

# Uterine torsion in relation to blood calcium concentration in dairy cattle



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## Abstract

An important reason for dystocia in cattle during parturition is a uterine torsion. When a uterine torsion is present, there is a rotation of the uterus around the longitudinal axis.

For every animal calcium homeostasis is of great importance. The blood calcium concentration of cows is between the 2,3 and 3,2 mmol/L. During parturition the calcium concentration of the blood decreases. Research will be conducted to test whether hypocalcaemia can be related to the etiology of a uterine torsion.

Various veterinary practices in the Netherlands have collected blood samples for this study. When a cow with uterine torsion is noticed, a blood sample from the jugular vein or the median caudal vein will be taken by the local practitioner. In this sample the calcium level will be measured by the practitioner. A blood sample was also collected for the control cow, this is the first pregnant cow on the same farm that will give birth a short period of time after having taken the blood sample.

A major finding of the study is that cows with a uterine torsion have significant ( $p=0,05$ ) lower calcium concentrations of  $1,92 \pm 0,35$  mmol/L compared with the control cows of  $2,21 \pm 0,20$  mmol/L.

To conclude, in this study lower blood calcium concentrations in animals with a uterine torsion compared to control animals is found. However, based on this study the relationship with a uterine torsion is difficult to explain. There are several reasons for the calcium concentration to be low in cows with a uterine torsion. During parturition, cows experience stress, high oestrogen concentrations and a reduced feed intake. Maybe the calcium concentration around parturition from this study must be adapted. Based on our study normal blood calcium concentrations in cows around parturition could be set to 1,81 – 2,61 mmol/L.

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## Introduction

An important reason for dystocia in cattle during parturition is a uterine torsion. When a uterine torsion is present, a rotation of the uterus around the longitudinal axis can be found (E.Erteld, 2012). Due to the torsion, the calf cannot be born through the narrowing birth canal. Without any rapid obstetrical intervention both the cow's and calf's life are in danger (Monika Kruse, 2004). In cattle, torsions to the left are more often diagnosed compared to torsions to the right. A uterine torsion usually arises in cattle during parturition or at the end of gestation. The blood supply of the uterine wall is blocked due to the rotation resulting in diminished blood flow through the uterus and formation of oedema in the uterine wall. This also results in oxygen deficiency for the calf. This situation cannot endure for too long (Bemers, 2009). In order to diagnose a uterine torsion, rectal palpation is performed. To determine the direction of the torsion, the direction of the broad ligaments is important.

Lyons et al. (2013) noticed that the incidence of a uterine torsion during parturition was 0,24%. Up to 23% of all the dystocia were due to uterine torsions (Lyons et al., 2013). Faria and Simoes (2015) investigated Holstein-Friesian cows and found that 24,4% of all dystocia was caused by uterine torsions (Faria & Simões, 2015). In dairy cattle the frequency of uterine torsions is higher compared to beef cattle (Aubry et al., 2008). In dairy cattle the incidence is 24,4% of all dystocia's and by beef cattle by 3% of all dystocia's (Faria & Simões, 2015; Sloss & Johnston, 1967).

Currently, the pathogenesis of the uterine torsion is unclear. This creates a gap in the existing literature. There is general agreement on the fact that due to the anatomy of the cow, the cow is predisposed to developing a uterine torsion. Several etiological factors have been mentioned as explanatory variables. Recently, it has been suggested from a veterinary practice that there might be a relationship between low calcium concentrations and the occurrence of uterine torsions. This possible relationship between blood calcium concentration and a uterine torsion have never be reported or is however never investigated.

For every animal calcium homeostasis is of great importance. Different reference values of calcium are used in literature, ranges from 2,3 -3,2 mmol/L and 2,11-2.75 mmol/L (JPHM Vossen, 2016; Lumb & Jones, 1996). During parturition, blood calcium concentrations are lower than normal reference ranges from literature (Hernández-Castellano et al., 2017).

In this study, the possible role of calcium in the etiology of uterine torsion is investigated. First a literature review will be provided with respect to the current understanding of the etiology of uterine torsions. This review aims to provide a deeper understanding about the etiology of uterine torsions. Furthermore, it aims to provide an insight into the occurrence of hypocalcaemia around parturition. In order to further investigate the occurrence of hypocalcaemia in combination with uterine torsions a field study is performed. Research will be conducted to test whether hypocalcaemia can be related to a uterine torsion under Dutch circumstances.

## Literature review

In this study at first a literature review was performed. The used databases were Pubmed, Scopes, CAB Abstract, books and other educational materials. The following keywords were used independently or in combination with other key words: uterine torsion, dairy cattle, calcium, hypocalcaemia, uterine torsion related to calcium, therapy of uterine torsion, beef cattle, incidence, season, parity, stress, oestrogen, calcium absorption, parturition, fasting, prevention, dystocia, pathogenesis, etiology, bovine uterus, age, foetal fluids, large abdomen, weight, sex of the calf, lactation number, primiparous cows, multiparous cows, blood calcium concentration, calcium metabolism, ionized calcium, PTH, vitamin D, hypercalcemia, lactogenesis, mammary glands, intestines, osteoclasts, osteoblasts, serotonin, calving, metabolic acidosis, feed intake, oxidative stress, starvation, infusion, inhibit bone response, calcium measurements, SPSS, statistics, equipment, nutrition, muscle contraction and milkfever.

The articles were selected based on relevance, publication date and publication in a scientific journal. References used in cited articles were also examined.

## Uterine torsion

There are different types of dystocia in cattle. One of the causes is a uterine torsion which is seen in all domestic species. Between species there is a wide variation in incidence due to the differences in suspension of the tubular genital tract, which affects the stability of the gravid tract. The most common appearance of a uterine torsion in a cow is rotation of the uterus around its longitudinal axis with twisting of the anterior vagina (E. Noakes & J. Parkinson, 2009). Most common is a degree of torsion between 180 and 360 degrees. In 60 till 90 percent of the cases the direction of the torsion in the cow is counter-clockwise (Mock et al., 2015).

At first, symptoms of a uterine torsion are not well recognised. In the first stage of parturition the cow will be likely to act in a normal way. Signs of first stage labour are shown such as restlessness, abdominal pain due to myometrial contractions and cervical dilatation. The first symptoms of a uterine torsion are that the period of restlessness endures abnormally long or the restlessness wanes and there is no progression into the second stage of labour. The symptoms seen are depression of the lumbosacral spine, tachycardia, pyrexia, tachypnoea, anorexia and vaginal discharge. On palpation of the anterior vagina a stenosis is noticeable. To indicate the direction of the uterine torsion, the walls must be palpate, which feel like oblique spirals. When the twist is precervical, before the cervix, the diagnosis can be made by palpating the uterus per rectum. Several treatments of a uterine torsion are possible such as rotation of the foetus per vaginam, rotation of the cow's body due to rolling and surgical correction (E. Noakes & J. Parkinson, 2009), but these corrections methods of a uterine torsion will not be discussed in this paper.

A uterine torsion is likely to happen due to instability of the bovine uterus. This results from the uterus being disposed cranially to its subileal suspension by the broad ligaments and the greater curvature of the organ being dorsal. There are some contributory factors during the first stage of labour. One of the factors during the first stage of parturition is the fetal movements that occur in response to the increasing frequency and amplitude of uterine contractions. Another factor is excessive fetal weight and oversized male foetuses (E. Noakes & J. Parkinson, 2009; Frazer et al., 1996). Also the age of the cow, small amount of foetal fluids, large abdomen, weight and sex of the calf are reported to be associated with uterine torsions (Mock et al., 2015).

