# Impact of metabolism and production diseases on reproductive function in dairy cows

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#### Summary

The increase of milk yield in dairy cows during the last decades has been accompanied by a reduction in fertility. This decline in reproductive performance is most likely caused by the pronounced negative energy balance typically observed in high vielding dairy cows and in cows suffering from production diseases. Often, both phenomena occur coincidentally. Cows with a profound negative energy balance and production diseases show hormonal alterations compared to healthy cows which impair follicular development and oestrous behaviour, delayed resumption of ovarian cyclicity, increased risk of a persistent corpus luteum and decreased peripheral progesterone concentrations, with negative impacts on oocyte guality and embryonic development. However, until now it has not been proven that inflammatory diseases induce embryonic mortality. There is, on the other hand, strong evidence that a negative energy balance causes disturbances in uterine involution by an impairment of immune defense mechanisms. The underlying pathophysiological mechanisms are not yet completely clarified. It has been demonstrated, however, that an early ovulation after parturition delays the process of uterine involution. Taken together, both metabolic disorders as well as inflammatory processes have diverse negative effects on reproductive function and are some of the reasons for the decline of fertility in high vielding dairy cows.

# Introduction

The critical challenge in intensive dairy production systems is to achieve a high milk yield, while simultaneously maintaining an appropriate level of animal health. Clearly, genetic selection for greater milk production is a risk factor for a variety of production diseases. Longevity of high-producing dairy cows is, however, economically feasible as it maximizes the life efficiency of dairy cows (expressed as kg milk per day of lifetime). In addition, longevity addresses society's concerns regarding animal welfare. Fertility represents a prerequisite for longevity. Thus, the interactions between milk yield, health and fertility have been a focus of researchers worldwide.

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Fertility, defined as conception within a defined period of time after the previous calving leading to the delivery of a fully developed calf, is affected both by milk yield in early lactation and the health status of the cow – importantly, these factors interact with each other. The transition from late pregnancy to early lactation represents the period of highest impact on subsequent fertility for several reasons.

First, independently of their specific etiology, any type of dystocia (e.g., twins, posterior presentation, oversized calf) increases the risk for retained placenta, puerperal metritis and subsequent chronic endometritis, lowering the probability for a successful subsequent insemination.

Second, negative energy balance (NEB) is common in high-producing dairy cows as the initial milk production is too high relative to feed intake and increases in feed intake lag behind those of milk production (Ingvartsen & Andersen 2000, Bobe *et al.* 2004). In this situation, precursors for hepatic gluconeogenesis, protein and minerals are lacking (Bobe *et al.* 2004, Duffield *et al.* 2008). NEB tends to be more pronounced in high yielding dairy cows than in lower yielding animals. Accordingly, peripheral and abdominal fat, as well as muscular tissue, are mobilized. The elevated levels of non-esterified fatty acids (NEFA) are well-known triggers of insulin resistance, which is characterized by reduced biological effects of insulin due to a decreased peripheral response (Hayirli *et al.* 2002, Kaske *et al.* 2004, de Koster & Opsomer 2013). Further triggers of insulin resistance, such as catecholamines and inflammation mediators, provoke elevated NEFA concentrations. Under these conditions, however, the onset of ovarian activity is delayed. Moreover, even the metabolic status throughout the dry period affects ovarian activity post partum (Castro *et al.* 2012).

Third, cows suffer from production diseases such as hypocalcemia, retained placenta, endometritis, ketosis, mastitis, abomasal displacement and claw diseases, predominantly throughout the first weeks of lactation (Fleischer *et al.* 2001). This is due to immunosuppression which has been demonstrated for cows around calving (Ster *et al.* 2012). Furthermore, factors related to NEB, such as elevated concentrations of ketone bodies and NEFA, increase the risk of production diseases (Mulligan & Doherty 2008, Roberts *et al.* 2012). As all production diseases enhance the risk of reduced feed intake, a vicious cycle may be implemented which further increases the risk of infertility particularly due to cystic ovarian follicles. Thus, not surprisingly, the risk for pregnancy loss was found to be markedly higher in cows experiencing a combination of poor body condition and mastitis at 70 days post partum compared to cows which had solely either mastitis or a lower body condition score (Hernandez *et al.* 2012).

