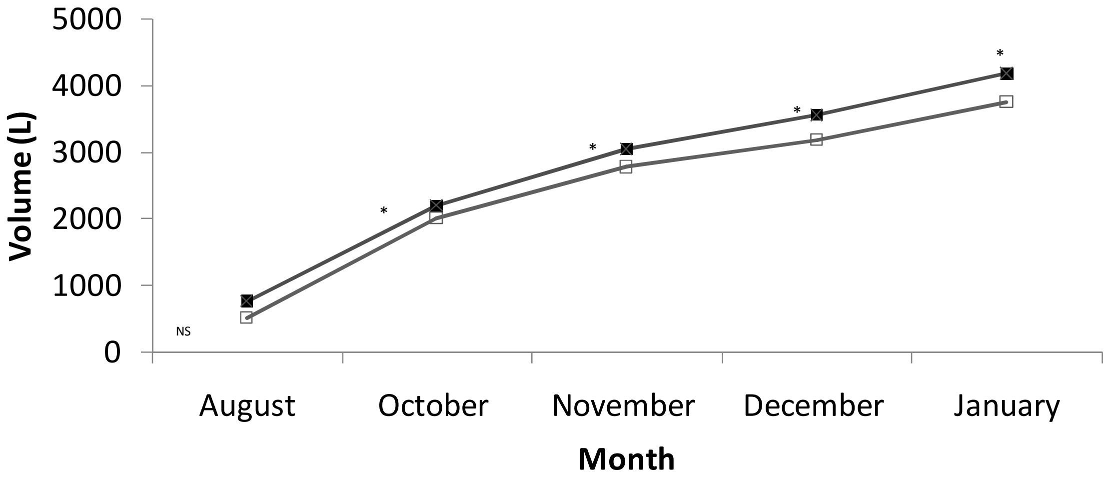

Figure 3.4 Mean (+ s.e.m) individual cumulative milk yield (L) for cows managed in Organic and Conventional farming systems between August 2006 and January 2007.Data from September 2006 were not supplied. Differences between means within months are significant where indicated (*P<0.05, ** P<0.01).

—■— conventional —□— organic

Similarly, cumulative milk yield was higher in Conventional cows (P=0.014; Figure 3.4). As expected, days in milk had a significant effect on cumulative milk yield (P<0.001); cumulative milk yield, corrected for days in milk. This was lower in the Organic cows (P<0.01). The Conventional cows had a significantly different pattern of increase in cumulative milk yield to Organic cows. (P=0.006).

3.2.4 ***Milksolids***

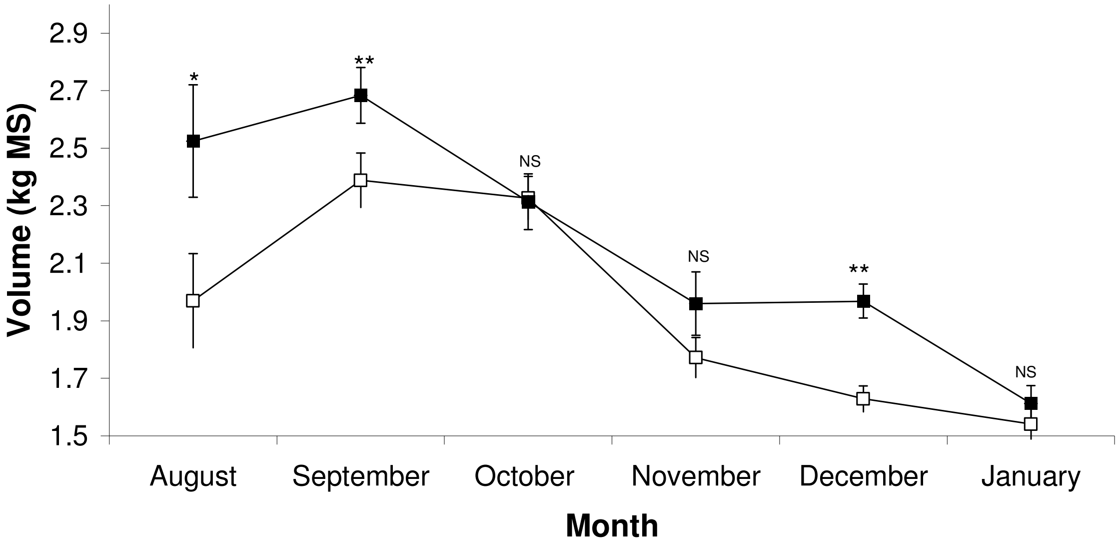

Figure 3.5 Mean (+ s.e.m) milksolids production for cows managed in Organic and Conventional farming systems. Differences between means within months are significant where indicated (*P=<0.05, ** P=<0.01). conventional organic

Overall, Conventional cows produced more milksolids than Organic cows (P=<0.001; Figure 3.5). The Conventional cows had higher milksolids production in every month between August 2006 and January 2007 except in October. Milksolids production when adjusted for days in milk (P=<0.001) was significantly lower in Organic cows (P=<0.001). Moreover, Conventional and Organic cows showed a different pattern of change in milksolids production throughout the year (P=<0.01), with the Conventional cows having high milksolids early postpartum with a slower decrease in milksolids production.

3.2.5 ***Somatic cell counts***

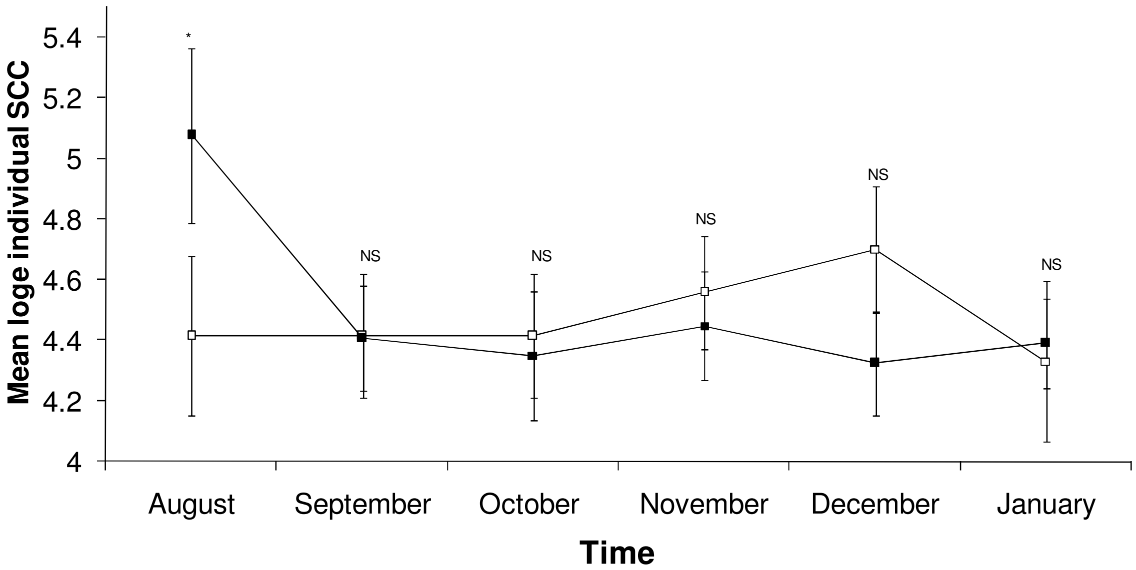

Figure 3.6 Mean (+ s.e.m) individual cell counts for cows managed in Organic and Conventional farming systems. Differences between means within months are significant where indicated (*P<0.05, ** P<0.01). —■— conventional —□— organic

Overall, there was no significant difference in somatic cell count between the Conventional and Organic herds (P=0.925; Figure 3.6). The effect of days in milk, upon somatic cell counts was significant (P<0.02). After correction for this effect, the patterns of somatic cells over time differed between Organic and Conventional cows (P<0.001). The Conventional cows began the season with a higher somatic cell count (August, 461.68 ± 183.64) but throughout the season the somatic cell count of Organic cows increased, until, January when the conventional cows had higher somatic cell counts (192.54 ± 50.33, P<0.001)