In research performed by G.S. Frazer (1996) there was no effect of season on the occurrence of uterine torsions. During the peak of the calving season, most uterine torsions occurred. This is the period from August to November (Mock et al., 2015). There is a higher risk to develop a uterine torsion in the age of 2 to 4 years, compared to cows between 7 and 10 years of age (Frazer et al., 1996). This suggest that there is a decreased risk of uterine torsion for older cows. Probably, the fetal movement, fetal size and amount of uterine asymmetry in pregnancy are more important in the etiopathogenesis of uterine torsion than any cow specific factors such as age and parity (Frazer et al., 1996).

The etiopathogenesis of uterine torsion remains open to speculation and poorly understood. Charolais, Holstein and Brown Swiss cows have a higher risk of developing a uterine torsion, especially the Brown Swiss. Predisposing factors of the Brown Swiss are large fetal birth weights and a deep, capacious abdomen. The Jersey, Hereford and Angus breeds, cows have a lower risk of developing a uterine torsion, possibly due to the relative small fetal birth weights (Frazer et al., 1996). In dairy cattle the frequency of uterine torsions is higher compared to beef cattle (Aubry et al., 2008). Namely, in dairy cattle the incidence is 24,4% of all dystocia's and by beef cattle is 3% of all dystocia's (Faria & Simões, 2015; Sloss & Johnston, 1967). Both studies are done by cows with dystocia from different farms. The study with dairy cattle is done in Portugal and the beef cattle is done western Victoria.

The most common cause of dystocia in beef cattle is fetomaternal disproportion. A study by Sloss and Hohnston (1967) focussed on the causes of dystocia in 635 beef cattle (Sloss & Johnston, 1967).

Cause	% of all dystocia's
Fetomaternal disproportion	46
Faulty fetal disposition	26
Incomplete cervical and vaginal dilatation	9
Uterine inertia	5
Uterine torsion	3
Cervical prolapse	3
Pelvic fracture	2
Uterine rupture	2
Cervical neoplasia	0,5
Fetal abnormalities	5

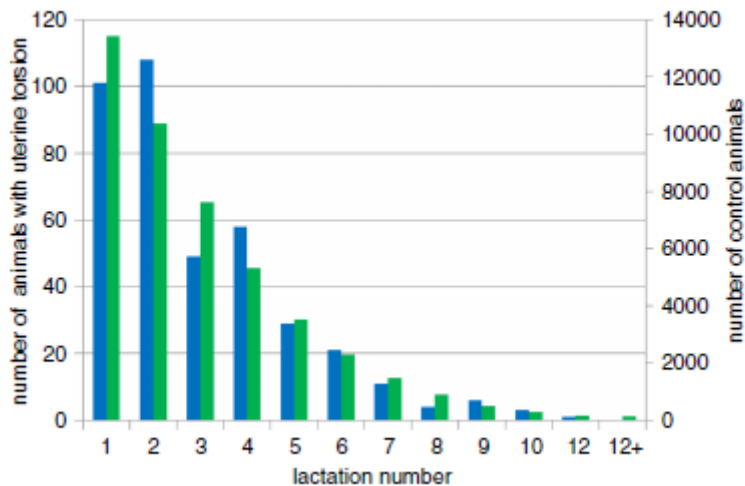
**Table 1** Causes of dystocia in 635 beef cattle (Sloss & Johnston, 1967)

In the UK, research is done on Holstein Friesian cattle with a uterine torsion, the incidence of a uterine torsion during parturition was 0,24% (Lyons et al., 2013). Up to 23% of all cases of dystocia are due to a uterine torsion. Most of them, namely 93% of the uterine torsions were corrected. 43% of the cows had further obstetrical difficulties after correction of the uterine torsion. 96% of the calves were born successfully after correction, however only 59% of the calves were born alive (Lyons et al., 2013). Another research on Holstein-Friesian cows found that 24,4% of all dystocia was caused by a uterine torsion. In this study 119 cows were evaluated, of which 29 cows had a uterine torsion (Faria & Simões, 2015).

The incidence depends on different feeding practices amongst different countries. An influence of husbandry practices probably reflects this, most notably nutrition. Cows on a concentrate diet have a smaller rumen volume than those at pasture. With a smaller rumen there is more space in the abdominal cavity for the unstable gravid uterus to occupy (Mock et al., 2015).

## Parity and uterine torsion

In an article of Mock (2015) is shown that cattle with a lower lactation number and also lower parity, have a bigger chance of developing a uterine torsion. In the first and second lactation most cases were reported, the median lactation number was two. In the lower parity more cows have a uterine torsion.



**Figure 1: Cows suffering from uterine torsion (blue bars) and control animals that calved without dystocia (green bars) distributed to lactation numbers (Mock et al., 2015)**

Frazer et al. (1996) indicated that cattle between seven and ten year of age were significantly less at risk to have a uterine torsion compared to the risk in the two to four year old group. In a study 28, 22, 24 and 14% of all the cases were first, second, third and fourth gestations. In these cases only 12% had a parity number equal to or higher than five (Frazer et al., 1996).

In the article of Aubry (2008) multiparous cows had a bigger chance to be diagnosed with a uterine torsion than primiparous cows compared to control dystocia's. Of all dystocia's, 32% were multiparous cows and 10% were primiparous cows with a uterine torsion. Of all the uterine torsions approximately 30% occurred in heifers and 70% in cows. This could be due to the larger abdominal cavity, decreased uterine tone and stretched mesometrium of cows compared to heifers (Aubry et al., 2008).

It is possible that the above figure corresponds to the parity number of the at risk cows in the general population. This means that this does not conclusively demonstrate that a previous pregnancy reduces the likelihood of a cows developing in condition (Mock et al., 2015).

In conclusion from literature currently, the pathogenesis of the uterine torsion is unclear.

There is general agreement on the fact that due to the anatomy of the cow, the cow is predisposed to developing a uterine torsion. Several etiological factors have been mentioned as explanatory variables such as fetal movements, excessive fetal weight, oversized male foetuses, age of the cow, small amount of foetal fluids, large abdomen, sex of the calf and lack of exercise (E. Noakes & J. Parkinson, 2009; Frazer et al., 1996; Mee, 2004). This means that there are several factors that could influence the developing of a uterine torsion.

In a study with buffalo serum with respect to a uterine torsion, the calcium level decreased with increasing duration of the parturition (Swelum et al., 2012). The concentration of calcium was higher when the foetus stayed alive compared to a dead foetus and calcium levels were within reference range again 24 hours after calving. This indicates that there is a correlation between

blood calcium concentrations and uterine torsions, the calcium concentration will be influenced by the duration and the severity of uterine torsion (Swelum et al., 2012).

## Calcium

For every animal calcium homeostasis is of great importance, because of the chances of extremely positive and extremely negative fluctuation of blood calcium concentrations. Extreme fluctuations can have lethal consequences. The primary functions of calcium are neurotransmitter release, membrane permeability, muscle contraction, enzyme activation, intracellular second messenger, hormone release, bone formation, blood coagulation, cell motility, cellular differentiation, apoptosis and cell secretion (Larry R. Engelking, 2012).

There are physiological mechanisms to regulate blood calcium. These mechanisms respond by modulation of enhancement of gastrointestinal absorption, absorption of bone and urinary excretion. At the end of gestation, dairy cows start producing colostrum whereby calcium is necessary. During calving, calcium is used as well, for example for the contractions of the uterus. This represents a problem for the calcium homeostasis, which can result in hypocalcaemia during calving (Martín-Tereso et al., 2016). Cows that are unable to maintain normal blood calcium levels during calving have hypocalcaemia, a metabolic disorder. Hypocalcaemia is associated with multiple postpartum disorders such as uterine prolapse, mastitis, endometritis, retained fetal membranes, dystocia, poor fertility and reduced abomasum and rumen motility. Hypocalcaemia in multiparous cows can occur in 54% of the cows (Benzaquen et al., 2015).