In general, however, it has to be emphasized that cows are able to cope with NEB (Bauman & Currie 1980, Herdt 2000). Thus, there is no inevitable relationship between milk yield, the incidence of metabolic disturbances and infertility *per se*. Instead, there is general consensus that metabolic disturbances develop as a result of the insufficient adaptation capacity of the affected cows, which underscores the impact of individual disposition (Bauman & Currie 1980, Herdt 2000, Ingvartsen & Andersen 2000). The lack of clear evidence for a direct relationship between milk yield and production diseases may be due to the importance of the herd management for the health status of the herd. At present, the incidence of production diseases seems to be influenced more by the expertise of the farm manager than by the level of milk yield (Fig. 1).

#### Impact on ovarian follicles after parturition

In most dairy cows, several follicles appear within the first week and a dominant follicle develops in the second week after parturition (Savio *et al.* 1990a, Savio *et al.* 1990b). However, only about 50 % of all cows ovulate during the first three weeks after parturition (Lucy *et al.* 1992,



**Fig. 1.** Fertility (percentage of cows pregnant after first service in relation to all pregnant cows) of dairy cows with different milk yield per lactation (black bars: ≤6000 kg; gray bars: 6001-8000 kg; white bars: 8001-10000 kg milk / lactation) on two farms with differences in herd milk yield per lactation (farm A: 9800 kg; farm B: 9100 kg). The data show that the effect of farm management on fertility was higher than the effect of milk yield (A Wangler, unpublished observations 2006).

Darwash *et al.* 1997b, Lamming & Darwash 1998). In the remaining cows, the dominant follicle during the first postpartal follicular wave becomes either atretic or cystic, and the first ovulation occurs at about the fourth follicular wave after parturition (Kawashima *et al.* 2012). The duration between parturition and resumption of luteal activity is related to the extent of the NEB in the first weeks of lactation. Cows with a more pronounced NEB between the first and second week postpartum ovulate later compared to cows with a moderate NEB (Staples *et al.* 1990).

The metabolic alterations during the transition period affect the follicular activity after parturition for several reasons. First, decrease of dry matter intake towards the end of pregnancy and the homeorrhetic changes typical during the onset of lactation (Bertics *et al.* 1992, Vazquez-Anon *et al.* 1994) provoke an uncoupling of the somatotropic axis characterized by low IGF-I concentrations irrespective of increased growth hormone (GH) secretion from the pituitary gland (Kunz *et al.* 1985). IGF-I is well-known as a sensitive signal between metabolism, NEB and reproduction (Konigsson *et al.* 2008).

However, IGF-I levels do not only change within cows but also differ between animals. IGF-I concentration in blood plasma is a heritable trait, with heritability estimates ranging from 0.23 to 0.52 (Davis & Simmen 1997, Grochowska et al. 2001, Davis & Simmen 2006, Swali & Wathes 2006). Cows developing production diseases post partum exhibit significantly lower IGF-I concentrations several weeks before calving compared to cows remaining healthy in the transition period (Piechotta et al. 2012).