3.3 Reproductive outcomes

Data for reproductive outcomes are shown in Tables 3.1 and Figures 3.7, 3.8, 3.9, and 3.10

Table 3.1: The effect of management system on fertility outcomes

	Calving to 1st AI (days)	Calving to conception (days)	Planned start of mating to 1st AI (days)	Planned start of mating to conception (days)	Number of services (n)	Conception rate to 1st insemination (%)
Organic (mean ± SEM)	72.9 ± 2.4	86.9 ± 3.0	12.8 ± 1.3	26.8 ± 3.0	1.7 ± 0.1	0.5 ± 0.1
Conventional (mean ± SEM)	85.2 ± 5.1	92.7 ± 5.0	15.4 ± 2.1	30.8 ± 4.9	1.7 ± 0.1	0.4 ± 0.1
Difference (P value)	0.017	0.163	0.146	0.244	0.461	0.248

3.3.1 ***Interval from Calving to 1st artificial insemination***

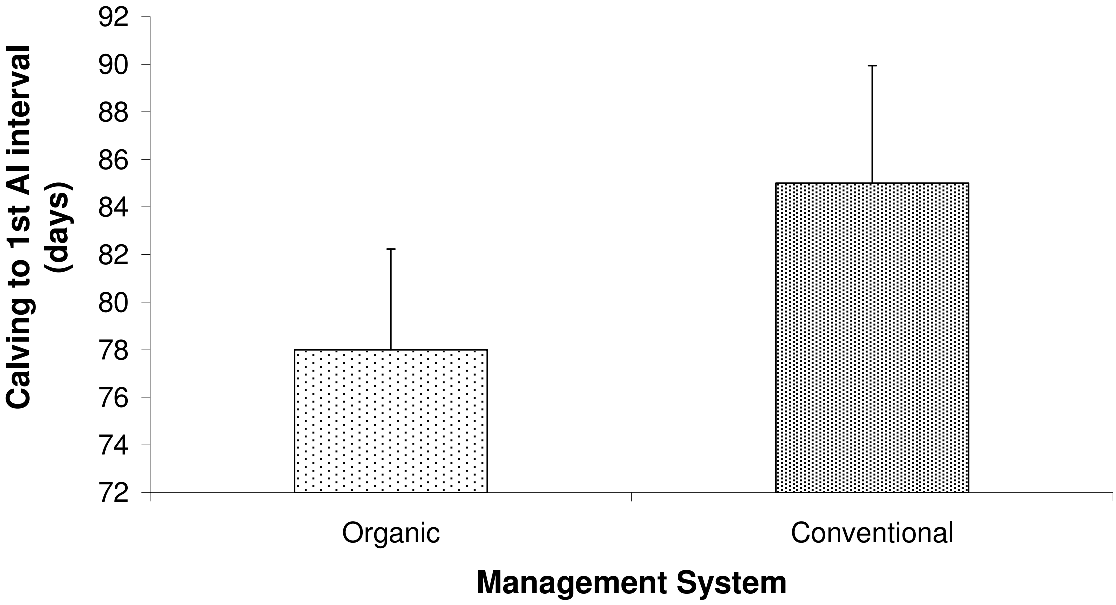

Figure 3.7 Mean (+ s.e.m) of the interval from calving to first AI insemination for cows managed in Organic and Conventional farming systems.

Organic cows had a shorter interval from calving to first AI insemination (P=0.017). The mean interval from calving to first AI insemination was 72.9 ± 2.4 days in the Organic cows and 85.2 ± 5.1 days in the Conventional cows (Table 3.1, Figure 3.7).

3.3.2 ***Interval from Planned Start of Mating to First Insemination***

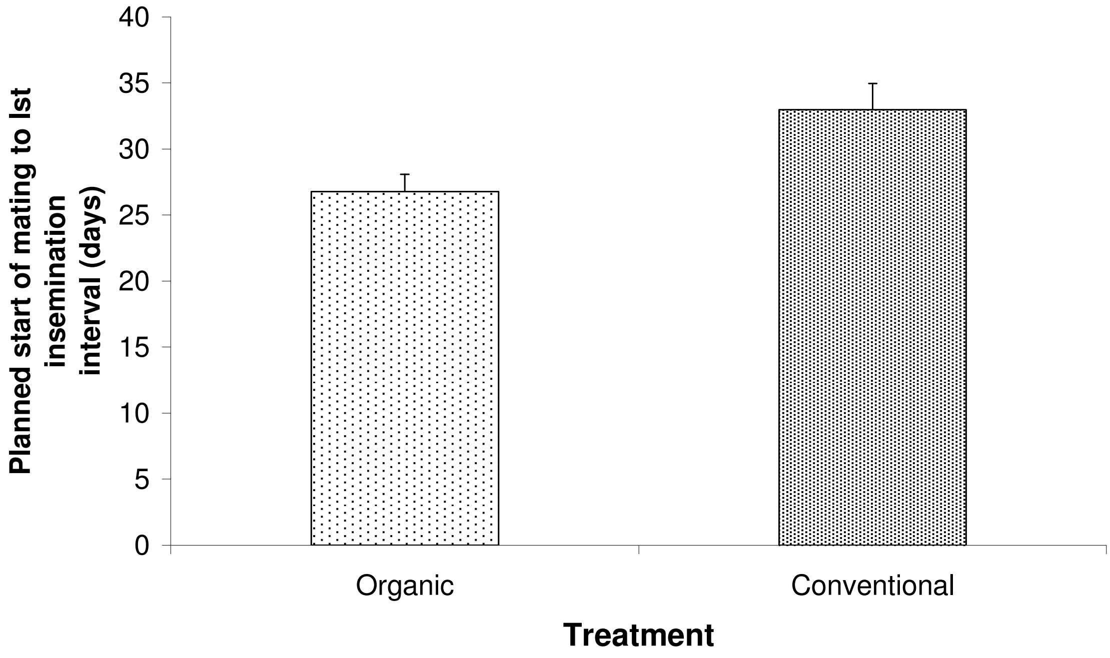

Figure 3.8 Mean (+ s.e.m) of the interval from planned start of mating to first AI insemination for cows managed in Organic and Conventional farming systems. Organic Conventional

However, there was no significant difference in the interval between the planned start of mating and first insemination between Organic and Conventional cows (Table 3.1, Figure 3.8). The mean number of days from planned start of mating to first insemination in Organic cows was 12.8 days and in Conventional cows 15.4 days (P=0.146).

3.3.3 ***Interval from Calving to Conception***

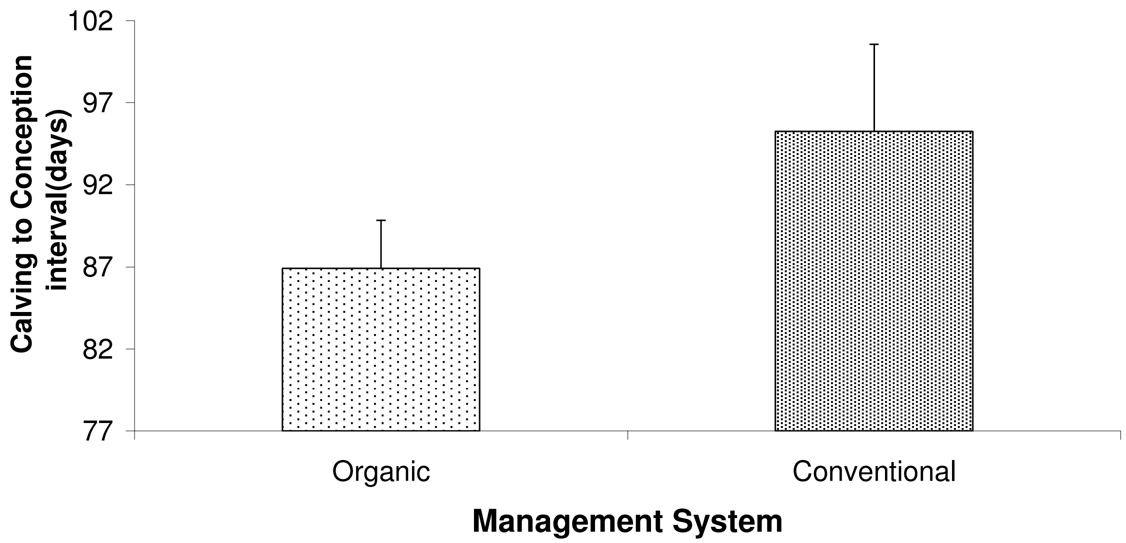

Figure 3.9 Mean (+ s.e.m) of the interval from calving to conception for cows managed in Organic and Conventional farming systems.