## Calcium metabolism

Calcium can be found in the blood in three different forms: ionized calcium, complexed calcium (with citrate, bicarbonate or phosphate) and calcium bound to serum proteins (mainly albumin, minor  $\alpha$ - and  $\beta$ - globulins). If the albumin concentration changes by 1 gram/dL, there is a change of the calcium level of 1 mg/dL (Bigras-Poulin & Tremblay, 1998).

The ionized calcium influences the secretion of PTH, blood coagulation and maintenance of neuromuscular excitability (Larry R. Engelking, 2012). To maintain normal body functions, the calcium concentration of the cell's cytoplasm and extracellular fluid must be regulated within a narrow range (Larry R. Engelking, 2012). The changes in ionized calcium are the most important. The normal total blood calcium concentrations in cattle is 2,3-3,2 mmol/L and for ionized calcium this is 1,0-1,3 mmol/L (JPHM Vossen, 2016). Calcium can be released by circulating storage forms such as protein bound and complexed forms (Larry R. Engelking, 2012).

The fifth most abundant element in the body is calcium. Most of the calcium, namely 99%, is stored in the skeletal system. Calcium and phosphate in the skeletal system are a storage to replenish serum deficits and are essential for bone strength. In adult animals, less than 1% of skeletal calcium is available for exchange with the extracellular fluid (Larry R. Engelking, 2012).

In the animal, the metabolic route of calcium involves the intake, digestion, intestinal passage due to trans-epithelial membrane that calcium absorbed and endogenous faecal excretion. The calcium is absorbed in the intestine, where it mixes quickly with body fluids (Lopes et al., 2010). In the rumen, abomasum and omasum small amounts of calcium are absorbed and only play a little role in the calcium homeostasis (Veum, 2010).

When normal plasma calcium levels are present, 50% of the calcium goes to bones. A constant exchange exists between the blood and bone tissues. About 50% of the calcium in plasma that



circulates in the kidneys, is filtered in the tubules. In different parts of the kidneys calcium can be reabsorbed (Lopes et al., 2010).

Calcium in food and liquid is absorbed by the intestines due to digestive secretions, desquamated mucosal cells and through tight junctions (Larry R. Engelking, 2012). The absorption in the intestines is a three-step mechanism. In neutral pH calcium is not ionized. When calcium gets in contact with the HCl in the stomach, the calcium salts solubilize and frees calcium from dietary protein. This makes absorption in the small intestine possible. The first step in this mechanism is the opening of calcium channels in mucosal cell membranes. Vitamin D is responsible for the increase of calcium influx from the lumen. Vitamin D increases the efficiency of calcium absorption in the duodenum with 40-70%. The buffer action of calbindin, a calcium binding protein, is maintained for the gradient of calcium influx, which is the second step in the mechanism. The last step is calcium ATPase facilitating the pumping of calcium out of the mucosal cells (Larry R. Engelking, 2012). Not all of the calcium in the intestines is absorbed, thus a part of it is excreted in the faeces.

When an animal suffers from alkalemia, plasma pH is high and plasma proteins are ionized, thus more proteins can bind calcium. In acidaemia, plasma pH is low, the proteins are less ionized and there is less calcium binding capacity of these proteins. During acidaemia it is possible that hypocalcaemia arises. In cases of hypercalcemia a decreased neuromuscular excitability is manifest. Opposite, in hypocalcaemia there is an increased neuromuscular excitability (Larry R. Engelking, 2012).

In an adult non-pregnant animal there is a balance between the renal calcium excretion and the intestinal absorption to maintain normal calcium levels. This is achieved by increasing intestinal calcium absorption, renal tubular calcium reabsorption and bone resorption. When an animal is growing or pregnant, the intestinal absorption exceeds urinary excretion. In the newly formed fetal tissues and bone calcium accumulates (Larry R. Engelking, 2012).

In all domestic animal species the serum calcium, phosphate and magnesium levels are regulated due to PTH, vitamin D and calcitonin on the bone, gastrointestinal tract and kidneys. PTH and the active form of vitamin D are responsible for raising serum calcium levels. The counterbalance is calcitonin. Glucocorticoids, glucagon, oestrogens and growth hormone are hormones that play a minor role in the regulation of calcium homeostasis (Larry R. Engelking, 2012). Parathyroid (PTH) is an endocrine secretion of the parathyroid glands. It interacts with vitamin D and calcitonin to maintain phosphate, magnesium and calcium homeostasis. The greatest influence on PTH release is a low serum ionized calcium concentration. There is a positive feedback on the parathyroid gland. Osteoblast have receptors for Vitamin D and PTH. A high dose of PTH inactivates the osteoblast, indirectly this activates the osteoclasts and these will break down bone tissue. When the calcium concentration is too low, there must be a coordination between the renal mechanism and the bone degradation to increase the plasma concentration ionized calcium (Larry R. Engelking, 2012).

### Calcium concentration in cows around parturition

A cow around calving has to perform two important tasks namely parturition and lactogenesis. The cow must increase its energy intake and calcium metabolism. The normal calcium concentration of cows is 2,3 – 3,2 mmol/L (JPHM Vossen, 2016). Lactogenesis is a high demand on calcium metabolism. For the flow of calcium to the mammary gland there needs to be an increase in intestinal absorption and a greater mobilisation of calcium from the bone. This means that around parturition there is a drop in serum calcium. Calcium is important for muscle

contraction and this affects the function and motility of the rumen, intestine, abomasum and uterus (Hernández-Castellano et al., 2017).

There is a relationship between the serum calcium values and the cow's age. The total serum calcium values were negatively correlated with age (Bigras-Poulin & Tremblay, 1998). Possible explanations are the decrease in bone resorption efficiency, or the dry matter intake and the efficiency of a decrease in intestinal absorption (Anderson, 1991). In human the number of osteoclasts and osteoblasts reduce with age (Anderson, 1991). Recently these same phenomenon is seen in cows (Smith, 2014).

There are other factors in periparturient cows that can explain the lower calcium concentrations. The calcium homeostatic mechanisms in general have a low response, this means that cows cannot react fast enough when calcium concentrations decrease. It takes time for the calcium concentration to be within reference ranges (Bigras-Poulin & Tremblay, 1998).

During the last five days prepartum the oestrogens concentrations are higher (P.L. Senger, 2005). Oestrogens reduce feed intake and inhibit bone resorption, which results in a decrease in calcium absorption from the intestine and in bone responsiveness to parathyroid hormone (Bigras-Poulin & Tremblay, 1998). In skeletal homeostasis oestrogen, estrone and estradiol-17 $\beta$  play an important role. These are potent inhibitors of bone resorption. Osteoclasts are targeted directly by the hormone oestrogen. The osteoclast activity is suppressed when oestrogen concentration is high (Devkota et al., 2015; Hollis et al., 1981).

In the last days before parturition the demand of the mammary glands on calcium increase (Bigras-Poulin & Tremblay, 1998). These effects could explain the lower calcium concentrations around parturition in cows. The higher demand of calcium in the circulation, means that the output of calcium from the circulation must be balanced. This is possible by increasing the intestinal absorption of calcium and by increasing bone resorption (Yousuf et al., 2016). Because the route of calcium in the placenta is unidirectional, the foetus cannot be used as a calcium source. This means that for an optimum homeostasis the absorption from gastrointestinal tract and resorption of calcium from the bone are essential (Yousuf et al., 2016). Cows with higher estradiol concentrations at parturition have a greater risk to develop hypocalcaemia. After calving the estradiol concentration decrease rapidly, osteoclasts are activated and the bone resorption starts (Devkota et al., 2015). The blood calcium concentration depends on the milk productivity of the animal (Yousuf et al., 2016).