GH stimulates lipolysis in adipose tissues (Dominici & Turyn 2002, Andersen et al. 2004) to provide the cows with sufficient energy (Herdt 1988). If lipolysis is too high, NEFA levels will increase. In the face of postpartal NEB, the liver becomes GH resistant (Thissen et al. 1994, Lang & Frost 2002), i.e., the GH receptors in the liver are down-regulated and the stimulating effect of GH on the synthesis of IGF-I is reduced. Therefore, plasma concentrations of IGF-I decrease from about 3 weeks prepartum to 1 week postpartum despite an increase in GH (Andersen et al. 2004, Meikle et al. 2004, Taylor et al. 2004). In addition, plasma concentrations of insulin decrease before and after parturition, except for an acute surge at parturition (Holtenius et

*al.* 2003, Taylor et *al.* 2004). Insulin plays a central role in the homeostatic control of energy metabolism and its concentration is positively correlated with energy intake (Chilliard *et al.* 1998). This endocrine constellation around parturition affects ovarian function. Bovine follicles possess IGF-I- and insulin receptor mRNA (Spicer *et al.* 1994, Sudo *et al.* 2007, Shimizu *et al.* 2008). Liver-derived IGF-I is a factor in regulating the final maturation of the dominant follicle during the first postpartal follicular wave (Beam and Butler 1998), and regardless of parity, circulating IGF-I in ovulatory cows at the first follicular wave postpartum is higher than that in anovulatory cows (Beam & Butler 1998, Kawashima *et al.* 2007a, Kawashima *et al.* 2007b) (Fig. 2). Hence IGF-I, as well as insulin, directly stimulates both the proliferation of granulosa cells and steroidogenesis. Thus, low IGF-I- and insulin concentrations being typical for the first weeks of lactation reduce the probability of ovulation (Armstrong *et al.* 2001, Armstrong *et al.* 2003, Gong 2002).



Moderate NEB during the peripartal period in cows:

**Fig. 2.** Schematic presentation of the effect of the degree of peripartal negative energy balance on development of the dominant follicle within the first three weeks after parturition in dairy cows (mod. from Kawashima et *al.* 2012).

Low plasma glucose concentrations are frequently found in recently calved cows due to the high glucose demand of the mammary gland to support milk synthesis (Harrison *et al.* 1990, Andersen *et al.* 2004, Schlamberger *et al.* 2010) while elevated plasma NEFA concentrations reflect peripheral lipolysis. The serum concentrations of these metabolites are also reflected in the follicular fluid of the dominant follicle. *In vitro* studies showed that NEFAs have negative effects on the quality of oocytes, cumulus cells and resultant blastocysts (Leroy *et al.* 2006, Van Hoeck *et al.* 2013). In fact, higher plasma NEFA concentrations during the peripartum period are associated with delayed ovulation in postpartum dairy cows (Giuliodori *et al.* 2011). Therefore,

448

postpartal changes of serum concentrations of these metabolites alter the pattern of ovarian follicle growth and development, resulting in reduced reproductive performance in dairy cows.

High yielding dairy cows experiencing pronounced NEB during the transition period do not only exhibit a prolonged period of anoestrus. Remarkably, even when these cows initiate oestrous cycles following a period of anovulation or anoestrus, conception rate is compromised (Gumen *et al.* 2003, Rhodes *et al.* 2003, Cerri *et al.* 2004, Galvao *et al.* 2004). Lack of progesterone (P4) and lower concentrations of oestradiol (E2) during prooestrus in the oestrous cycle preceeding AI might result in shortened oestrous cycles due to premature luteal regression (Mann & Lamming 2000, Shaham-Albalancy *et al.* 2001). According to the hypothesis of Britt (1991) damage to primary follicles induced by a NEB during the first weeks after parturition may cause disturbances of the ovulatory follicles developing around 60 to 80 days later. Such follicles may contain oocytes of lower quality and a reduced capacity to synthesize steroid hormones. These disturbances could be another reason for lower conception rates in cows exhibiting a NEB during the transition period.