The mean number of days from calving to conception in Organic cows was 86.9 days and 92.7 days in Conventional cows (Table 3.8; Figure 3.9). However, this difference was not significant (P = 0.163).

3.3.4 ***Interval from Planned Start of Mating to Conception***

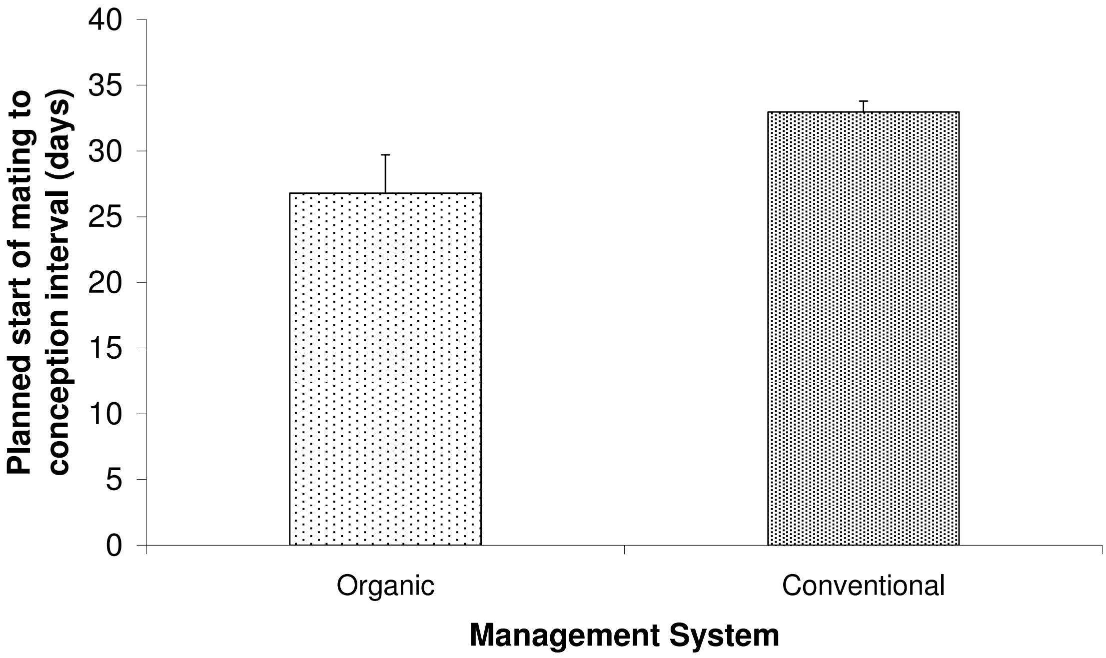

Figure 3.10 Mean (+ s.e.m) of the interval from planned start of mating to conception in Organic and Conventional farming systems. □ Organic ▩ Conventional

The mean number of days from planned start of mating to conception in Organic cows was 26.8 days and 30.8 days in Conventional cows (Table 3.1, Figure 3.10). Again, this difference was not significant (P = 0.244).

3.4 Cervical Diameter

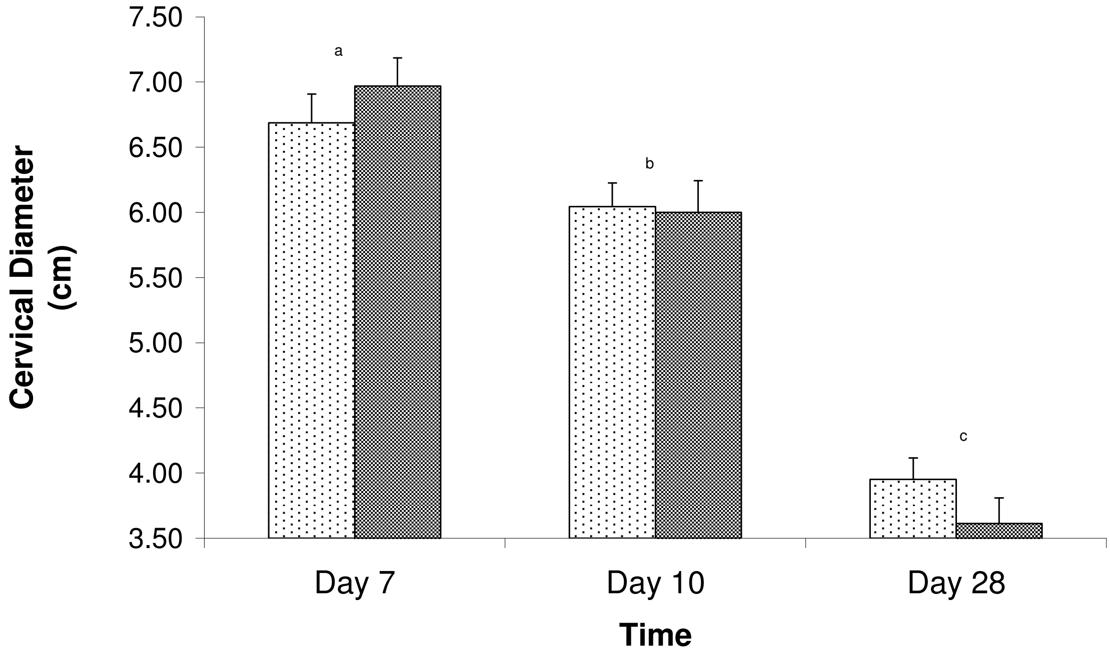

Figure 3.11 Mean (+ s.e.m) cervical diameter cows managed in Organic and Conventional farming systems. Differences between means within days are significant where indicated (a,b,c P<0.01). Organic Conventional

Changes in cervical diameter over time after calving approached a statistical significance between the two groups of cows (P=0.06). However, time postpartum (P<0.01) had a highly significant effect on cervical diameters across both management systems (Figure 3.11). The mean cervical diameter was on Day seven postpartum was 6.7 ± 1.4 cm for the Organic cows and 7 ± 1.3 cm for the Conventional cows. By Day ten postpartum, the mean cervical diameter had decreased to 6.0 ± 1.2 cm for the Organic cows and 6.0 ± 1.5 cm for the Conventional group. On Day 28 postpartum, the mean cervical diameter was only 4.0 ± 1.0 cm in the Organic cows and 3.6 ± 1.2 cm in the Conventional cows.

3.5 Microbiology

3.5.1 *Aerobic bacteria*

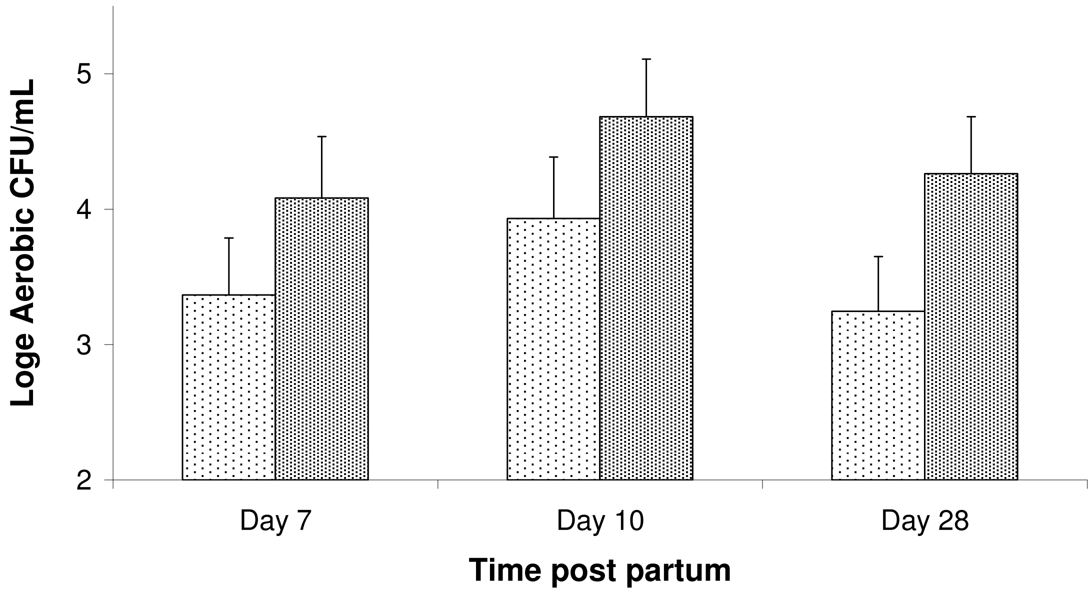

Figure 3.12 Mean (+ s.e.m) of aerobic bacterial counts over time for cows managed in Organic and Conventional farming systems.

