Physiological and clinical aspects of uterine contractility during the postpartum period in cows

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Physiological and clinical aspects of uterine contractility during the postpartum period in cows

Fysiologische en klinische aspecten van de contractiliteit van de baarmoeder bij de koe tijdens de periode na het afkalven

(met een samenvatting in het Nederlands)

A méhkontrakciók vizsgálatának élettani és klinikai vonatkozásai a tehenek ellést követő időszakában

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„Felix, qui potuit rerum cognoscere causas.”

Vergilius: Georgica
(29 BC)

Szüleimnek, továbbá
Előднek, Ildikónak, Dánielnek, Bálintnak

Contents

CHAPTER 1

General introduction

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1. Introduction to the scope of the thesis

This thesis focuses on different aspects of uterine contractility in dairy cows during the first few days after calving.

The physiological events that take place during the early postpartum period are of special interest in the light of a possible next conception and pregnancy. The first and main function of uterine contractions during the first hours and days after calving is to expel the uterine contents, which include both the fetal membranes and the lochia.

Reliability and repeatability of measurements that will accurately quantify uterine contractility changes during the early postpartum period are of crucial importance. Although numerous techniques are available to measure uterine contractions, only a few of these do not involve a surgical intervention. The advantage of using non-invasive methods is that they can also be applied under practical conditions, allowing an analysis of either physiological or pathological events associated with uterine contractility on farms, and to test the efficacy of various treatments that aim to improve uterine contractility during the puerperal stage. Therefore, there is an obvious need to develop proper, accurate and efficient techniques for recording and quantifying uterine contractions in postpartum cows that can be used on dairy farms.

The few data that have been published so far on postpartum uterine contractility in cows still do not provide accurate quantitative information as to physiological changes of uterine function after normal calvings in cows kept at large scale farms. For many years, drugs like oxytocin and prostaglandins have been routinely used to stimulate uterine contractility, either following local veterinarian customs or taking over the recommendations of the drug producing companies. So far, scientific studies that could justify such a use by demonstrating their effect on uterine contractions in postpartum cows, are missing.

The studies, presented in the following chapters of this thesis, were designed to fill some of the above-mentioned gaps in our methods and knowledge on myometrial function in postpartum dairy cows.

2. Scientific background

2.1. Events in the uterus after the calf is born

The parturition process is not completed with the delivery of the calf. A final stage -the third stage of labor-, which includes the expulsion of the fetal membranes, terminates the parturition. By definition, this postpartum period starts immediately after the birth of the calf, and it represents the first part of the puerperal phase, during which a very intense period of uterine involution takes place. During involution, the size of the uterus diminishes and both the myometrium and endometrium are restored, so that the uterus will be prepared for a next

conception. The involution is completed within 5 to 7 weeks, during which a series of physiological processes take place that could also sometimes deviate from normal pathways, thereby prolonging the duration of uterine involution.

After the expulsion of the calf, the next step is the shedding of the afterbirth. Cows have a bicornuate uterus and the placenta of the cows histologically is of the synepitheliochorial type [1], meaning that there are five tissue layers between the maternal and fetal circulation during pregnancy. Based on the distribution and arrangement of allantochorionic villi, the bovine placenta can be classified as a so-called cotyledonary placenta [2]. The allantochorionic membrane makes intimate connection with the uterine wall at areas where finger-like structures or villi form outgrowths, called cotyledons. These groups of villi interdigitate with crypts of specialized areas of the uterine endometrium, called caruncles. Together these contact areas of cotyledons and caruncles are called placentomes. There are a total number of approximately 120 functioning placentomes in a pregnant bovine uterus. Because an intimate connection of the uterus and the allantochorion membrane is only possible at the site of the placentomes, the intercaruncular endometrium is not attached but only apposed to the intercotyledonary parts of the fetal membranes. Therefore, separation of the fetal parts of the placenta only takes place within the placentomes [3]. Purely because of this type of connection and the concomitant enzymatic processes [4,5], a certain amount of time is needed for the expulsion of the placenta. In cows that do not develop a retention of their fetal membranes within 24 hours after birth of the calf (cows without retained fetal membranes; NRFM cows), expulsion of the placenta usually takes place within 6 hours after calving (in more than 75% of such cows), and only less than 5% of the remaining NRFM cows release the placenta between 12 and 24 hours [6]. Although a wide range (between 8 and 48 hours) has been reported for this physiological shedding [7], with the age of the cow significantly influencing the process [6], cases in which shedding either exceeds a period of 12 [8] or 24 hours after calving [9] are usually considered as a placental retention (retained fetal membranes; RFM) with a pathological background, in which numerous factors can be involved [10,11].