However, follicular activity in postpartum dairy cows is not only affected by metabolites and hormones, but also by inflammatory mediators. Metritis and mastitis are considered to be the most common infectious diseases during the postpartum period. Within three weeks after parturition, up to 40% of cows develop metritis (Sheldon *et al.* 2009) and mastitis is found in approximately 20–50% of all dairy cows (Wilson *et al.* 1997, Pitkala *et al.* 2004). Infectious diseases often perturb normal ovarian cyclic activity, resulting in abnormal folliculogenesis, development of cystic ovarian follicles or prolonged anoestrus (Opsomer *et al.* 2000, Mateus *et al.* 2002). In fact, in cows with metritis the first postpartum dominant follicle had reduced growth and peripheral plasma E2 were found to be lower (Williams *et al.* 2007).

Escherichia coli (*E. coli*) is an important infectious agent causing metritis and mastitis. Much of the tissue pathology is associated with endotoxins from gram-negative bacteria, the lipopolysaccharides (LPS). LPS has been detected in plasma, uterine fluid (Mateus *et al.* 2003), and follicular fluid (Herath *et al.* 2007) of cows with metritis and in the plasma and milk of cows with *E. coli* mastitis (Hakogi *et al.* 1989). It has been reported that LPS act at either hypothalamus or pituitary gland to suppress gonadotrophin release and perturb follicle growth and function (Suzuki *et al.* 2001).

Moreover, LPS are assumed to have a direct effect on the ovary, including follicular components such as the theca and granulosa cells and the oocyte. In bovine follicles, granulosa cells express toll-like receptors 4 (TLR4) which are activated through LPS, indicating that follicular cells are capable of responding to LPS. *In vitro* studies have shown that LPS suppressed E2 production by down-regulation of transcripts for P450 aromatase in granulosa cells from both large and small follicles (Herath *et al.* 2007, Shimizu *et al.* 2012).

Recently it has been shown, that LPS also reduce the primordial ovarian follicle pool in the bovine ovarian cortex ex vivo and in the murine ovary in vivo (Bromfield & Sheldon 2013). These findings may provide further explanation as to why cows affected by infectious production diseases during the first weeks after parturition exhibit lower fertility.

## Impact on ovarian follicles of offspring

Strikingly, metabolic and inflammatory triggers do not only affect ovarian follicles during the puerperal period in the cow, but also ovarian activity in the developing foetus (Evans *et al.* 2012, Mossa *et al.* 2013). Mossa *et al.* (2012) studied the effect of restrictive feeding of pregnant cows (60% of their maintenance energy requirements shortly before conception until the end

of the third month of pregnancy). Although birth weight of the offspring was unaltered, antral follicle number in calves born from restrictively fed dams were 60% lower compared with calves born from controls. Cows with a higher number of antral follicles show higher pregnancy rates, a shorter calving to conception interval and fewer services during the breeding season compared to cows with a low number of antral follicles (Mossa et al. 2012). Thus, the results of this experiment provide evidence that maternal NEB during pregnancy may have an important trans-generational impact on the size of the ovarian reserve and thereby the fertility of the offspring. The same research group examined the effects of mastitis occurring during pregnancy on ovarian development of their offspring and observed that dairy cows with a high number of somatic cells in the milk (> 200 000 cells/ml) several (4 or 5) times during pregnancy gave birth to female calves with relatively low circulating concentrations of anti-Müllerian hormone (AMH) as adults. Anti-Müllerian hormone is a glycoprotein produced exclusively by granulosa cells of healthy growing follicles (La Marca & Volpe 2006) and circulating AMH concentrations are highly correlated with the number of antral follicles and the size of the ovarian reserve in cattle and other species (Ireland et al. 2011). Therefore, these results indicate that chronic infections during pregnancy of dairy cows diminish the size of the ovarian reserve, with potentially negative effects on the future reproductive performance in their offspring.

#### Impact on oestrus behaviour

In the last five decades the percentage of cows that have been observed in oestrus and stand to be mounted has declined from 80% to 50%. Moreover, the duration of oestrus became shorter from 15 h to 5 h over the past 50 years (Dobson *et al.* 2008) due to a negative relationship between milk yield and duration of oestrus (Lopez *et al.* 2004). A possible reason are reduced E2 concentrations as a result of a higher metabolism of this hormone (Wiltbank *et al.* 2006). Lactating cows have a higher liver blood flow and increased metabolic clearance of E2 compared to non-lactating cows (Sangsritavong *et al.* 2002). Another reason for lower E2 levels may be lower IGF-I levels in high yielding dairy cows (Lucy 2000).