Organic cows had significantly lower aerobic bacterial counts than the Conventional cows (P=0.042; Figure 3.12); the Organic cows had an overall mean of 615 ± 186 CFU/ml and the Conventional cows of 2641± 1117 CFU/ml. However, there was no effect of time or any interaction between treatment and time in the numbers of aerobic bacteria present in Organic and Conventional cows (P= 0.304 and 0.964, respectively).

3.6 Anaerobic Bacteria

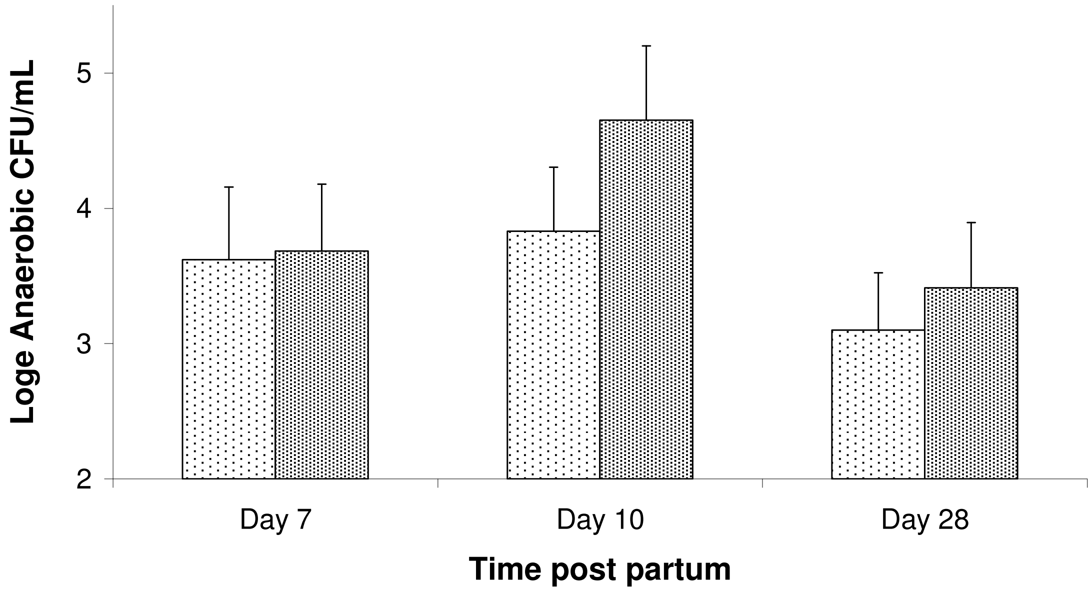

Figure 3.13: Mean (+ s.e.m) of anaerobic bacterial counts over time for cows managed in Organic and Conventional farming systems. ☐ Organic ▩ Conventional

Conversely, there was no significant difference in anaerobic bacterial counts between Organic and Conventional cows (P = 0.537; Figure 3.13). Nor was there a significant effect of time or interaction between management system and time in Organic and Conventional cows (P= 0.143 and 0.953, respectively).

3.6.1 ***Total Bacteria***

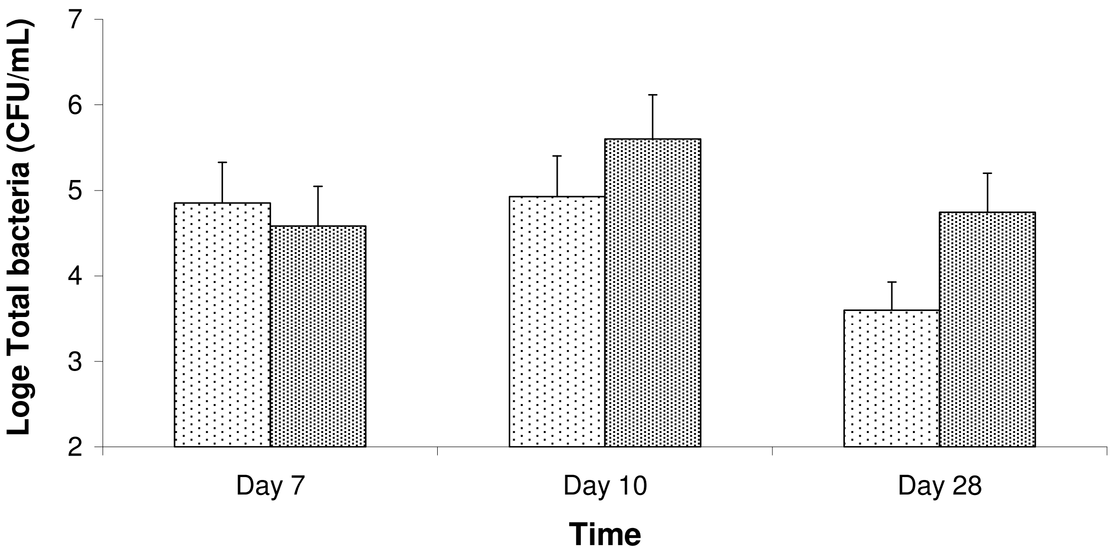

Figure 3.14 Mean (+ s.e.m) of total bacterial counts over time for cows managed in Organic and Conventional farming systems. ☐ Organic ▩ Conventional

The total bacterial counts, calculated as the arithmetic sum of aerobic and anaerobic plate counts, did not differ between the two groups with time (P=0.267; Figure 3.14). Again there was no significant effect of time nor the interaction between management system and time (P= 0.094 and 0.232, respectively).

The proportion of cows that had no bacteria present was also calculated (Table 3.2). The uterus of only one Organic cow was sterile on Days seven, ten and 28. Likewise, only one Organic cow was uncontaminated (less than 20 CFU/ml) on Days 10 and 28. Finally, eight Organic and eight Conventional cows were sterile on Day 28; 11 Organic and nine Conventional cows were uncontaminated (less than 20 CFU/ml) at Day 28.

Table 3.2 The percentage of positive samples over time from Organic and Conventional cows

	Management System	
Day	Organic	Conventional
7	84.2	82.1
10	84.2	92.3
28	89.5	89.7
Mean	86.0	88.0

3.7 Cortisol concentrations

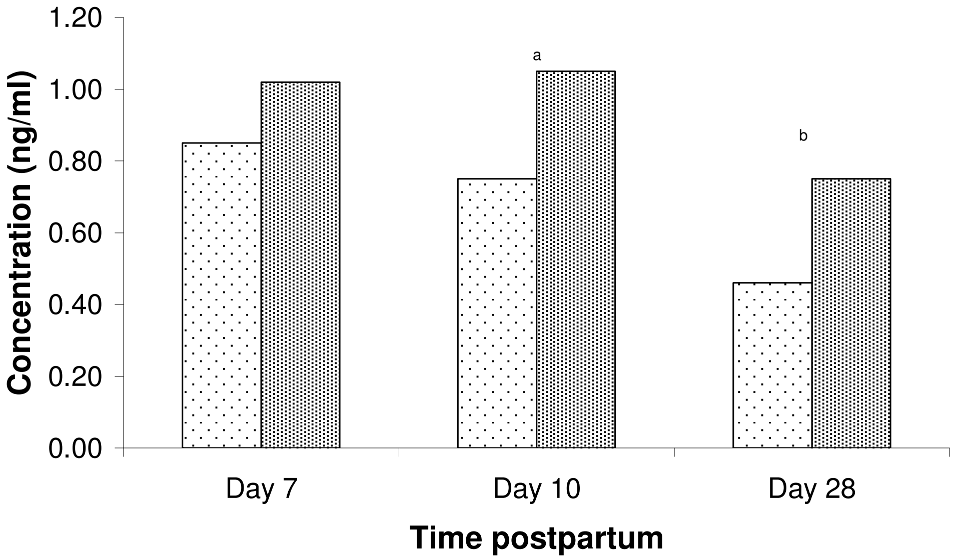

Figure 3.15: Mean (+ s.e.m) of cortisol concentrations over time in Organic and Conventional cows. Differences between means within days are significant where indicated (a,b P<0.01). ☐ Organic ▤ Conventional