2.2. The role of myometrial contractions in evacuation of the uterine content after calving

The removal of the uterine content after calving is an important prerequisite for a proper involution is. It seems obvious that uterine contractions are actively involved in emptying the uterine cavity [12], including the expulsion of the afterbirth [11]. However, for cows, the exact role of uterine contractility in expelling the fetal membranes has not yet been clarified. The increased level of uterine activity, as observed in cows with RFM [12-14], suggests that the decrease of uterine contractility is probably not a causal factor in RFM during the first days postpartum.

Several factors and conditions are required for contraction of a muscle cell. Availability of intracellular calcium is one of the essential components and, therefore, plasma calcium levels are of clinical importance in postpartum animals. If not disturbed by abnormalities, such as a

steep decrease in blood Ca^{2+}-concentrations, the pattern of myometrial contractions abruptly changes upon the expulsion of the calf, but contractions do remain [15] and show a regular pattern [16], characterized by powerful and frequent individual pressure cycles [17,18]. However, if a cow suffers from milk fever, due to a severe reduction in the blood Ca^{2+}-concentration during the immediate post-calving stage, a flaccid uterus can be palpated and in such cases intrauterine pressure (IUP) changes can not be measured [19]. A direct negative effect of an experimentally reduced blood Ca^{2+}-concentration on myometrial activity during parturition and after expulsion of the calf has also been reported [20]. Hypocalcemia has also been associated with a higher incidence rate of RFM [21,22]. Substantial evidence suggests that in cows with subclinical hypocalcemia, involution may be delayed [23,24].

2.3. Possibilities to measure uterine contractility in postpartum cows

There are several techniques to register uterine contractility, as will be reviewed in detail in Chapter 2. Although in this thesis we also used electromyography, our studies concentrated mainly on the use of the so-called open tip type intrauterine pressure recording technique. The accuracy and practicability of using electromyography and two different intrauterine pressure recording techniques will be compared in a separate study (Chapter 3).

Electromyography (EMG) has been used in several laboratory and domestic animal species to quantify uterine activity [25]. The basic principle of EMG is the measurement of electric potential changes that are associated with the polarization and depolarisation of the membranes of myometrial cells during the contraction and relaxation periods of these cells. One of the earliest in vivo studies in farm animal species described an EMG recording technique for studying changes in myometrial activity during the ovine oestrous cycle [26]. Later experiments applied this same technique for studies in periparturient cows, providing preliminary data on myoelectrical activity during the postpartum stage as well [14,27]. Although electromyography is often used in chronic experiments, allowing continuous and repeated recordings in the same animal, during periods up to several months, it usually requires a surgical intervention for implanting the electrodes [14,27]. This factor limits its application to experimental laboratory conditions.

A possible alternative method to investigate uterine motility is the measurement of intrauterine pressure (IUP). The principle for this technique is based mainly on two major laws of physics. One of them is Laplace's Law, stating that pressure development in a closed spheroid depends on the radius of the object as well as on the tension and thickness of its wall. The other is Pascal's Law, which states that in a fluid-filled, closed system, the pressure is equal at all points. It is also known that pressure values depend on temperature changes, but this is especially true for gas-filled systems. However, even in the case of gas accumulation within the postpartum uterus, this phenomenon probably plays a minor role in changing the internal pressure values, because the temperature is regulated within a very narrow range in living organisms.

Pascal's Law assumes the involvement of fluid-filled closed systems with a solid wall. The immediate postpartum uterus of the cow only partly meets these criteria. After having expelled the calf, it contains the fetal membranes and a variable amount of fluid. Further components involved are gases originating mainly from the external air and might have entered the uterus during the parturition process.

Another relevant question is to what extent the postpartum uterus can be considered as a closed system. The cervical canal is still widely opened immediately after calving, but elements of the soft birth canal still provide a certain degree of closing, because in a physiological situation the vulva lips, the vagina and in the initial postpartum phase, the cervix are compressed by the surrounding tissues and organs of the abdominal and pelvic cavity. As puerperium advances, the involutional processes also involve regressive changes in the cervix, so that the structure of the collagen-based folds will gradually develop again. But even during these changes the uterus most likely remains closed, due to the tension, the surrounding organs exert on the soft birth canal. However, at the same time, this type of closure is only temporary and intermittent in nature, because uterine contents are slowly, but continuously and intermittently discharged. This includes the expulsion of the fetal membranes, the lochia and also gases from the uterine cavity. Resorption of fluid also occurs, but this is not considered to be a determinant factor in eliminating uterine contents during the postpartum stage in cows. Therefore, while expelling these substances, the uterine lumen obtains an open connection with outside, probably only for short periods (some seconds?) of time.