Production diseases also have a negative effect on oestrus behaviour. For example, lame cows may be unable to mount herdmates. However, other production diseases such as mastitis induce acute as well as chronic stressors which affect the hypothalamus-pituitary-adrenal gland axis and the hypothalamus-pituitary-ovarian axis (Dobson & Smith 2000). Hence, GnRH and LH pulse frequency are reduced leading to short–term decreases in follicular E2 production, as well as delaying and reducing of the magnitude of the LH surge (Dobson *et al.* 2007).

### Impact on progesterone

Progesterone has a key role in reproductive performance, especially during pregnancy. For example, in the oestrous cycle preceeding insemination, low systemic P4 concentrations have been shown to be detrimental to conception rate (Inskeep 2004, Lonergan 2011). Although not all mechanisms responsible for this phenomenon are fully understood, it is well known that the pulsatile release of GnRH and thus of LH is suppressed by P4 (Kinder *et al.* 1996). The frequency of LH pulses has a significant influence on the development and ovulation of the dominant follicle (Fig. 3). If P4 concentrations are high, LH pulse frequency is low and the dominant follicle undergoes atresia during dioestrus. In contrast, if P4 values are low during dioestrus, LH pulse frequency is higher. As during dioestrus this increase in LH never reaches follicular-phase-type frequencies that are necessary for the final maturation of the preovulatory follicle and ovulation,



Fig. 3. Schematic presentation of the effect of the concentration of progesterone levels on follicular waves during dioestrus and on oocyte quality of the ovulatory follicle (mod. from Inskeep 2004).

the dominant follicles show a longer persistence. Cows with follicles persisting more than four days have been shown to have lower pregnancy rates (Mihm et al. 1994, Mihm et al. 1999).

After insemination, the postovulatory P4 rise between days 4 and 7 is important (Stronge et *al.* 2005, Diskin & Morris 2008). P4 alters secretion of histotrophs by the endometrium enhancing the growth and development of the embryo. Thus, cows with a steep increase in P4 concentrations between days 4 and 7 after insemination have a greater chance of maintaining a pregnancy than animals with a delayed rise (Diskin & Morris 2008). Accordingly, a fivefold increase in systemic P4 concentrations early post conception was associated with an increase in the size of the embryo on days 13 and 16 of pregnancy (Carter et al. 2008).

One reason for low peripheral P4 concentration is a reduced secretion of the CL. The synthesis of P4 by the CL can be affected by several mechanisms, especially by disturbances in the development of the ovulatory follicles during the previous oestrous cycle (Robinson *et al.* 2006). A smaller ovulatory follicle develops a smaller CL which synthetizes lower amounts of P4 (Vasconcelos *et al.* 2001). Another reason is higher metabolism of P4. Like E2, P4 is metabolized in the liver. Therefore, the increased feed intake and hepatic blood flow of high yielding dairy cows results in an elevated clearance of P4 and thereby lower peripheral P4 concentrations. Thus, negative correlations exist between P4 concentrations in the blood during the luteal phase and milk yield, as well as dry matter intake (Wiltbank *et al.* 2006).

P4 concentrations in dairy cows can also be negatively affected by inflammatory diseases. For example, cows with severe bacterial uterine contamination have smaller CLs and lower P4 concentrations than healthy cows (Williams et al. 2007, Struve et al. 2013). The pathogenesis of luteal impairment caused by metritis is unclear. Metritis is accompanied by increased PGF<sub>2α</sub> plasma concentrations (Thompson et al. 1987, Del Vecchio et al. 1994, Mateus et al. 2003), which possibly disturb luteal development. Other inflammatory mediators, such as tumor necrosis factor- $\alpha$ , which may be released during metritis, are cytotoxic to bovine luteal cells (Petroff et al. 2001). Furthermore, as stated above, endotoxins inhibit the responsiveness of the pituitary gland to GnRH (Williams et al. 2001), which in turn could affect luteal development.