Cortisol concentrations varied with time postpartum (P=<0.01; Figure 3.15); such that the values were lower on Day 28 than on Days and 10 (0.60 ± 0.06 compared to 0.94 ± 0.09 and 0.90 ± 0.10 respectively). Cortisol concentrations also varied with management system such that the overall mean was significantly (P=0.01) lower in Organic (0.68 ± 0.08 ng/ml) than Conventional cows (0.95 ± 0.06 ng/ml). There was no significant difference in the changes of cortical concentrations over time between the two groups of cows (P=0.559).

Table 3.3: Correlation matrix of reproductive outcomes and bacterial counts

	Calving to first AI	Calving to conception	Number of Services	Conception rate to first service	Planned start of mating to conception	Planned start of mating to first AI
Day 7: Aerobic Bacteria Log_e CFU/ml	0.554	0.147	0.249	0.064	-0.143	0.28
Day 10: Aerobic Bacteria Log_e CFU/ml	0.57	-0.158	-0.237	-0.348	0.32	-0.266
Day 28: Aerobic Bacteria Log_e CFU/ml	0.615	-0.074	0.465	0.149	-0.165	0.405
Day 28: Anaerobic Bacteria Log_e CFU/ml	0.296	0.286	0.044	-0.181	-0.205	0.095
Day 28: Anaerobic Bacteria Log_e CFU/ml	0.437	-0.366	-0.298	-0.415	0.475	-0.331
Day 28: Anaerobic Bacteria Log_e CFU/ml	0.57	-0.087	0.376	0.194	-0.205	0.318
Day 7: Total Bacteria Log_e CFU/ml	0.413	0.214	0.232	0.001	-0.148	0.268
Day 10: Total Bacteria Log_e CFU/ml	0.465	-0.239	-0.295	-0.401	0.403	-0.327
Day 28: Total Bacteria Log_e CFU/ml	0.586	-0.004	0.417	0.121	-0.159	0.354

3.8 Relationships between cervical diameter, bacterial counts, cortisol concentration, milk production characteristics, management system and reproductive performance.

A correlation matrix was created to examine the associations between the data.

Simple correlations between reproductive outcomes and bacterial counts were very weak (Table 3.3). There were also weak correlations between reproductive outcomes and cortisol concentrations, although cortisol concentrations on Day seven were correlated with the interval from calving to conception (-0.611). Similarly, cortisol concentrations and milk production characteristics were weakly correlated including somatic cell count.

A stepwise partial regression analysis, plus analysis of variance, was performed and created several predictive equations. Cervical diameter, aerobic and anaerobic bacterial counts on Day 28, and milksoilds production in November could be used to predict the interval from calving to first artificial insemination (Table 3.4).

Table 3.4: Stepwise regression analysis on interval from calving to first AI insemination

	d.f.	s.s.	m.s.	v.r.	F pr.	estimate
Constant						62.86
Milksoilds November	1	606.73	606.73	14.63	0.004	7.3
Cortisol Day 7 ($\log_e$+1)	1	182.88	182.88	4.41	0.065	-22.83
Cervix diameter Day 28	1	87.31	87.31	2.11	0.181	3.95
Anaerobic bacterial count Day 28 ($\log_e$+1)	1	83.67	83.67	2.02	0.189	2.42
Aerobic bacterial count Day 28 ($\log_e$+1)	1	71.07	71.07	1.71	0.223	-1.56
Residual	9	373.27	41.47			
Total	14	1404.93	100.35			

Milksolids production in November could be predicted through cortisol concentrations on Day seven and Day 28, condition score in Week four postpartum and somatic cell count in August (P= 0.07; Table 3.5). Similarly, somatic cell count in October was able to be predicted by condition score and cortisol concentrations on Day seven (P=0.025; Table 3.6).

Table 3.5: Stepwise analysis of milksolids production in November

	d.f.	s.s.	m.s.	v.r.	F pr.	estimate
Constant						5.04
Cortisol concentration Day 28 (log_e+1)	1	0.6622	0.6622	4.37	0.055	2.078
Condition score in week 4 postpartum	1	0.5431	0.5431	3.58	0.079	-0.65
Cortisol concentrations Day 7 (log_e+1)	1	0.2109	0.2109	1.39	0.258	-0.972
Somatic cell count August (log_e+1)	1	0.2587	0.2587	1.71	0.212	-0.1145
Residual	14	2.1219	0.1516			
Total	18	3.7968	0.2109			

Table 3.6: Stepwise regression analysis on somatic cell count log in October

	d.f.	s.s.	m.s.	v.r.	F pr.	estimate
Constant						2057
Condition score Week 4	1	213531	213531	9.86	0.006	-308
Condition score Week 1	1	21989	21989	1.02	0.329	-144
Cortisol concentrations Day 7 log (e) + 1	1	25059	25059	1.16	0.298	-156
Residual	16	346572	21661			
Total	19	607152	31955			

4 Discussion

4.1 Introduction

The main objective of this study was to compare lactational and reproductive parameters of Organic and Conventional herds, with particular focus upon the process of uterine involution and its relationship with reproductive outcomes. The key findings of this study showed some significant differences in lactational and reproductive characteristics between the Conventional and Organic cows. The Conventional cows had a higher milk and milksoilds production but the Organic cows had a shorter interval from calving to first AI. Organic cows were also found to have lower aerobic bacterial contamination as well as lower cortisol concentrations postpartum.

Organic farming has the philosophy that all elements of the system are linked and interdependent, and focuses on creating a balanced system with an optimal environment and healthy animals. One of its fundamental principles is to ensure healthy soil as every other elements are dependant on this. Organic farming focuses on prevention of illness and injury through good hygiene and stress reduction on the cows, so genetic selection of the “ideal” organic cow plays an important role in maintaining optimal cow health.

The philosophy of organic farming impacts on the farm’s production. Organic farming does not use synthetic chemicals such as fungicides, herbicides, antibiotics and soluble fertilisers. Organic farming attempts to identify natural materials that can be used to achieve the same results, such as homeopathy, organic fertilisers and herbal remedies. Homeopathy and natural remedies can be used effectively for the treatment of many ailments but they are limited in their ability to treat certain illnesses particularly mastitis and parasites (Thatcher 2007).

The use of only approved fertilisers often means that pasture production is lower than on a conventionally-managed farm where artificial nitrogen can be applied.

The extra pasture production on the conventionally-managed farms results in higher milk production per cow but it also means higher milk production per hectare because the extra pasture allows a higher stocking rate.

4.2 Lactation characteristics

The literature that directly compares the productivity of cows managed under organic and conventional systems is still relatively sparse (Reksen *et al.* 1999; Byström 2002; Roesch *et al.* 2005), although there is a reasonable amount of research on cows managed in solely organic systems (Busato *et al.* 2000; Bennedsgaard *et al.* 2003; Nauta *et al.* 2004; Valle *et al.* 2007). In comparative studies, conventionally-managed cows have higher milk yields when compared with those in organically-managed systems (Byström 2002; Hovi 2002). Byström (2002) found that while cows managed in organic systems had an overall lower daily milk yield, if milk yield is divided into sub-periods, then milk yield is significantly different only in early lactation. Previous research on the Dairy Cattle Research Unit (where the present study was conducted) found Conventional cows had higher milk yields than Organic cows (Kelly *et al.* 2007). This study is consistent with this previous research.