It is also of theoretical importance that the wall of the uterus has elastic properties. Laplace's Law is represented by an equation that takes elasticity into account, but it can be questioned, how that should be adjusted to the uterine wall of a contracting uterus of a postpartum cow? It is obvious that uterine muscle contractions that cause a pressure increase are associated with a reduction of the volume of the organ, because when a large population of myometrial cells shorten at the same time, the entire surface area of the uterine wall is shrinking. On the other hand, if pressure rises within the abdominal cavity, due for example to abdominal straining or coughing of the animal, the uterus will also be compressed and this results in a pressure rise within the uterine cavity. Considering all these circumstances together, it should be realized that the postpartum uterus can not always be considered as a closed system. Moreover, not only does the shape and size of the uterus change gradually due to the involutional processes that start immediately after calving, but the physical properties of the components of the uterine wall also vary.

Despite all the limitations and theoretical implications mentioned above, the measuring of IUP is an often applied tool for monitoring uterine contractility under in vivo circumstances.

2.4. The need for a system for non-invasive, accurate recording and analysis of intrauterine pressure changes in postpartum cows

In general, there are two main ways to acquire pressure signals directly from the uterine cavity of an early postpartum cow. When fluid- (or air-) filled plastic catheters, with a closed or open tip are placed inside the uterus [12,13,28], the pressure is transformed into electrical signals by a pressure

transducer, which is usually located extra-corporally. However, when catheters with a built-in miniature pressure sensor are used, pressure transformation takes place at the pressure acquisition site, i.e. within the uterus. Such microtransducer systems were first introduced for studies in women [29,30] and were at a later stage also applied in cows, although more often in non-pregnant [31-33] than in pregnant, parturient or postpartum animals [15]. Based on their construction, the microtransducer systems that are especially suitable for cardiovascular studies, are less sensitive to artefacts originating from external disturbances, such as frequent steps, tail wagging or any other body movements. Because of their accuracy, such systems are still rather expensive, which is of relevance when the higher risk for damage under farm circumstances is taken into account. In contrast, some of the catheter types, which are manufactured in a disposable form, are less expensive and could be more suitable for studies in the field. Yet, a thorough comparison of the accuracy of both systems for measurements of uterine contractility under in vivo circumstances remains to be carried out.

If the measuring catheters are not fixed inside the uterus, they might be expelled from the organ or displaced within its cavity, resulting in either the termination of the recordings or unreliable recordings. In order to achieve standardization of recordings, a surgical fixation of the catheters to the uterine wall is preferred [15,18]. Although surgically fixed devices enable even prolonged measurements, the need of an operation restricts their use to experimental conditions. Such experiments also require much more labour and the experimental cows usually need to be culled after the completion of the study. These conditions therefore, imply that such studies can generally not be performed on the farm.

For the recording and analyzing of IUP changes, either analogue or digital methods can be used. Analogue data acquisition and analyzing techniques have numerous limitations and are subjected to errors, which cannot properly be kept under control. Analogue recordings are usually made on paper and although there are technical possibilities to adjust them in an optimal way, the quality of the signal, once on paper, cannot be changed anymore afterwards [34]. Therefore, digital methods are to be preferred, not only for evaluation of original analogue paper recordings (for example by using a planimeter [35]), but also throughout the complete process, including the initial data acquisition phase [34,36]. Digital records enable a more thorough analysis of pressure cycles, especially with respect to the defining of criteria to distinguish between signals, representing real myometrial activity and those produced by artefacts.

2.5. Uterine contractility in cows during the first days after calving

Accurate information about spontaneous physiological changes in uterine contractility has to provide a basis for any measure to improve the efficacy of uterine involution in dairy cows. Measurements of IUP during the early postpartum period are supposed to give a good estimation about uterine mechanical activity [25]. Although several studies have been conducted in cows [13,15,17,18,37-39], as will be reviewed in Chapter 2, they provide hardly any quantitative information about spontaneous changes in uterine contractions during the early normal puerperium.

2.6. The use of drugs to alter uterotonic activity

There are no obvious clinical indications for the use of uterine relaxants in postpartum dairy cows. However, such drugs can be beneficial for several obstetrical interferences in parturient animals [40-43]. On the other hand, the use of uterine stimulants has more frequently been reported. Their application is believed to facilitate placental shedding after calving (yet, a determinant role of uterine contractions in this process is still under debate, as has been already mentioned in Section 2.2), and to facilitate the emptying of the uterus during the puerperium. Nowadays, mainly oxytocin, oxytocin-like drugs, and natural and synthetic analogues of prostaglandin $F_{2\alpha}$ are used to enhance uterine contractility.