A study done by Yousuf (2016) focussed on the nutritional status of cows around parturition. The blood calcium concentration was measured in three periods. Period one was a month before the estimated date of parturition, the second period was 7 days after parturition and period three was measured two months after parturition (Table 2) (Yousuf et al., 2016).

	Period one	Period two	Period three
Ca (mg/dL)	9,3 $\pm$ 1,8	6,5 $\pm$ 1,0	7,2 $\pm$ 0,9
Ca (mmol/L)	2,33 $\pm$ 0,45	1,63 $\pm$ 0,25	1,8 $\pm$ 0,23

**Table 2** Measured calcium in three different periods around parturition (Yousuf et al., 2016)

In period one significant higher calcium level were found. The lowest calcium value was found in the second period. One week after parturition the serum calcium level decreased significantly. Two months after parturition the serum calcium level increased, but the concentration did not reach the value of period one. This can be explained because the cows need more calcium for colostrum and milk production. The serum levels of calcium, compared

to one month before and after calving, decreased significantly during calving (Yousuf et al., 2016).

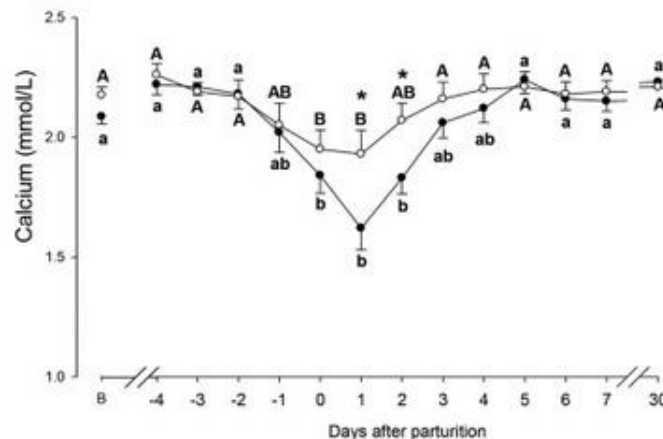
The book Veterinary Medicine describes that the calcium concentration during parturition is between 2,1 and 2,6 mmol/L (O.M. Radostits et al., 2007).

In a research with multiparous Holstein cows with dystocia, calcium levels were measured. There were two groups, namely the cows with dystocia and the cows without dystocia. In this study, it has been found that cows with dystocia had a decreased plasma calcium concentration compared to cows with a normal parturition (Benzaquen et al., 2015).

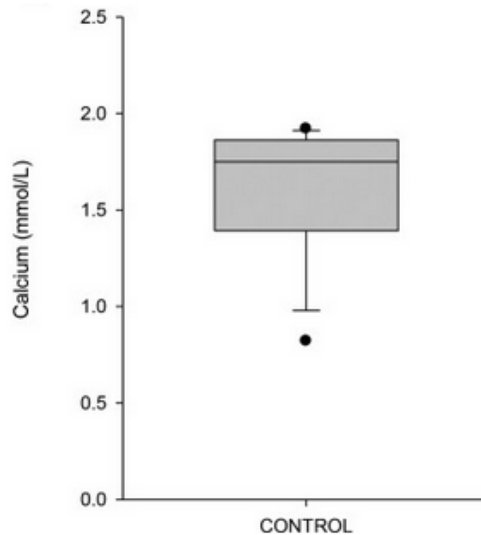
In 2017 Hernández-Castellano did a research on calcium homeostasis in dairy cows. One of the hormones this research focussed on is serum serotonin, a key factor for calcium homeostasis that modulates the blood calcium concentration. Serotin (5-hydroxytryptamine) is synthesized in many peripheral tissues as the mammary gland and in the central nervous system. It is related to mood or appetite (Hernández-Castellano et al., 2017).

Every morning, until seven days after parturition, blood samples were collected before calving- There were two groups consisting of ten animals, one control group (a,b) and the research group (A,B) that were given serotonin (figure 2). The chart with the black circles in figure 2 shows the control group (Hernández-Castellano et al., 2017). The boxplot (figure 3) shows that a lot of cows have a calcium concentration below the normal value of 2,3-3,2 mmol/L (JPHM Vossen, 2016).

In the research is found that cows that receive serotonin before parturition seemed to be able to mobilize more calcium from their bones compared to the control group. The difference between the two groups can be explained due to other physiological mechanism that regulate calcium homeostasis such as renal retention and intestinal absorption (Hernández-Castellano et al., 2017). Maybe this shows that the decline of calcium concentration around parturition is physiological and not pathophysiological.

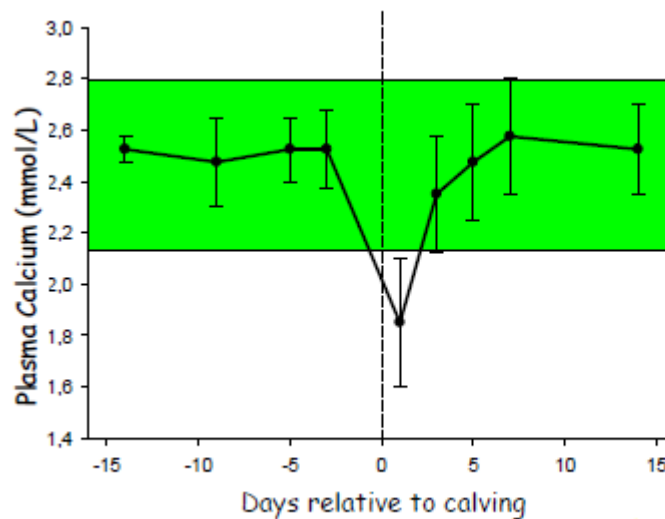


**Figure 2** Calcium concentrations of cows (control; ●) or administered prepartum 1 mg/kg of BW of serotonin (5-HTP; ○) during the experimental period. Different lowercase letters (a,b) indicate significant ( $P < 0.05$ ) differences between time points within control group. Different uppercase letters (A,B) indicate significant ( $P < 0.05$ ) differences between time points within 5-HTP group. \*Indicates a significant ( $P < 0.05$ ) difference between control and 5-HTP groups within each time point (Hernández-Castellano et al., 2017).



**Figure 3** Boxplot of control group of serum calcium (Hernández-Castellano et al., 2017)

In a study conducted by Grünberg et al. (2011) the calcium concentration around calving was lower too and during one day after calving lower than the normal values for calcium (figure 4). This was due to metabolic acidosis, that arises by lack of energy and sustained effort, and a decreased calcium flux caused by a decreased calcium absorption from the intestinal tract, increased calcium excretion in urine and mobilization of calcium from bone. The metabolic acidosis can result from several deleterious metabolic effects such as a decreased feed intake, increased protein catabolism and decreased insulin responsiveness and sensitivity (Grünberg et al., 2011).



**Figure 4** plasma concentration in mmol/L in days relative to calving. The green bar in the figure are the reference of calcium concentrations in the blood by a healthy cow (Grünberg et al., 2011)

### Calcium and parity

A decrease in the plasma calcium concentration is related to parity. Research conducted by Martín-Tereso (2016) showed that the need of receiving blood calcium infusion after parturition depended on the parity and milk production level from the preceding lactation. Multiparous cows have been through more lactations and required calcium on a daily basis. In these cows a down regulation of gastrointestinal absorption of calcium was present. It required time in order for the calcium absorption to be up-regulated. In multiparous cows the delay of up-regulation of intestinal absorption is enormous, during calving there is also a reduced feed intake and the lactation starts. This can explain the fact that in higher parity cows the blood calcium concentration decreased more compared to lower parity cows (Martín-Tereso et al., 2016).