There are many different factors that are likely to contribute to the lower milk production by the organic cows (Byström 2002; Kelly *et al.* 2007 and the present study. One of the factors would be the different feeding systems between the Organic and Conventional cows. Byström considered that the lower milk yield was due to the lower ME intake of the organically-managed cows. In Kelly *et al.* (2007) suggested it may be due to a lower pasture production which leads to a lower herbage allowance or lower feed intake, different stocking rates, or the different environmental conditions that exist between the farmlets. The pasture production on the conventional farmlet in the current study was 9.3 t DM/ha and on the organic farmlet it was 8.5 t DM/ha, so it is likely that the difference in pasture production did indeed affect the milk yields (Kelly *et al.* 2007). The stocking rates were relatively similar, with the average stocking rate being 2.2 cows per hectare for the organic farmlet and 2.4 cows per hectare for the conventional farmlet, so the stocking rate was unlikely to have been a major contributing factor to the differences in milk production (Thatcher 2007). Peak milk yield generally occurs six to eight weeks postpartum with a slow decrease until drying-off (Holmes *et al.* 2001). In the present study, peak milk yield occurred in September/October (see Figure 3.3). However, whilst there were differences in the milk yields in early lactation between Organic and Conventional cows, there were no major differences in the time of peak lactation or in the rate of decline of milk yield after its peak.

In the studies of Byström (2002) and Hovi (2002), cows managed in organic systems produced less milksolids than those managed conventionally. In the present study, yield of milksolids per cow was greater for Conventional cows than Organic cows, except during the month of October when this difference was not significant (Figure 3.5). Conventional cows presumably had higher milksolids production for the same reasons as for the milk yield, namely, higher pasture production. Since the stocking rate of Conventional cows was greater than that of Organic cows, it would be expected that the yield of milksolids per hectare would also have been greater in that group. However, the time of year is important in determining milksolids production. The colder and wetter spring of 2006 resulted in lower pasture growth rates than average, reducing the difference in pasture production that would usually be expected to have occurred between organic and conventional systems. This was the likely explanation for the similar milksolids production between the two groups provided by Kelly *et al.* (2007).

Somatic cell counts in cows managed in organic farming systems in the UK were similar to the national average (Blanshard 1999). In contrast, Nauta *et al.* (2006) found that somatic cell counts were significantly higher in organically-managed than conventionally-managed cows, although, as might be expected, somatic cell counts were dependant on the management of the individual herds (Bennedsgaard *et al.* 2003). The importance of good management to minimise somatic cell counts was illustrated in the Dairy Cattle Research Unit in the 2005/2006 milking season, when it was reported that teat spraying did not occur in the latter half of lactation allowing the spread of *Staphylococcus aureus* and causing a dramatic increase in somatic cell counts (McLeod *et al.* 2008). In the present study the somatic cell counts were not affected by the management system, during the period between September and December, although there were significantly higher somatic cell counts in the Conventional cows in August. Such results are in broad agreement with previous studies conducted on the same farm during the 2001/2002 and the 2003/2004 milking seasons (Lopez-Villalobos *et al.* 2003; Silva *et al.* 2005).

High cell counts at the start of lactation are commonly reported for cows calving on pasture (Holmes *et al.* 2002). The first 90 days of lactation are associated with the highest incidence of mastitis (McDougall 1998) and, of those cows that develop clinical or sub-clinical mastitis at that time; most became infected shortly after dry-off or around parturition (Schrick *et al.* 2001). This is supports the findings in previous study. Nonetheless, it is unclear why somatic cell counts were higher in the Conventional cows than in the Organic cows in August, unless means were skewed by the effects of individual animals within the overall small number of cows that had calved during that month.

Overall, Conventional cows had higher milk yields and higher production of milksolids than Organic cows, but these yields were affected by time of year and days in milk. Somatic cell counts were similar in the two management groups, except for higher values in the Conventional cows at the start of lactation.

4.3 Reproductive outcomes

Sub-fertility, resulting in failure to conceive or conception late in the breeding season, is a major cause of loss to the New Zealand dairy industry. It is an important issue because of both its financial and animal costs. It reduces genetic gain, because cows are being culled for failing to become pregnant instead of production characteristics, meaning cows with poor production or health issues are retained. It also reduces genetic gain by reducing the number of replacement heifers born thus allowing less choice when selecting the replacements. There are also additional veterinary treatment costs and a reduced milk income (Xu and

Burton 2001). In New Zealand, Xu and Burton (2000) found that almost 50 percent of cows culled were culled for reproduction-related problems. The New Zealand dairy cow is expected to conceive when its milk production is at its peak in order to calve at a similar time the next year. It is necessary to mate during this period because, in a pastoral system, calving should ideally occur when pasture production is best able to meet the cow's feed demand (Holmes et al 2001). However, high-producing dairy cows are often sub-fertile during this stage of lactation.

Before conception can occur, cows must have completed uterine involution. Normally, uterine involution should be completed by 40 to 50 days postpartum (Gier and Marion 1968), although this can be extended by dystocia, retained foetal membranes, endometritis or inadequate protein nutrition (McDougall and Murray 2000; Morton 2000) The level of bacterial contamination that cows suffer during the post partum period is therefore likely to be related to their risk of developing endometritis (Mateus *et al.* 2002; Williams *et al.* 2005) and, in the work of Daoud (2008) was related to the reproductive outcomes.

There has been only a small volume of published research on reproduction and fertility in the organically-managed dairy cow, most of which has originated outside New Zealand. There are number of key differences between organically-managed cows in New Zealand and organically-managed cows overseas. Overseas cows are housed inside in barns for most of the year, in much smaller herds (usually less than 50), are fed a diet which contains concentrates and are calved all year around (Rosati and Aumaitre 2004; Roesch *et al.* 2005). Overseas organically-managed cows had a mean calving interval to first service which was slightly lower than the average interval for conventionally-managed cows (Blanchard 1999; Fall *et al.* (2008; Hovi 2002). However, the organically-managed cows required more services per conception than conventionally-managed cows (Blanchard 1999; Hovi 2002).

In contrast, other studies have found no consistent differences in the mean calving interval and the number of services per conception (Reksen *et al.,* 1999; Rosati and Aumaitre 2004; Roesch *et al.* 2005; Fall *et al.* 2006). Also, no significant difference was found in the interval from calving to first insemination (Reksen *et al.* 1999; Rosati and Aumaitre 2004; Roesch *et al.* 2005)

In the present study, the management system had little effect on reproductive outcomes. However, Organic cows had a shorter interval from calving to first artificial insemination (AI), therefore it is not unreasonable to assume that the significant difference is most likely due to both biological and physiological reasons. On the other hand, there was no significant difference in the interval from planned start of mating to first AI, the interval from planned start of mating to conception and the interval from calving to conception. The shorter interval between calving and first AI suggests that the Organic cows have a shorter postpartum anoestrus period. However, if this were correct, then the interval of calving to conception and the interval from conception to first AI would also be significantly different. As these intervals were not significantly different, the shorter calving to first AI interval might have been due to more careful observation by the farm staff in the detection of oestrous behaviour in the Organic cows compared with Conventional cows, or that the Organic cows were exhibiting oestrous behaviour earlier in the postpartum period. There is a possibility that the Conventional cows may also have been experiencing silent heats. The latter idea is supported by Menge *et al.* (1961) who found a trend towards corpus luteum formation in the absence of oestrus behaviour (silent heat); the average interval from calving to first corpus luteum formation being 13.5 days less than to the first oestrus. It is unlikely that the shorter interval is due to the Organic cows calving later than Conventional cows as the mean calving dates are not significantly different. However, the high proportion of two year olds in the organic herd may be a contributing factor (Figure 3.1). There is also the possibility that that the Conventional cows had short-term uterine infections early postpartum or their milk production affected their ability to ovulate or show oestrus behaviour. However, further research would be needed to substantiate this idea.

In contrast, the management system did not affect the average number of services per conception. This is important because it likely indicates that overall reproductive outcomes are not affected by the management system. However, it does suggest that the management system affects fertility in the early postpartum period.