Several studies report on the use of oxytocin to stimulate uterine contractility in cows during the early postpartum days [44-46]. Its effect does not seem to depend on the method of administration [45,47] and pre-treatment with oestradiol [48]. A treatment with 30 IU of oxytocin exerts a positive effect on uterine contractility up to the second day postpartum [47], but it has been reported in beef cows, that if a large dose of oxytocin (200 IU, im.) was administered even on the third day after calving, uterine contractility still increased significantly [49]. A positive effect of oxytocin becomes less evident during the 4^{th} to 6^{th} days after calving [47]. There is presently no published data on changes in oxytocin receptor (OTR) concentrations in the uterine wall of cows during the first few days after calving that would explain any changes in myometrial sensitivity to (exogenous) oxytocin.

The relatively short half-life of oxytocin [50] forms also part of the explanation for the rather short uterotonic effect of this drug [45]. Efforts to prolong the effect lead to the development of oxytocin analogues, such as carbetocin [51]. It is of clinical relevance to compare the uterotonic effects of the therapeutic dosages of oxytocin and carbetocin during the first day postpartum. If the uterotonic effect of carbetocin lasted longer than that of oxytocin, treatment protocols, which use repeated or slow rate infusion oxytocin therapies, could be simplified.

Oxytocin elicits myometrial contractions directly by stimulating the myometrial oxytocin receptors [52,53]. An additional, indirect pathway may also exist, in which occupation of endometrial OTR's leads to the release of prostaglandins (PG), which on their turn act on the myometrium by a paracrine route [54].

In a few in vitro and in vivo studies, a uterine release of prostaglandins upon treatment of cows with various doses of OT has been reported. Endometrial tissue taken from heifers in oestrus responded to oxytocin with $PGF_{2\alpha}$ secretion in a dose-dependent way, but if endometrial tissue was collected on Days 19-20 of the oestrous cycle, a stimulatory response failed to occur [55]. When very low but physiological doses of OT were intravascularly administered to late pregnant cows, $PGF_{2\alpha}$ concentrations did not increase in uterine venous blood [56]. However, when much higher, pharmacological doses were applied, the resulting $PGF_{2\alpha}$ release became dose-, and stage-dependent [57]. At Days 10, 20 or 30 after calving, intravascular treatments with 30, 150 and 300 IU OT resulted in an elevation of the peripheral plasma prostaglandin $F_{2\alpha}$-metabolite (see below) concentrations. The magnitude of this response decreased with the advancing of the postpartum period [58]. Presently, no information is available about an OT-induced prostaglandin response on the first day after normal calving.

Prostaglandin production and release by the uterus can be directly estimated by determination of the $PGF_{2\alpha}$ concentration in utero-ovarian venous blood [59]. Based on the good correlation between this central prostaglandin $F_{2\alpha}$ concentration and the peripheral plasma level of one of its major metabolites, 15-ketodihydroprostaglandin $F_{2\alpha}$ (PG-metabolite), the more complicated blood collection from the utero-ovarian vein can be substituted by peripheral blood sampling [60-62].

Experiments with cows, in which a hysterectomy was performed on the day of parturition, demonstrated that the increase in peripheral plasma PG-metabolite concentrations that normally takes place during the postpartum period, did not occur. The low PG-metabolite levels during the postoperative days in such cows clearly demonstrated that the uterus is the main source of F series prostaglandins ($PGF_{2\alpha}$ and/or PG-metabolite) during the postpartum period [63,64].

Several in vitro studies, including those with uteri of pregnant rats, have shown a close association between uterine PG release and uterine muscle activity [65,66]. However, with cows, both the in vitro [67] and the in vivo data [68,69] on the effects of PGs on myometrial activity are rather conflicting. In vivo treatments with recommended clinical (luteolytic) doses of $PGF_{2\alpha}$ (25 mg, im.) either report absence of an uterotonic effect in cows during oestrus [68,70], or an increase in uterine motility during all stages of the oestrous cycle [69], or the effect only consisted of a sustained (6 to 8 min) contracture on Day 7 of the oestrous cycle [68]. However, in early postpartum cows, no uterotonic effect could be observed after $PGF_{2\alpha}$ treatment [71].

In conclusion: despite the fact that treatment with exogenous prostaglandins does not always stimulate myometrial activity in cows, a role of OT-induced prostaglandin release can still be part of the mechanism by which myometrial contractility is affected by oxytocin in early postpartum cows.

3. Aims of the studies described in this thesis

In the first, introductory chapter **(Chapter 1)**, after introducing the scope of the thesis, background information is given about some theoretical and practical aspects of uterine contractility during the early postpartum period. Finally, the aims of the different chapters are briefly summarized.