### The influence of stress on calcium

The period around calving is accompanied by metabolic and oxidative stress (Bühler et al., 2017). An acute state of stress is induced during exercise. The process of calving is comparable to exercise. In a variety of tissues the presence of oxidized molecules increases. The intensity, duration of exercise and mechanical stress can have impact on the extent of oxidation (Fisher-Wellman & Bloomer, 2009). Oxidative stress occurs due to an imbalance between production of nitrogen species and reactive oxygen. The elimination of nitrogen species and reactive oxygen due to protective mechanisms leads to oxidative stress. This causes cellular dysfunctions, a loss of energy metabolism, gene mutations, impaired cellular transport mechanisms and an altered cell signalling and cell cycle. Normally the absorption of calcium in the intestines depends on the physiological needs of calcium. During stress the intestine responds inadequate. In the intestines the calcium absorption is inhibited. Stress induces a decrease in calcium concentration.

This means that around parturition, when the cow experiences stress, the amount of calcium absorption is insufficient. There is a loss of calcium homeostasis. This can contribute to a reduced concentration of calcium around calving (Barboza et al., 2017). When the cow has a uterine torsion, the duration of stress increases. The longer the duration of stress, the greater the deviation of the calcium homeostasis, the lower the blood calcium concentration (Fisher-Wellman & Bloomer, 2009).

### Decrease of calcium concentration after fasting

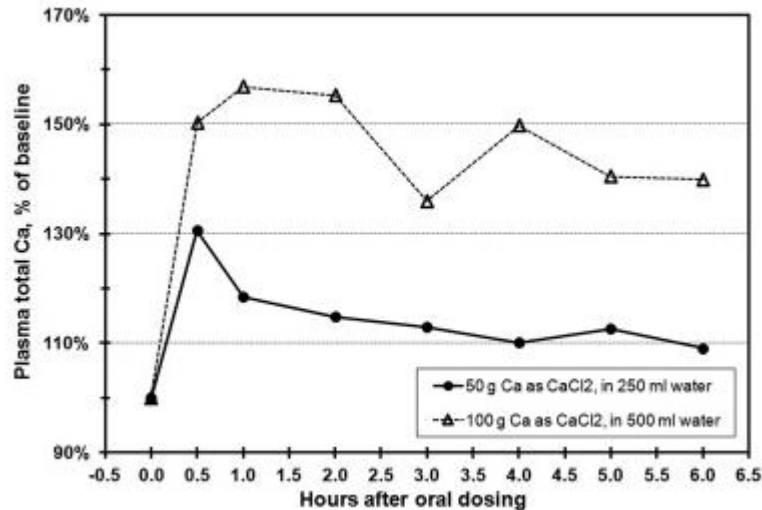
Short-term starvation influences calcium ion homeostasis. The effect of starvation on the total plasma calcium concentration and blood ionized calcium concentration has been determined in a study on rats. In the rat three days of starvation caused a reduction of the ionized calcium concentration and a significant drop in total calcium concentration. In another study with rats, a decline in plasma calcium concentrations was observed after 6 hours of fasting. The fall in total calcium concentration was paralleled by a fall in the blood ionized calcium concentration. This fall of calcium concentration occurred due to a fall in blood pH associated with starvation. The most likely cause of the decreased calcium concentration, was the reduced calcium intake due to starvation (Thompson et al., 1989).

Fasting influences the regulation of production and degradation of calcium-regulating hormones such as insulin-like growth factor, PTH and  $1,25(\text{OH})_2\text{D}_3$ , the bone formation and resorption decrease. Results of a study done by Kawane in 1997 on rats show that the longer the rats starved, the lower the blood calcium concentration. Already after one day, there was a decrease in calcium concentration measured (Kawane et al., 1997).

Another publication mentions that after a period of starvation, the chance of hypocalcaemia in cows increases. The calcium concentration decreases and the parathyroid hormone increases. So it is essential for the cow's appetite to remain high around the time of parturition, even brief starvation may result in imbalance (Allen & Sansom, 1985; Tollman & Gautvik, 1980). The parathyroid hormone increase is reversible, so it is considered secondary to the low calcium concentration. After refeeding, the plasma parathyroid hormone level decreased and the plasma calcium concentration reached a value within reference ranges (Tollman & Gautvik, 1980). Other research has been carried out to determine the effect of two days starving period in two cows on their calcium concentration. After two days, in both cows the calcium concentration reached a value lower than 7mg/dL, which corresponds to 1,75 mmol/L. The cows did not show any clinical disturbance, except for a low pulse and lower respiration rates (Halse, 1958). Their feed intake during parturition was lower too, but the cows did not starve for two days. Due to the reduced feed intake, the calcium intake was lower, so the calcium concentration in the blood were lower.

### Prevention of hypocalcaemia

There are some methods to prevent hypocalcaemia after calving such as a low calcium diet ante-partum, calcium binders, oral calcium around calving, vitamin D, anionic salts (DCAD diets) and calcium infusion intravenous or subcutaneously (Walter Grünberg, 2015). It is essential that the calcium content is low during the non-lactating period. In dairy cows 14 days ante partum calcium requirements are around 30 gram calcium per day. During the last ten days of pregnancy the daily calcium intake should not exceed 20 gram calcium per day in order to trigger the negative calcium balance. However, this is difficult to apply under field conditions. Calcium binders, for example natrium-Aluminimsilicate, need to be administered a few days before parturition. This binds the dietary calcium, which makes the calcium unavailable for absorption. When the negative calcium balance is induced, PTH release and activation of vitamin D3 is triggered. Although this stimulates the absorption of calcium, it has a negative effect on feed intake, for it reduces dry matter intake for 20 to 50%. It is successful on serum calcium around parturition in dairy cows in third and plus lactation (Grünberg, 2015). Administering oral calcium to cows around calving results in an increase in calcium concentration. The oral calcium influences the calcium absorption and blood calcium responses. The most effective way to increase the blood calcium concentrations is to use calcium chloride. It is preferred to give a small oral dose (50 gram CaCl<sub>2</sub>), because this provides the best absorption. When 100 gram CaCl<sub>2</sub> is given, it is possible for the blood calcium concentration to be excessively high (figure 5). This can result in a decrease of the cow's own calcium homeostatic mechanisms. The oral calcium supplements are used for cows with early clinical signs of hypocalcaemia and lessens the negative impact of hypocalcaemia (Garrett R. Oetzel, 2013).



**Figure 5** Effect of two different doses of oral calcium chloride on plasma total calcium concentrations (Garrett R. Oetzel, 2013)

Another way to prevent hypocalcaemia is to give vitamin D parenteral. This vitamin D needs to be given between ten and four days before the expected calving in a dose of 10 to 15 million I.U. The parenteral vitamin D has a narrow therapeutic range. Doses higher than 17 million I.U. cause death within days. It is ineffective if the dose is below the recommended dose. It gives a higher calcium concentration in the serum for a short time after calving and increases the incidence of subclinical hypocalcaemia. Vitamin D can also be administered orally. This has a similar effect to parenteral treatment, but it is safer. It needs to be given five days before calving (Grünberg, 2015).

To prevent hypocalcaemia it is also possible to feed dairy cows low dietary cation-anion differences during late gestation, because this reduces the incidence of hypocalcaemia. This diet needs to be given three weeks before the expected calving date. The fully compensated metabolic acidosis increased calcium influx, this resulted in increased plasma calcium concentration one day after calving (Grünberg et al., 2011).

Intravenous calcium infusion is a treatment for recumbent hypercalcaemic cows. Infusion can change hypocalcaemia into hypercalcaemia (figure 6). It interrupted the PTH secretion and activate vitamin D. Due to these two changes there is a decreased bone mobilization and decreased intestinal calcium absorption (Grünberg, 2015).