In addition, persistent CLs are frequently found in cows suffering from metritis (Opsomer et al. 2000, Mateus et al. 2002, Taylor et al. 2003). The persistent CL has been determined as one of the most frequent abnormal ovarian activities in dairy cows, with a prevalence of 11 to 35% (Fonseca et al. 1983, Lamming & Darwash 1998, Opsomer et al. 2000, Mateus et al. 2002, Zulu et al. 2002, Gumen et al. 2005, Pollott & Coffey 2008). Although the pathogenesis of a persistent CL is not clearly understood, some studies have provided evidence that the luteotropic prostaglandin, PGE, which is elevated in cows showing uterine inflammation, might be involved (Henderson et al. 1977, Gimenez & Henricks 1983, Reynolds et al. 1983, Akinlosotu et al. 1986, Thibodeaux et al. 1992). The impact of persistent CLs on bovine fertility is largely unknown. While some authors (Taylor et al. 2003) found no difference in reproductive competence between cows with persistent CL and normal cyclic cows, another study showed that persistent CLs was followed by a higher level of late embryonic and early foetal mortality (Lamming & Darwash 1998). In our own study performed recently, we reduced the prevalence of persistent CLs by an exogenous injection of  $PGF_{2\alpha}$  in cows showing a CL five weeks after parturition (Kögel et al. 2014). This treatment led to a significant increase in first service conception rate, a decrease in the AI per conception ratio and a shortened calving-toconception interval.

It is a widely accepted hypothesis that elevated  $PGF_{2\alpha}$  synthesis induced by inflammatory diseases may be responsible for higher embryonic loss rate in pregnant cows (Hansen *et al.* 2004). In fact, it has been demonstrated that cows affected by clinical mastitis within the period between first AI and pregnancy require more inseminations and have a longer calving-to-conception interval compared to healthy cows (Barker *et al.* 1998). The authors stated that the inflammation-related induction of complete luteolysis was responsible for the termination of pregnancy. However, in further experimental studies a single intravenous administration of LPS between days 7 and 9 of the oestrous cycle in cows caused only a transient decrease of luteal size and P4 concentrations (Fig. 4). Although the cows infused with LPS showed lower P4 values for some days compared to untreated control cows, the duration of the oestrous cycle was not significantly altered (Gilbert *et al.* 1990, Herzog *et al.* 2012).



**Fig. 4.** Plasma progesterone concentration (mean +/- S.E.M.) in cows given saline (blue) or E. coli lipopolysaccharides (red) on day 9 of the oestrous cycle. \*Values differed between saline- and LPS-treated cows ( $P \le 0.05$ ) (mod. from Herzog et al. 2012).

Furthermore, after an intrauterine infusion of LPS, systemic P4 concentrations were found to be decreased over several days (Williams *et al.* 2008). In a recently completed trial we infused LPS intravenously in eight pregnant cows between days 29 and 38 after successful insemination. In these cows LPS induced reduced P4 concentrations, but no embryonic loss was observed in any of the cows (Herzog *et al.* 2014). The results of these studies suggest that a short term exposure to endotoxins provokes a temporary depression in luteal function, but does not necessarily cause further negative consequences. It cannot be excluded, however, that a chronic, long-lasting exposure to either endotoxins or other components of bacteria might induce complete luteolysis leading to embryonic mortality.