Following the general introduction, in **Chapter 2**, a brief overview is given of the development of various techniques, which can be used for measuring, analyzing and evaluating uterine activity (uterine contractility). In addition, a more detailed description is provided about recording and analyzing procedures by means of different intrauterine pressure (IUP) recording devices. In a comparative overview, the most frequently used parameters of IUP recordings are described and summarized in a table. The use of both analogue and digital techniques are compared with each other. After a description of the chronological changes in uterine contractility that have been reported so far in postpartum cow, this review chapter finally summarizes the effects of some drugs that have been used to influence myometrial contractility in early postpartum cows.

The first main goal of the entire project is to establish a system, by which uterine contractility measurements and their evaluation will be performed in a standardized and objective way, so that methodological errors, including the subjective ones, occurring during analysis, are minimized. The envisaged computer-assisted, digital methods for the registration and analysis of simultaneous uterine IUP and electromyographic (EMG) signals were also designed to provide results that would enable to select an IUP system for future on farm studies with untreated and treated postpartum dairy cows. Therefore, in **Chapter 3**, the digitally recorded IUP data from two different, simultaneously used devices are compared with each other, and they are also tested against quantified digital EMG signals. Measurements took place during the first 48 hours postpartum in cows after prostaglandin-induced calvings.

Because the transcervically inserted open tip IUP catheter system was found to be accurate and suitable for on-farm studies, this non-invasive technique is applied for a field study **(Chapter 4)**, during the first 48 hours after spontaneous, normal calving in cows without retention of the fetal membranes (NRFM). The aim of the study described in Chapter 4 is to provide quantitative data on physiological changes in uterine contractility after uncomplicated calvings in dairy cows that are kept on a large-scale farm. Possible relationships between the characteristics of uterine contractility and blood ionized calcium (Ca^{2+}) concentrations are also investigated.

After having obtained data on spontaneous changes in uterine contractility during the first two days after calving, the next study **(Chapter 5)** was designed to document the effects of two related uterotonic drugs that are available on the veterinary market. At this purpose, a single treatment with either 50 IU of oxytocin or 0.35 mg of the long-acting oxytocin analogue carbetocin was applied between 14 and 16 h after normal, uncomplicated calvings in NRFM dairy cows. Both the short-term (within 4 h), and the long-term (between 12 and 36 h after treatment) uterotonic characteristics and effectiveness of the two treatments are evaluated and compared.

The study described in **Chapter 6** aims to investigate if oxytocin treatment in postpartum cows results in enhanced uterine prostaglandin release that is mirrored by increased PG-metabolite levels in peripheral plasma. In this experiment, we apply the same single dose of oxytocin (50 IU) by the same (intramuscular) route as used in the study of Chapter 5. In addition, two cows are treated intravenously. Treatments take place between 13 and 15 h after normal parturition in NRFM cows. The response of this oxytocin treatment on synthesis and release of prostaglandin $F_{2\alpha}$ is evaluated by measuring its main metabolite, the 15-ketodihydroprostaglandin $F_{2\alpha}$ (PG-metabolite) concentration in peripheral plasma. With this study it is anticipated to obtain evidence for an additional indirect stimulatory effect of oxytocin on myometrial activity, namely an effect mediated by enhanced uterine prostaglandin synthesis and release. Such an effect would be supplementary to the direct stimulation by oxytocin as found in Chapter 5.

Finally, **Chapter 7** presents and summarizes the major findings of this thesis against the background of their possible relevance for the clinical management of uterine involution.