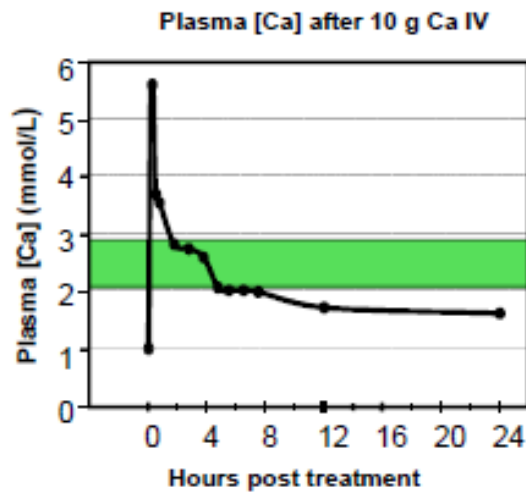


Figure 6 Calcium blood plasma concentration after 10 gram calcium intravenous (Walter Grünberg, 2015)

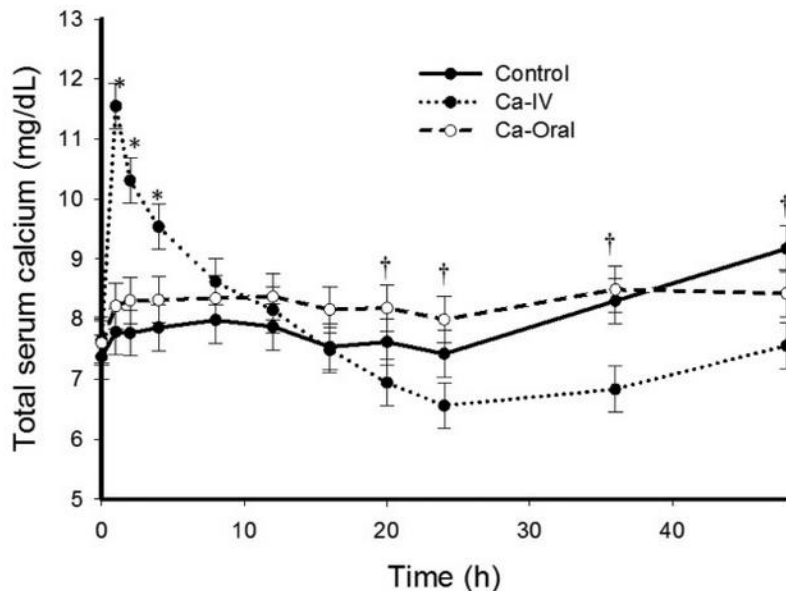


Figure 7 Total serum calcium levels after postpartum treatment of subclinical hypocalcemia in control, intravenous and oral calcium (Blanc et al., 2014)

Sometimes calcium infusion is administered on a preventive base. When a farmer or veterinarian suspects hypocalcaemia, they are quickly inclined to give some calcium intravenously. This disturbs the regulation mechanism of the calcium homeostasis and increases the risk of subclinical hypocalcaemia for at least 48 hours after treatment (Blanc et al., 2014). When someone suspects hypocalcaemia, it is preferred to give them an oral calcium bolus instead of calcium infusion (Blanc et al., 2014). Cows with calcium infusion (Ca-IV) show a rapid increase in serum calcium concentration ten minutes after treatment (figure 7). But from 5 to 24 hours after treatment cows were already in a hypocalcaemic stage. Due to the hypercalcemia that occurs directly after the infusion, there is a decrease in blood parathyroid hormone levels and a decrease in renal and bone calcium reabsorption. The negative consequence is the longer period of hypocalcaemia in cows a few hours after treatment. Cows receiving oral calcium have higher blood calcium concentrations during the first 24 hours after



treatment compared to control cows with no treatment (figure 7). This could be due to the reduced feed intake (Blanc et al., 2014).

### Uterine torsion related to hypocalcaemia

In an article from Aubry et al. (2008) 47 cows with an uterine torsion were evaluated, twelve of them had clinical signs of hypocalcaemia and received calcium. Twenty cows were given calcium preventively after being diagnosed with a uterine torsion, so two-thirds of the cows with a uterine torsion were treated with calcium. These were older cows and these cows had a history of hypocalcaemia in a previous lactation, so these cows were considered at risk and were given calcium. Cows with hypocalcaemia had a higher chance of developing a uterine torsion compared to the heifers that did not have hypocalcaemia. Milk fever in primiparous animals is rare. Heifers with a uterine torsion show more severe signs than cows with other types of dystocia (Aubry et al., 2008).

In conclusion, the calcium concentration around calving was lower than normal values 2,3-3,2 mmol/L (Grünberg et al., 2011; JPHM Vossen, 2016). There are a few reasons why calcium concentrations is lower during parturition such as stress, starvation, age of the cow, start of lactogenesis, uterine contraction during parturition, higher oestrogens concentrations around parturition and inhibit bone resorption. When the cow has a uterine torsion, the duration and intensity of stress increases. The longer the duration of stress, the greater the deviation of the calcium homeostasis, the lower the blood calcium concentration (Fisher-Wellman & Bloomer, 2009). Cows with a uterine torsion has a longer period of starvation. Cows with hypocalcaemia had a higher chance of developing a uterine torsion (Aubry et al., 2008). Maybe there is a relation between uterine torsion and calcium.

## Materials and methods

There might be a relationship between low calcium concentrations and the occurrence of uterine torsions. In this study, the possible role of calcium in the etiology of uterine torsion is investigated.

### *Animals*

In this research five various veterinary practices in the Netherlands collected blood samples from the jugular vein or the median caudal vein from cows with a uterine torsion. In this sample the calcium level was measured by the practitioner.

Calcium values were reported in an Excel file. The local practitioner noted the date when the sample was retrieved, cow identification number, its parity and the expected date of parturition of the patient. The same information including a blood sample was collected for the control animal too. The control animal is the first pregnant cow on the same farm that will give birth soon.

Maybe the season and farms have an influence on the calcium concentrations or the uterine torsion. Because of that, blood samples of the control group are also taken on the same farms as the blood samples of the uterine torsion cows. Before the study starts, one veterinary practice already collected blood samples from cows with a uterine torsion, not from control cows. During the study there were a few cows with a uterine torsion, so there were a few control cows too. Therefore the control group is very small, namely 17. Blood samples were available from animals around parturition from the teaching farm that belongs to the University Utrecht. In the blood samples from the University Utrecht cows with clinical symptoms were present too. These blood samples are combined with blood samples of the control cows from this study. To get the best conclusion about the relationship between low blood calcium concentrations and the occurrence of uterine torsions different control groups were used.

### Group 1: General control cows (GCC)

During the prospective part of the study the incidence of the uterine torsion was low, therefore the control group was very small, namely 17 cows. To increase the control group, other sets of animals during parturition were included from the teaching farm of the University Utrecht. This control group existed of animals with clinical phenomena such as hypocalcaemia and cows that were not yet at the end of the gestation.

	General control cows	N
Ca	Uterine torsion	69
	Control	75

**Table 3 Group 1: General control cows (GCC)**

### Group 2: Healthy control cows (HCC)

In this group all the cows without clinical symptoms such as hypocalcaemia and all cows that were calving in one day and cows two day after calving, were collected from group 1. This method was based on the study of Hernández-Castellano et al. 2017.