4.4 Uterine involution

Uterine bacterial contamination occurs in the first two to seven weeks postpartum when a wide variety of bacteria invade the uterus. Most cows will have some degree of bacterial contamination in the first few weeks postpartum but most cows are able to eliminate the bacteria within two to four weeks (Griffin *et al.* 1974a,b; Foldi *et al.* 2006). The uterus is continually being reinfected due to spontaneous contamination, elimination and recontamination of bacteria (Griffin *et al.* 1974a). For uterine bacterial contamination to become an infection, bacteria need to persist, colonise and replicate within the tissues of the uterus. In pastoral dairying systems, significant uterine infections only occur in a small number of cows, although they are much more common in housed / intensively managed cows (Daoud 2008). Uterine infections often prolong postpartum uterine involution, whilst severe uterine infections can be detrimental to general health and to milk production (Konigsson *et al.* 2001). Uterine infections generally affect reproductive performance, by reducing conception rates and increasing embryonic mortality, thereby adversely affecting the calving to conception intervals and the chances of an individual cow becoming pregnant (McDougall and Murray 2000; Morton 2000; Parkinson 2007).

The present study found that Organic cows had significantly less aerobic bacterial counts than Conventional cows, but conversely, there was no significant difference in anaerobic bacterial counts between the two groups. There was no effect of time on bacterial counts which was consistent with the idea that the bacterial contamination in the uterus was constantly changing in the first few week postpartum.

It is interesting to consider why these differences between Organic and Conventional cows might have occurred. Organic farming has a philosophy of preventing disease and illnesses rather than treating them when they occur. Yet it is difficult to understand how this philosophy might have been translated into differences in bacterial counts between the two managements systems. On one hand, it could be suggested that the Organic cows had less bacterial contamination as a result of being less stressed because of their different management system. Some evidence for this exists, in as much as the Organic cows in the present study had significantly lower cortisol concentrations throughout the period between Days seven and 28 postpartum than did the Conventional cows. There is a possibility that the Organic cows could be less stressed because their lower milk production. If so, lower cortisol concentrations and the possibility of diverting energy away from milk production towards other processes such as immune system function, could have allowed the Organic cows to be better equipped to minimise bacterial contamination than were the Conventional cows. However, it is not clear to what extent any or all of these factors affect uterine bacterial contamination; the present data provides only modest evidence that this was the case, since no parameters of milk yield were directly related to measures of bacterial counts although Day seven cortisol concentrations were correlated with aerobic bacterial counts on that day.

The difference between aerobic and anaerobic bacterial counts is of note and may be due to the different environmental conditions required for growth of anaerobic bacteria compared to those for aerobic bacteria (Tortora 2010). In addition, time did not affect the amount of anaerobic bacteria present. However, on Day 28, the Organic cows had significantly fewer total bacterial counts than the Conventional cows. Whether this could be taken to indicate that some of the Conventional cows had started to develop mild endometritis is open to conjecture, but the present study provides no direct evidence that this was the case.

Cervical involution involves the restoration of the cervix to its prepartum state. Cervical involution is affected by parity and postpartum discharge (Oltenacu *et al.* 1983). Cervical width is regarded as a good indicator of the progression of uterine involution particularly in the first three weeks postpartum as the uterus is difficult to palpate during this period. The cervix contracts between Days five and ten, then relaxes back to its prepartum state (Moller 1970; Wehrend *et al.* 2003). The involution process of the cervix is important because it protects the uterus while allowing lochia to drain out early postpartum; the rapid reorganisation of the cervix is necessary to prevent bacterial contamination (Wehrend *et al.* 2003).

The present study found that cervical width decreased over time: a finding which is supported by other research (Oltenacu *et al.* 1983; Del Vecchio *et al.* 1994; Wehrend 2003). It is important to note that cervical involution is very much an individual process with large variations between each cow. Evidence to support this is that on Day seven, the cervical diameter ranged greatly from 3.5 to 10 cm in the Organic cows and in the Conventional cows, it ranged from 4 to 10.5 cm. The variation continued on Day ten, the range was 3.5 to 8 cm in the Organic cows and 3 to 9.5 cm in the Conventional cows. Similarly on Day 28, cervical diameter in the Organic cows ranged from 2.5 to 6.5 cm compared with 2 to 8 cm in the Conventional cows. While Daoud (2008) found larger mean diameters in his research, the conclusions of the present study are consistent with the Daoud (2008) study. This difference could be explained by the low sample size in both studies and the large natural variation between individual cows or the fact that cervical involution is relatively unaffected by systems. This idea can be supported by the relatively small standard errors of mean for cervical diameters (~ 0.2 over the sampling period).

A previous study on organic cows (Reksen *et al.* 1999) found that they had fewer days open postpartum. However, these cows were mated naturally and cows that are mated naturally will have fewer days open than cows mated to AI. In the present study, the difference in cervical width between the different management systems is almost significant. If the study was replicated with a larger sample size, it is most likely that the difference between the different management systems would become significant. Another factor that needs to be considered is that Organic cows may have been less stressed, as indicated by their lower cortisol concentrations. Stress reduces the ability of the immune system to function and may impair the rapid reorganisation of the cervix (Gwazdauskas 2002). Further research would be required to evaluate the effect of stress on the immune system and therefore its effect on uterine involution.

4.5 The interactions between reproductive outcomes, bacterial counts and cervical diameter.

This study was unable to produce principal components using bacteriology, management systems and cervical diameter that were predictive of reproductive outcomes. It is interesting to note that similar research was done with some of these cows and their ancestors and that research was able to produce a principal component using prostaglandin metabolite, collagen breakdown products, bacteriology and cervical diameter (Daoud 2008). It is therefore possible that while cervical width is a good measurement of reduction in uterine size, it is not a good predictor of the progression of the sloughing and regeneration of the uterine wall. Daoud (2008) found that the involution breakdown products are a more efficient predictor of reproductive outcomes as cows with subclinical endometritis can appear to have a normal uterus but are in fact still in relatively early stages of the involution process.

This present study found a number of weak correlations between reproductive outcomes and bacterial counts, and reproductive outcomes and cortisol concentrations. On Day 28, aerobic, anaerobic and total bacterial counts were correlated with the interval from calving to first AI. The effect of bacterial contamination on reproductive outcomes is well documented; high bacterial counts have a detrimental effect on reproductive outcomes (Bonnet *et al.* 1993; Lewis 1997; Daoud 2008). Bacteria produce endotoxins which are potent inducers of prostaglandins and cytokines that disrupt uterine involution and the resumption of uterine activity (Foldi *et al.* 2006). The mere presence of *A. pygogenes* in the first three weeks postpartum affects the first service conception rate and the interval from calving to first AI (Hartigan *et al.* 1974). The stronger correlation between reproductive outcomes and bacterial counts on Day 28 was likely to be related to the fact that the cows that still had bacteria present on Day 28 would probably be those cows that had difficulty eliminating bacteria and had, or would likely develop,endometritis (Sheldon and Dobson 2004). However, as no other reproductive outcomes are strongly correlated with bacterial outcomes, it was likely that any uterine contamination was transient and quickly eliminated by the cow.

Predictive equations were created using a stepwise partial regression analysis and analysis of variance. These equations allowed the prediction of certain factors investigated by using the data from this study. The interval from calving to first AI could be predicated using cervical diameter, aerobic and anaerobic bacterial counts on Day 28, and milksolids production in November (Table 4.2). The link between the interval from calving to first AI seems logical because increased cervical diameter and high aerobic and anaerobic bacterial counts would likely lead to a uterine infection and increase the postpartum anoestrus period. Cortisol concentrations on Day seven and Day 28, condition score in Week four postpartum and somatic cell count in August could be used to predict milksolids production in November (Table 4.3). These factors are linked because cortisol concentrations and condition score in early lactation can affect milk production later in lactation, as cows with low body condition and with high cortisol concentrations are likely to become sick which may result in reduced milk production. Similarly, somatic cell count in October was able to be predicted by condition score and cortisol concentrations on Day seven. Again, condition score and cortisol concentrations are likely to be indicative of the cow's health in early lactation and high somatic cell counts occur in cows that have infections (Table 3.4).