4. References

1. Wooding FBP. Current topic: the synepitheliochorial placenta of ruminants: binucleate cell fusions and hormone production. *Placenta* 1992;13:101-113.
2. Noakes DE. Part Two: Pregnancy and Parturition; Chapter 2. Development of the Conceptus. In: Noakes DE, Parkinson TJ, England GCW (eds): *Arthur's Veterinary Reproduction and Obstetrics.* 8th ed. London: WB Saunders, 2001;57-68.
3. Bjorkman N, Sollen P. Morphology of the bovine placenta at normal delivery. *Acta Vet Scand* 1960;1:347-362.
4. Eiler H, Hopkins FM. Bovine retained placenta: effects of collagenase and hyaluronidase on detachment of placenta. *Biol Reprod* 1992;46:580-585.
5. Gross TS, Williams WF, Manspeaker JE, Russek E. In vitro proteolytic activity of the late pregnant and peripartum bovine placenta. *J Anim Sci* 1985;61:391-392.
6. Van Werven T, Schukken YH, Lloyd J, Brand A, Heeringa HT, Shea M. The effects of duration of retained placenta on reproduction, milk production, postpartum disease and culling rate. *Theriogenology* 1992;37:1191-1203.
7. Arthur GH. Retention of the afterbirth in cattle: a review and commentary. In: Grunsell CSG, Hill FWG (eds): *The Veterinary Annual.* Bristol: Scientechnica, 1979;26-36.
8. Eiler H. Chapter 44. Retained Placenta. In: Youngquist RS (ed): *Current Therapy in Large Animal Theriogenology.* Philadelphia: WB Saunders, 1997;340-348.
9. Esslemont RJ, Peeler EJ. The scope for raising margins in dairy herds by improving fertility and health. *Br Vet J* 1993;149:537-547.
10. Grunert E. Ätiologie, Pathogenese und Therapie der Nachgeburtsverhaltung beim Rind. *Wien Tierärztl Monatsschr* 1983;70:230-235.
11. Laven RA, Peters AR. Bovine retained placenta: aetiology, pathogenesis and economic loss. *Vet Rec* 1996;139:465-471.
12. Zerobin K, Spörri H. Motility of the bovine and porcine uterus and fallopian tube. *Adv Vet Sci Comp Med* 1972;16:303-354.
13. Venable JH, McDonald LE. Postparturient bovine uterine motility - normal and after experimentally produced retention of the fetal membranes. *Am J Vet Res* 1958;19:308-313.
14. Taverne MAM, van der Weyden GC, Fontijne P. Preliminary observations on myometrial electrical activity before, during and after parturition in the cow. In: Hoffmann B, Mason IL, Schmidt J (eds): *Calving Problems and Early Viability of the Calf. Volume 4*. The Hague: Martinus Nijhoff, 1979;297-311.
15. Kündig H, Thun R, Zerobin K, Bachmann B. Die Uterusmotorik des Rindes während Spätgravidität, Geburt und Puerperium. I. Die Spontanmotorik. *Schweiz Arch Tierheilkd* 1990;132:77-84.
16. Burton MJ, Dziuk HE, Fahning ML, Zemjanis R. Myometrial activity during natural and dexamethasone-induced parturition in the cow. *Am J Vet Res* 1987;48:37-44.
17. Gillette DD, Holm L. Prepartum to postpartum uterine and abdominal contractions in cows. *Am J Physiol* 1963;204:1115-1121.
18. Zerobin K. Die Uterusbewegungen bei Kühen während der Geburt und der Nachgeburtsphase. *Schweiz Arch Tierheilkd* 1970;112:544-560.
19. Bajcsy ÁC. Uterine contractility in a cow suffering from milk fever. Unpublished data. 2001.
20. Al-Eknah MM, Noakes DE. A preliminary study on the effect of induced hypocalcaemia and nifedipine on uterine activity in the parturient cow. *J Vet Pharmacol Ther* 1989;12:237-239.
21. Pelissier CL. Herd breeding problems and their consequences. *J Dairy Sci* 1972;55:385-391.
22. Muller LD, Owens MJ. Factors associated with the incidence of retained placentas. *J Dairy Sci* 1974;57:725-728.