	Healthy control cows	N
Ca	Uterine torsion	69
	Control	12

**Table 4 Group 2: Healthy control cows (HCC)**

### Group 3: Calved healthy control cows (CHCC)

From another study, calcium samples were available too. These samples came from six animals between one and two days after parturition, from University Utrecht. These samples are taken as a control from animals that are perfectly healthy and not showing clinical hypocalcaemia. These samples were taken from a teaching dairy farm from the University Utrecht in the period of November 2016 till May 2017.

	Calved healthy cows	N
Ca	Uterine torsion	69
	Control	6

**Table 5 Group 3: Calved healthy control cows (CHCC)**

This research was approved by Animal Care and Use Ethical Committee of the Utrecht University.

#### *Calcium measurements*

The calcium concentration was measured by the veterinarian. Most of the samples have been taken in the months April 2017 till June 2017. One veterinary practice collected the samples since April 2016. Calcium levels of the uterine torsion cows and the three control groups were measured by Idexx Catalyst One, Spotchem and Fugix 500 I. In Utrecht the AU 680 Beckman Coulter was used for the calcium measurement.

In the results section the calcium concentration of cows with a uterine torsion were compared to the concentration of cows that were included by each of the three control groups.

#### *Statistics*

To execute the statistics part of this study, the calculations program SPSS was used.

First of all were tested whether there are significant differences in the calcium levels between the different groups. This was tested by conducting Independent samples t-test, in which the means of the calcium concentration are compared. An independent samples test determined whether this discrepancy in average is significant and whether the average calcium of the cows with a uterine torsion significantly deviates from the calcium concentration of the control group. Before conducting an independent sample test, a Levene's equality of variances test is conducted. The standard deviation is the difference between the average divided by the standard error difference.

Second, a regression analysis on the calcium concentrations of cows with and without uterine torsion was conducted. The regression analyses tests if an independent variable affects a dependent variable.

In group 1 (GCC) the regression analysis was expanded with parity because in literature a relationship between parity and uterine torsion was found. Alongside this analysis a scatterplot of this relation will be shown from group 1 (GCC). A significance level of  $\leq 5\%$  was taken into account.

## Results

### Group 1: General control cows (GCC)

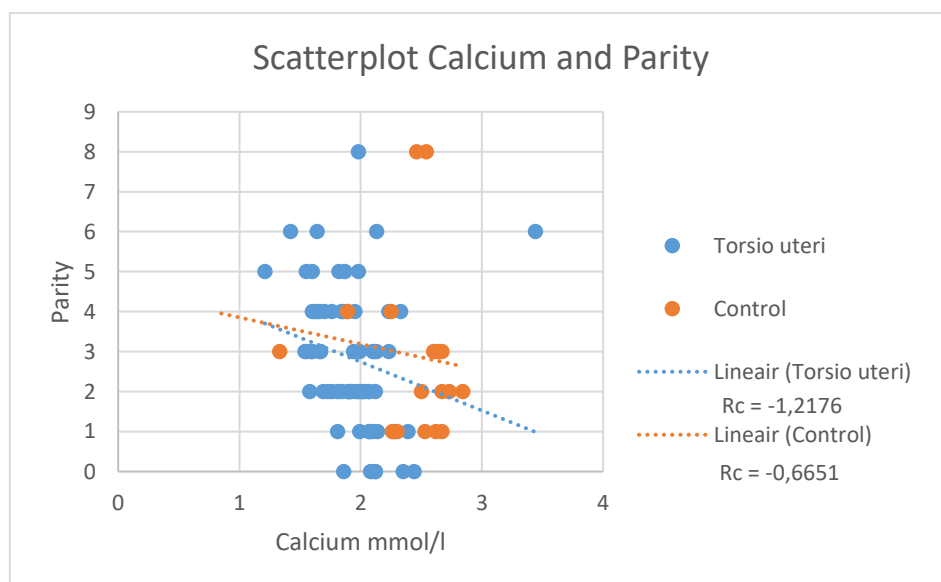
This control group included animals with clinical phenomena and cows that were not yet at the end of the gestation.

	Control	N	Mean	Std. Deviation
Ca	Uterine torsion	69	1,92	0,35
	General control cows	75	2,12	0,44

**Table 6** Descriptive statistics group 1 general control cows (GCC)

Uterine torsion cows have significant ( $p = 0,004$ ) lower calcium concentration of  $1,91 \pm 0,31$  mmol/L compared to control cows of  $2,12 \pm 0,44$  mmol/L.

Including parity to the comparison, using a regression analysis, it can be concluded that parity has no significant effect on the cow's calcium concentration ( $p = 0,83$ ).



**Figure 8** Scatterplot calcium and parity from group 1 general control cows (GCC). The blue dots are the cows with a uterine torsion and the orange dots are control group cows. The linear blue chart shows that there is a negative relationship between parity and calcium concentration.

In this scatterplot calcium and parity are compared. The lower the calcium concentration, the higher the parity. This can be explained due to the reduced numbers of osteoclasts and osteoblasts with age. Statistics, however, show that this relation is not significant, but cows with an uterine torsion have a stronger decline of calcium compared to the control group.

### Group 2: Healthy control cows (HCC)

In the second group healthy control cows (HCC) all cows without clinical symptoms such as hypocalcaemia and all cows that were calving in one day and cows two day after calving were collected, which was based on the study of Hernández-Castellano et al. (2017).

	Control	N	Mean	Std. Deviation
Ca	Uterine torsion	69	1,92	0,35
	Healthy control cows	12	2,13	0,23

**Table 7** Descriptive statistics group 2: Healthy control cows (HCC)

Also in this group, uterine torsion cows have significant ( $p=0,04$ ) lower calcium concentration of  $1,92 \pm 0,35$  mmol/L compared to the healthy control cows of  $2,13$  mmol/L  $\pm 0,23$ .

	Sig.	T	Mean difference	Std. Error Difference
Equal variances assumed	0,04	-2,05	-0,22	0,11

**Table 8** T-test calcium Group 2: Healthy control cows (HCC)

### Group 3: Calved healthy control cows (CHCC)

In this group the control group exists of 6 cows that have already calved. The cows one day before and cows two day after calving have been removed.

	Controle	N	Mean	Std. Deviation
Ca	Uterine torsion	69	1,92	0,35
	Calved healthy control cows	6	2,21	0,20

**Table 9** Descriptive statistics Group 3: Calved healthy control cows (CHCC)

Also in this group uterine torsion cows have significant lower ( $p=0,05$ ) calcium concentration of  $1,92 \pm 0,35$  mmol/L compared to the calved healthy control cows of  $2,21$  mmol/L  $\pm 0,20$ .

	Sig.	T	Mean difference	Std. Error Difference
Equal variances assumed	0,05	-1,98	-0,29	0,15

**Table 10** T-test calcium dat Group 3: Calved healthy control cows (CHCC)

## Discussion

In this study cows with uterine torsion during parturition have significant lower calcium concentrations ( $1,92 \pm 0,35$  mmol/L) compared to cows during parturition without uterine torsion ( $2,21 \pm 0,20$  mmol/L). In both groups the calcium concentration during parturition is lower compared to the normal calcium levels of 2,3-3,2 mmol/L (JPHM Vossen, 2016).

In this present study the calcium concentration in the control group is 1,81 – 2,61 mmol/L. These values were in line to the values found in literature by cows during parturition such as 2,1-2,6 mmol/L (O.M. Radostits et al., 2007), 1,0-1,9 mmol/L (Hernández-Castellano et al., 2017) and 1,6 - 2,1 mmol/L (Grünberg et al., 2011).. The range of calcium concentration is 1,29 – 2,84 mmol/L (figure 8).