## Effects on uterine involution

Uterine involution during the puerperal period is an important and critical process in the reproductive cycle of dairy cows (Frazer 2005). It includes the reduction of uterine size, elimination of bacterial contamination and regeneration of the endometrium representing preconditions for a subsequent pregnancy (Sheldon *et al.* 2008). During the first three weeks after parturition, the weight of the uterus decreases from 9 kg to around 1 kg (Gier & Marion 1968). Indeed, a variety of hormones play a crucial role in the regulation of uterine contractility (Frazer 2005). The muscle cells of the uterus express high-affinity receptors for oestrogens, P4, oxytocin and prostaglandins as well as adrenaline and norepinephrine (Kundig *et al.* 1990). Oestrogens have a contraction-promoting effect on the myometrium, while P4 immobilizes the myometrium (Garfield *et al.* 1988).

Cows suffering from uterine diseases due to disturbances of uterine involution show a calving-to-conception interval approximately 19 days longer and a 20% lower conception rate to first service compared to controls (Fourichon et *al.* 2000). Even after clinical resolution of a uterine infection, conception rates remain about 20% lower in affected cows. Finally, 3% of such cows remain infertile and have to be culled (Borsberry & Dobson 1989).

The main issues affecting uterine involution during the puerperium are a retained placenta and uterine infections (Fonseca *et al.* 1983, Borsberry & Dobson 1989), mostly associated with peripartal complications such as dystocia, twins or stillbirths. Dystocia is usually associated with human obstetrical assistance resulting in an increased contamination of the uterus and vagina. A retained placenta contributes to the pathogenesis of metritis by providing an ideal environment for bacterial growth, due to the large amount of necrotic tissue present, the delay of lochia expulsion and potential lesions to the uterus because of manual removal. Thus, bacterial uterine inflammation is a common consequence (LeBlanc *et al.* 2002, Dubuc *et al.* 2010a, Sheldon & Dobson 2004). However, it is important to note that during the first days after parturition the uterus is contaminated with a wide range of bacteria in nearly all cows. The development of metritis is dependent on the balance between immunity of the cow and pathogenicity of the bacteria (Sheldon *et al.* 2009).

There are conflicting results concerning the effects of metabolites and production diseases on uterine involution. LeBlanc (2012) reviewed the interaction between metabolism, inflammation and fertility and concluded that NEB contributes to immune dysfunction, a major factor in the establishment of reproductive tract inflammatory disease. Hammon *et al.* (2006) noticed that high serum concentrations of ß-hydroxybutyrate (BHBA) and NEFA reduce the activity of polymorphonuclear neutrophils (PMN), which play an important role in uterine immune defence and are involved in the pathophysiology of metritis. This finding is supported by another study showing that cows with uterine diseases had higher serum concentrations of NEFA and BHBA around calving (Galvao *et al.* 2010). It has also been suggested that cows with pronounced

NEB reveal an increased expression of inflammatory genes in the endometrium 2 weeks after calving (Wathes *et al.* 2009). However, other studies did not find an interaction between NEB and uterine disease. Similar concentrations of NEFA and glucose were observed in cows with and without endometritis (Burke *et al.* 2010), and neither NEFA nor BHBA during early lactation were found to have an association with metritis (Valergakis *et al.* 2011) or subclinical endometritis (Senosy *et al.* 2012). Markusfeld (1987) described an increased risk of retained placenta related to the body condition of cows with distinct BCS losses during the dry period. Also cows with a low BCS were more likely to have retained placental membranes. However, in another study no relationship between BCS and the incidence of metritis was observed (Kim & Kang 2003).

Hypocalcaemia causes a reduced uterine muscle tone, which could be responsible for retained placenta and a delay in uterine involution (Erb *et al.* 1985). Low blood calcium levels may also be associated with impaired immune function, but their association with uterine diseases is also equivocal. In one case-control study, with a relatively low number of cows (n = 38), clinical hypocalcaemia was associated with a higher prevalence of purulent vaginal discharge about three weeks after parturition (Whiteford & Sheldon 2005). However, in large field studies with several hundred cows, no relationship between the occurrence of hypocalcaemia and uterine diseases was found (Dubuc *et al.* 2010b, Cheong *et al.* 2011).