23. Kamgarpour R, Daniel RCW, Fenwick DC, McGuigan K, Murphy G. Post partum subclinical hypocalcaemia and effects on ovarian function and uterine involution in a dairy herd. *Vet J* 1999;158:59-67.
24. Jonsson NN. The effects of subclinical hypocalcaemia on postpartum fertility. In: *Proceedings* BCVA. 1999.Vol. 7, Part 3.255-260.
25. Finn CA, Porter DG. Part 3: The Myometrium. In: Finn CA, Porter DG (eds): *Reproductive Biology Handbooks. Volume 1. The Uterus.* London: Elek Science, 1975;133-274.
26. Naaktgeboren C, van der Weyden GC, Klopper PJ, Kroon CH, Schoof AG, Taverne MAM. Electrophysiological observations of uterine motility during the oestrous cycle in sheep. *J Reprod Fertil* 1973;35:511-518.
27. Hanzen C. Electrical activity of the bovine uterus prior to and post parturition. *Vet Res Commun* 1981;5:143-150.
28. Hays RL, Van Demark NL. Spontaneous motility of the bovine uterus. *Am J Physiol* 1953;172:553-556.
29. Csapo A. The diagnostic significance of the intrauterine pressure. Part I. General considerations and techniques. *Obstet Gynecol Surv* 1970;25:403-435.
30. Åkerlund M, Bengtsson LP, Ulmsten U. Recording of myometrial activity in the non-pregnant human uterus by a micro-transducer catheter. *Acta Obstet Gynecol Scand* 1978;57:429-433.
31. Schmid G, Stolla R. Intrauterine Druckmessung beim Rind mittels Mikrotransducern. *Tierärztl Umsch* 1988;43:439-444.
32. Hirsbrunner G, Küpfer U, Burkhardt H, Steiner A. Effect of different prostaglandins on intrauterine pressure and uterine motility during diestrus in experimental cows. *Theriogenology* 1998;50:445-455.
33. Hirsbrunner G, Knutti B, Burkhardt H, Küpfer U, Steiner A. Effect of two dosages of d-cloprostenol on intrauterine pressure and uterine motility during dioestrus in experimental cows. *J Vet Med A* 1999;46:345-352.
34. Braaksma JT, Veth AFL, Janssens J, Stolte LAM, Eskes TKAB, Hein PR, van der Weide H. A comparison of digital and nondigital analysis of contraction records obtained from the nonpregnant uterus in vivo. *Am J Obstet Gynecol* 1971;110:1075-1082.
35. Döcke F. Untersuchungen zur Uteruskontraktilität beim Rind. *Arch Exper Veterinärmed Sonderdr* 1962;16:1205-1307.
36. Braaksma JT, Veth AFL, Eskes TKAB, Stolte LAM: Digital evaluation of uterine contraction records. In: Josimovich JB (ed): *Uterine Contraction-Side Effects of Steroidal Contraceptives. Volume 1. Problems of human reproduction: A Wiley-Interscience series*. New York: John Wiley & Sons, 1973;9-18.
37. Jordan WJ. The puerperium of the cow: a study of uterine motility. *J Comp Pathol Ther* 1952;62:54-68.
38. Giama I. Erfassung der postpartalen Uterusmotilität des Rindes und der motilitätssteigernden Wirkung eines Oxytozinpräparates. 1. Mitt.: Spontane Uterusmotilität im Frühpuerperium des Rindes nach normalen und gestörten Geburten. *Monatsh Veterinärmed* 1975;30:850-852.
39. Martin LR, Williams WF, Russek E, Gross TS. Postpartum uterine motility measurements in dairy cows retaining their fetal membranes. *Theriogenology* 1981;15:513-524.
40. Jonker FH, van der Weijden GC, Taverne MAM. Effect of clenbuterol administered during the expulsive stage of bovine parturition on uterine activity and the fetus. *Vet Rec* 1991;129:423-426.
41. Putnam MR, Rice LE, Wettemann RP, Lusby KS, Pratt B. Clenbuterol (Planipart™) for the postponement of parturition in cattle. *Theriogenology* 1985;24:385-393.
42. Zerobin K, Kündig H. The control of myometrial functions during parturition with a ß$_2$-mimetic compound, Planipart®. *Theriogenology* 1980;14:21-35.
43. Arbeiter K, Thurnher M. Über die Wirkung des Sympathikomimetikums Planipart® (NAB 365) auf den Geburtsablauf beim Rind. *Tierärztl Umsch* 1977;8:423-427.
44. Zerobin K. Die uterusmotorischen Ablaeufe waehrend Geburt und Puerperium beim Rind und deren Beeinflussbarkeit. In: *Proceedings* XI. Int Congr Dis Cattle 1980. Tel-Aviv.Vol.II:1157-1164.
45. Eulenberger K, Wilhelm J, Schulz J, Gutjahr S, Wohanka K, Däberitz H. Uterotonika im Puerperium des Rindes. *Monatsh Veterinärmed* 1986;41:371-377.