In this pilot study a complete match on farm level between uterine torsion cows and control cows was not possible. Only a control sample could be taken from a cow as close as possible to calving. A revisit of the farm was not possible and the farms were anonymous, information on the actual calving date was not possible either. Control cows from the farm were therefore probably at a moment on which calcium levels were high during pregnancy (Yousuf et al., 2016). That is why group 3 (CHCC) is used in this pilot study. These cows came from one farm and where measured directly after parturition. The tendency of significance may have arisen by the small control group, namely 6 cows. For statistical analyses, a large data set is more reliable. There is a less likely chance of a random outcome. Looking at the veterinary aspect, there are some good reasons to remove some cows from the group. In this research only the calcium concentration around parturition was essential. Maybe the smaller group is not necessarily reliable, but more valid because the measure is more precise. This is the reason that in this study control group of group 3 (CHCC) is used.

The control group 3 (CHCC) exists of 6 cows from one farm. So one dairy farm will be compared to several dairy farms throughout the Netherlands. Blood calcium concentrations between farms might slightly differ, as every farmer has his own food and all have their own management what might influences the calcium level (Mock et al., 2015). When interpreting the calcium values, in an ideal setting one would control for dairy farms. In future studies farms have to be included in the statistics.

In this research all blood calcium concentrations in uterine torsion cows was determined by the veterinarian. Most veterinary practices have different measuring equipment. This might have resulted in slightly different calcium concentrations between practices, which means the calcium concentration must be interpreted with caution. Probably the differences in the calcium values are small and the influence on the conclusion did not have a great impact.

This problem can be solved by sending the same blood monster to every practice. This blood sample must be measured in all veterinary practices. To compare these calcium concentration, the deviation of the measuring equipment has to be determined. In these blood samples the laboratory of the University of Utrecht can measure the calcium concentrations and make it possible to correct the calcium concentrations of all practices, which makes the calcium values more accurate. Another way to prevent this difference between the outcomes, is to use standard calibrated devices.

For further research of this subject ‘uterine torsion in relation to calcium’ it is very important to know the blood calcium concentration during calving. In the first group (GCC) of this research control cows that were several days before parturition were incorporated as well.

Literature publication (Hernández-Castellano et al., 2017) stated that one day before calving and two days after calving the calcium concentrations are the lowest. To know the exact calcium concentration, it is necessary to collect samples during or directly after calving. One way to collect information about calcium is to measure the calcium concentration in the cow's saliva (Devi, Singh, Lathwal, Kumaresan, & Dudi, 2016), which can be collected by the farmer. In this case, it is not necessary to call the veterinarian and it is easier to collect. This could be a way to collect a lot of samples with calcium concentrations of control cows. Besides that, it is better to know what the normal blood calcium concentration from the cow is. This can be done by taking the cow's saliva daily around the parturition. This way a change in the blood calcium concentration around parturition can be measured. When the calcium concentration around calving is known, better conclusions can be drawn about the calcium concentrations in cows with uterine torsion.

This study is done by dairy cattle, but it is possible to reconnect it to beef cattle. The drop of calcium concentration arises due to stress, age, oestrogen concentration, lactogenesis, parity, preceding lactation and fasting. In general the beef cattle are not stressed so easily compared to dairy cattle, so maybe the intensity of stress is lesser. Beef cattle are not as stressed for milk production as dairy cattle and the preceding lactation is not as intensive compared by dairy cattle. Beef cattle are affected less frequently by hypocalcaemia than dairy cattle. Nonparturient hypocalcaemia occurs more by beef cattle (G. R. Oetzel, 1988).

Lower blood calcium concentrations in animals with a uterine torsion compared to control animals are found in this study. However, based on this study the relationship with a uterine torsion is difficult to explain.

There are several reasons for the calcium concentration to be low in cows with a uterine torsion. During parturition, cows experience stress, high oestrogen concentrations and a reduced feed intake. When blood calcium concentration is low it influences among other things the rumen and uterus. One of the primary functions of calcium is muscle contraction. When the blood calcium levels are low, uterine motility decreases. The bovine uterus is very instable, as mentioned earlier in this research. The uterus tilts a lot when cows walk, stand up, lay down and so on. In a healthy cow, the uterus returns to its original position. However, when the calcium concentration is low and the uterus contractions decrease, the uterus is unable to return back to its original position. This could cause a uterine torsion.

On the other hand a uterine torsion can lead to a lower blood calcium concentration. In addition food intake during parturition is in general decreased and parturition is considered as a stressful event for the mother, both possibly contributing to lower blood calcium concentrations around parturition. In a uterine torsion the parturition is longer (Mainau & Manteca, 2011), it leads to a longer period of low food intake and an longer and higher stress level of the animal compared to a normal parturition (Devkota et al., 2015; Halse, 1958). These conditions lead to changes in calcium metabolism. Therefore a uterine torsion leads a more severe decrease in blood calcium levels. In this case the lower blood calcium levels are a consequence of the uterine torsion and not its cause.

In a study with buffalo serum with respect to a uterine torsion, the calcium level decreased with increasing duration of the parturition. This indicates that there is a correlation between blood calcium concentrations and uterine torsions, the calcium concentration will be influenced by the duration and the severity of uterine torsion (Swelum et al., 2012). More plausible is that the

consequences of uterine torsion is the low blood calcium level due to the longer duration of parturition, more stress and longer period of starvation.

In literature is found that the normal calcium concentration of cows is around 2,3 – 3,2 mmol/L (JPHM Vossen, 2016). In this research is found that a cow's blood calcium concentration around parturition by dairy cattle is approximately  $2,21 \pm 0,40$  mmol/L, which is lower than the normal levels reported in literature. A publication by Hernández-Castellano et al. (2017) found that the calcium concentration in cows in parturition is lower than 2,3 mmol/L (Hernández-Castellano et al., 2017). In Lumb and Jones (1996) is described that hypocalcaemia exists when serum calcium concentrations are lower than 2,0 mmol/L. This means that most of the cows in parturition have hypocalcaemia. However clinical signs of hypocalcaemia around parturition, are not seen in every cow. Maybe the calcium concentration around parturition from this study must be adapted and probably accept that the decline of calcium concentration around parturition is physiological and not pathophysiological.

The standard deviation in this research in group 3 (CHCC) of cows without a uterine torsion was 0,20. Based on our study normal blood calcium concentrations in cows around parturition could be set to 1,81 – 2,61 mmol/L. In some literature is found that recently calved, mature cows become recumbent with serum calcium levels lower than 1,5 mmol/L. This means that in cows with a uterine torsion without paresis the calcium concentrations is probably higher than 1,5 mmol/L (Lumb & Jones, 1996). The values of blood calcium concentration by calving cows that are found in this study are amply above the concentration of 1,5 mmol/L. The control group from CHCC contains of 6 animals, so it is better to repeat this study with a larger control group.

## Conclusion

In this study cows with a uterine torsion have significant lower ( $p=0,05$ ) calcium concentration of  $1,92 \pm 0,35$  mmol/L compared to the control cows of  $2,21 \pm 0,20$  mmol/L. However, based on this study the relationship between calcium and a uterine torsion is difficult to explain. When the calcium concentration is low and the uterus contractions decrease, the uterus is unable to return back to its original position. So the low blood calcium concentration could cause a uterine torsion. Or the lower blood calcium levels are a consequence of the uterine torsion and not its cause.

More plausible is that the consequences of uterine torsion is the low blood calcium concentration. There are several reasons for the calcium concentration to be low in cows with a uterine torsion. The duration of the parturition is longer, cows experience more stress, high oestrogen concentrations and a longer period of reduced feed intake. However, based on this study the relationship with a uterine torsion is difficult to explain.

Maybe the calcium concentration around parturition from this study must be adapted. Based on our study normal blood calcium concentrations in cows around parturition could be set to 1,81 – 2,61 mmol/L. The control group contains of 6 animals, to be sure more research is needed.

## Acknowledgement

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