4.6 The effect of stress on reproductive outcomes

Physical and social stressors can disrupt HPA axis leading to endocrine, immune and neural imbalances that can influence productivity and health. These stressors cause the secretion of cortisol and catecholamines; the prolonged secretion of cortisol causes the suppression of the immune system (Gwazdauskas 2002). Stress reduces the efficiency of reproduction and any impediments to optimal reproductive performance are exacerbated under stressful conditions (Dobson and Smith 2000; Gwazdauskas 2002). A stressor postpartum is the cow's inability to ingest enough feed to meet its nutrient requirements. This may show no detectable effect of milk production but may show up as reduced fertility. Gwazdauskas (2002) believes that the selection for increased milk production has made cows highly susceptible to stress.

Cortisol concentrations peak around parturition and decrease back to prepartum values between 24 hours to nine days postpartum (Smith *et al.* 1973; Torres *et al.* 1997; Preisler 2000). Torres *et al.* (1997) found that cortisol concentrations decreased over time between Days seven and 28 postpartum. In the present study, the Organic cows had overall lower cortisol concentrations than Conventional cows. It should be noted that the cortisol concentrations in both the Conventional and Organic cows were relatively low. The lower cortisol concentration in the Organic cows could be attributed to the fact that the organic dairying system places emphasis on good cow health and welfare, and reducing exposure to infectious agents, perhaps thereby helping to reduce the risk of infection and impairment of immune function. It is therefore possible that Organic cows were less stressed because of their management system. Rosati and Aumatrie (2004) also believe that there may be some basis for this theory.

However, the lower cortisol concentrations and associated lower stress levels in the Organic cows did not appear to translate into better reproductive outcomes. Dobson and Smith (2000) using beta-hydroxbutrate (B-OHB) as a measurement of stress levels relating to the degree of negative energy balance did not find any difference in first service conception rate, or number of services per conception, or interval from calving to first AI, or interval from first AI to conception between cows with high concentrations of B-OHB (above 1 mM) and cows with normal levels (below 1 mM). To what extent stress affects reproductive outcomes is unclear, as the high B-OHB concentrations in this study may not have been sufficient to have had a negative impact on reproductive outcomes. In the present study, the Organic cows had a shorter interval from calving to first AI compared with the Conventional cows, however, there were no other significant differences in reproductive outcomes between the two management systems. Again, it is unclear whether cows in this study had cortisol concentrations that were sufficiently high to have negatively impacted on reproductive outcomes.

Cortisol concentrations were weakly correlated with several factors investigated in the present study. There was a weak correlation between cortisol concentrations on Day seven and the interval from calving to conception. It is possible that those cows with high cortisol at Day seven were those cows that had calving difficulties, twins or metabolic disorders such as hypocalcaemia around parturition. These cows may have had a longer anoestrus interval as their reproductive tract required longer to involute and their immune system was likely to have been compromised. Glucocorticoids have been found to increase the susceptibility of the uterus to infections (Gwazdaukas, 2002). It is of note that cows with abnormal puerperium had significantly higher basal cortisol during the first week postpartum and significantly high bacterial contamination than cows with normal puerperium (Torres *et al.* 1997).

Cortisol concentrations on Day seven were also weakly correlated with somatic cell counts in early lactation (September and October). Around parturition, the udder becomes distended and the teat cistern is open allowing bacteria to migrate up the teat canal; this will likely lead to the development of clinical or sub-clinical mastitis in early lactation (Smith *et al.* 1985). Cows that develop mastitis will have elevated somatic cell counts from all the activity of the bacteria and they are also likely to have elevated cortisol concentrations due to the additional stress on the immune system to eliminate the bacterial infection.

Further research is required to investigate the effect of cortisol concentrations on bacterial contamination, lactational characteristics and reproductive outcomes.

4.7 The effect of milk production and negative energy balance on fertility

In the current study, the Conventional cows had higher milk yields and milksolids. The Organic cows appear to have been exhibiting visual oestrus earlier (shorter interval from calving to 1st AI insemination) with Conventional cows having a later return to oestrous cycles compared with the Organic cows. Whether this difference was due to variations in the degree of negative energy balance between the Organic and Conventional cows is unclear as it is likely that many other factors would have affected fertility.

There is conflicting evidence on the effect of milk production on fertility. Some research indicates that high milk production negatively affects fertility (Butler and Smith 1989; Mann 2004; Pryce, 2004; MCDougall 2006) while other research had no such findings (Harrison *et al.* 1990; McMillian *et al.* 1996; Grosshans *et al.* 1997). There are many contributing factors that should be taken into consideration when examining the effect of milk production on fertility such as herd size and its management (McMillian *et al.* 1996). Many authors believe that milk production has only a minor effect on fertility compared with effects of other factors such as postpartum diseases including endometritis and postpartum nutrition (Lucy 2001a; McDougall and Murray 2000).

It is of note that there has been a general decline in reproductive efficiency in many parts of the world in recent years; this is supported by an increase in the intervals of calving to first AI, calving to first ovulation and conception rate to first insemination over time; while over the same time period milk production has greatly increased (McMillan *et al.* 1996; Roche *et al.* 2000).

Higher milk production has been linked to the later onset of oestrus behaviour (Harrison *et al.* 1990; Royal et al., 2000). The number of days from visual oestrus (66 vs. 43 days) and the number of ovulations (1.6 vs. 0.7) before the first visual oestrus were higher for cows that had high milk production than for those cows that did not (Royal *et al.* 2000). This offers a possible explanation for the shorter interval from calving to first AI in the Organic cows. Harrison *et al.* (1990) found that many of the metabolic differences occurring between cows with different levels of milk production occur before oestrus is visible.

However, Harrison *et al.* (1990) found no association between uterine involution and milk production. There were no significant differences between cows with high or average milk production in the interval from parturition to uterine involution, and days to first ovulation between cows with high or average milk production (Harrison *et al.* 1990). This supports the concept that milk production negatively impacts on the expression of oestrus behaviour but not on ovarian functions. This may explain why the Organic cows exhibited oestrus behaviour earlier (shorter interval from calving to AI) but there were no other differences in reproductive outcomes between the Organic and Conventional cows.

A period of severe negative energy balance has been found to adversely affect the fertility of the cow (Wathes *et al.* 2007). It is therefore likely that it is the degree of negative energy balance rather than high milk production itself is the reason for lower fertility. Often, high producing cows are the cows that are in severe negative energy balance and this is why high milk production is often associated with poor fertility. The negative effect of milk production on fertility is usually only evident at very high levels of milk production. Though the Conventional cows in this study had higher milk production than the Organic cows, they did not have high milk production compared to the average New Zealand cow so it is unlikely that their milk production negatively impacted on their reproductive outcomes.

5 Conclusions

A number of differences were found when the Organic cows were compared to Conventional cows which were likely related to the differences between the two management systems. The Conventional cows had a higher milk yield and milksolids production compared to the Organic cows. The difference in pasture production between the two farmlets is also likely to be a major contributing cause to the differences between the Organic and Conventional cows. While there was lower aerobic bacterial contamination postpartum in the Organic cows, the anaerobic and total bacterial counts were similar between the two groups. The reasoning behind this difference is unclear but is likely to be associated with physiological differences in uterine involution between the Organic and Conventional cows. Another finding was that Organic cows had a shorter interval from calving to first AI which was again likely linked to differences in the progress of uterine involution within the different management systems. This idea is supported by the significant correlation between the interval from calving and AI, and cervical diameter and bacterial contamination at Day 28 postpartum which are indicative of the progression of uterine involution. A further difference was that Organic cows had lower cortisol concentrations and thus in all likelihood lower stress levels. It is probable that the lower stress levels in the Organic cows caused physiological changes in the cow that affected the progression of uterine involution possibly by affecting their susceptibility to uterine bacterial contamination and infection leading to the differences in bacterial contamination. However, the mechanisms through which this occurs are unclear and even the concept itself is open to conjecture.

This study found that Organic dairying does not adversely impact on the fertility of the dairy cow in New Zealand. However, further research is required to increase the understanding of the effect of organically-managed dairying systems on uterine involution and reproductive outcomes.

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