The reasons for these discrepancies have not been clarified and need further investigation. Different factors are intricately interlinked, which makes assessment of these single effects difficult (Williams 2013). Recently it has been shown that different risk factors are associated with different forms of uterine disease. Factors that increase uterine trauma and bacterial contamination, such as twinning and dystocia, are more likely to result in purulent vaginal discharge without an endometrial inflammation. Factors associated with either immune suppression or metabolic disturbances during the transition period, such as a thin body condition at calving or ketosis, result more often in endometritis as diagnosed by cytology (Dubuc et *al.* 2010b). Perivaginal discharge and cytological endometritis both reduce the probability of a subsequent pregnancy. As the effects are cumulative in cows showing both conditions, the authors of these studies concluded that the effects have a different origin (Dubuc et *al.* 2010a).

There are contradictory statements on the effects of the time of first ovulation after parturition on reproductive performance in cows. Some studies demonstrated that a short postpartum anovulatory period is associated with improved fertility (Thatcher & Wilcox 1973, Darwash et al. 1997a, Kawashima et al. 2006, Gautam et al. 2010), whereas others reported reduced fertility (Smith & Wallace 1998) or did not find any effect (Sakaguchi et al. 2004). Previous experimental studies on the effect of the postpartum anovulatory interval on fertility of dairy cows used either oestrogens or GnRH analogues for postpartum suppression of ovulation (Haughian et al. 2002, Padula & Macmillan 2002, Silvestre et al. 2009a, Silvestre et al. 2009b, Silvestre et al. 2009c). However, whether and to what extent uterine effects were caused indirectly by either the suppression of ovulation or directly by the administered hormones, is not known. Oestrogens have a positive effect on myometrial contractility and enhance uterine immune response (Killingbeck & Lamming 1963, Rodriguez-Martinez et al. 1987, Herath et al. 2007, Silvestre et al. 2009a, Shimizu et al. 2012), whereas P4 induces a relaxation of uterine muscles (Rodriguez-Martinez et al. 1987, Bonafos et al. 1995) and suppresses immunity (Lander Chacin et al. 1990, Subandrio et al. 2000). GnRH analogues also enhance uterine motility (Giammarino et al. 2009). In our own study we inhibited ovulation during puerperium in healthy dairy cows and in cows with postpartum uterine disease without hormonal treatments by repeated transvaginal follicular punctures (Heppelmann et al. 2013). Suppression of ovulation by transvaginal follicular punctures had a positive effect on uterine involution especially in cows with uterine disease. This supports the suggestion that postpartum suppression of ovulation, and thus delayed secretion of P4, enhances the reduction in uterine size and the elimination of inflammation and bacterial contamination. These findings advocate against early induction of ovulation in cows with postpartum uterine disease.

## Conclusions

Reduced fertility in dairy cows is not an inevitable consequence of high milk yield. Instead, production diseases, as well as infertility, may be caused by a variety of different factors including the consequence of an insufficient adaptation capacity of the respective cows to cope with the metabolic challenge during the transition period. Both, metabolic disorders and inflammatory processes have multifarious negative impacts on the onset of ovarian activity after parturition, uterine involution and oestrus behavior (Fig. 5). Until now, research has focused mainly on either the effects of NEB or production diseases on reproductive function. In future, more investigations should be performed to understand what happens to the reproductive system in cows experiencing NEB while suffering from a production disease at the same time.



Fig. 5. Schematic outline of the interaction between metabolism and production dieases and their effects on reproductive performance of dairy cows.

Moreover, there is a lack of knowledge about the influence of robustness in dairy cows. Many high-yielding dairy cows remain healthy and fertile resulting in longevity. The differences and influence of the metabolic constellation contributing to fertility between higher yielding and lower yielding cows need specific attention and further clarification.

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