46. Armstrong-Backus CS, Hopkins FM, Eiler H. The uterotonic effect of prostaglandin $F_{2\alpha}$ and oxytocin on the postpartum cow. In: *Abstracts* 64th Ann Meet Conf Res Workers Anim Dis 1983. Chicago. (93.abstract) 17.
47. Giama I, Elze K, Eulenberger K. Untersuchungen zur postpartalen Uterusmotilität des Rindes. 2. Mitt.: Uterusmotilität im Frühpuerperium des Rindes nach Oxytozinapplikation. *Monatsh Veterinärmed* 1976;31:940-942.
48. Burton MJ, Dziuk HE, Fahning ML, Zemjanis R. Effects of oestradiol cypionate on spontaneous and oxytocin-stimulated postpartum myometrial activity in the cow. *Br Vet J* 1990;146:309-315.
49. Eiler H, Hopkins FM, Armstrong-Backus CS, Lyke WA. Uterotonic effect of prostaglandin $F_{2\alpha}$ and oxytocin on the postpartum cow. *Am J Vet Res* 1984;45:1011-1014.
50. Wachs EA, Gorewit RC, Currie WB. Half-life, clearance and production rate for oxytocin in cattle during lactation and mammary involution. *Domest Anim Endocrinol* 1984;1:121-140.
51. Barth T, Slaninová J, Lebl M, Jošt K. Biological activities and protracted action of carba-analogues of deamino-oxytocin with O-methyltyrosine in position 2. *Collect Czech Chem Commun* 1980;45:3045-3050.
52. Ivell R, Fuchs A-R, Bathgate R, Tillmann G, Kimura T. Regulation of the oxytocin receptor in bovine reproductive tissues and the role of steroids. *Reprod Domest Anim* 2000;35:134-141.
53. Fuchs A-R, Periyasamy S, Alexandrova M, Soloff MS. Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effects of ovarian steroids. *Endocrinology* 1983;113:742-749.
54. Lye SJ, Challis JRG. Paracrine and endocrine control of myometrial activity. In: Gluckman PD, Johnston BM, Nathanielsz PW (eds): *Research in Perinatal Medicine (VIII). Advances in fetal physiology: Reviews in Honor of G.C. Liggins.* Ithaca, New York: Perinatology Press, 1989;361-375.
55. Lafrance M, Goff AK. Control of bovine uterine prostaglandin $F_{2\alpha}$ release in vitro. *Biol Reprod* 1990;42:288-293.
56. Taverne MAM, de Schwartz NCM, Kankofer M, Bevers MM, van Oord HA, Schams D, Gutjahr S, van der Weijden GC. Uterine responses to exogenous oxytocin before and after pre-partum luteolysis in the cow. *Reprod Domest Anim* 2001;36:267-272.
57. Fuchs A-R, Rollyson MK, Meyer M, Fields MJ, Minix JM, Randel RD. Oxytocin induces prostaglandin $F_{2\alpha}$ release in pregnant cows: influence of gestational age and oxytocin receptor concentrations. *Biol Reprod* 1996;54:647-653.
58. Del Vecchio RP, Chase CCJ, Bastidas P, Randel RD. Oxytocin-induced changes in plasma 13,14 dihydro-15-keto prostaglandin $F_{2\alpha}$ concentrations on days 10, 20 and 30 postpartum in the bovine. *J Anim Sci* 1990;68:4261-4266.
59. Fairclough RJ, Hunter JT, Welch RAS. Peripheral plasma progesterone and utero-ovarian prostaglandin F concentrations in the cow around parturition. *Prostaglandins* 1975;9:901-914.
60. Kindahl H, Edqvist L-E, Bane A, Granström E. Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ during the normal oestrous cycle and early pregnancy in heifers. *Acta Endocrinol (Copenh)* 1976;82:134-149.
61. Kindahl H, Granström E, Edqvist LE, Neely D, Hughes J, Stabenfeldt G. The advantages of measuring a prostaglandin $F_{2\alpha}$ metabolite in peripheral blood in studies of the physiological role of prostaglandin release during luteolysis in domestic animals. In: *Proceedings* VIIIth Int Congr Anim Reprod AI 1976. Krakow. Vol. III:145-148.
62. Guilbault LA, Thatcher WW, Foster DB, Caton D. Relationship of 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ concentrations in peripheral plasma with local uterine production of F series prostaglandins and changes in uterine blood flow during the early postpartum period of cattle. *Biol Reprod* 1984;31:870-878.
63. Guilbault LA, Thatcher WW, Drost M, Hopkins SM. Source of F series prostaglandins during the early postpartum period in cattle. *Biol Reprod* 1984;31:879-887.
64. Lindell J-O, Kindahl H, Edqvist L-E, Tufvesson G. Effect of hysterectomy on the postpartum prostaglandin levels in the cow. *Acta Vet Scand* 1982;23:144-146.

65. Vane JR, Williams KI. The contribution of prostaglandin production to contractions of the isolated uterus of the rat. *Br J Pharmacol* 1973;48:629-639.
66. Chan WY. Relationship between the uterotonic action of oxytocin and prostaglandins: oxytocin action and release of PG-activity in isolated nonpregnant and pregnant rat uteri. *Biol Reprod* 1977;17:541-548.
67. Patil RK, Sinha SN, Einarsson S, Settergren I. The effect of prostaglandin $F_{2\alpha}$ and oxytocin on bovine myometrium in vitro. *Nord Vet Med* 1980;32:474-479.
68. Cooper MD, Foote RH. Effect of oxytocin, prostaglandin $F_{2\alpha}$ and reproductive tract manipulations on uterine contractility in Holstein cows on days 0 and 7 of the estrous cycle. *J Anim Sci* 1986;63:151-161.
69. Rodriguez-Martinez H, Ko J, McKenna D, Weston PG, Whitmore HL, Gustafsson BK, Wagner WC. Uterine motility in the cow during the estrous cycle. II. Comparative effects of prostaglandins $F_{2\alpha}$, E_2, and cloprostenol. *Theriogenology* 1987;27:349-358.
70. Stolla R, Schmid G. Auswirkungen natürlicher und synthetischer $PGF_{2\alpha}$-Präparate auf die Uteruskontraktilität des Rindes. *Berl Münch Tierärztl Wochenschr* 1990;103:198-202.
71. Ko JCH, McKenna DJ, Whitmore HL, Chen CY, Gustafsson BK, Smith RP. Effects of estradiol cypionate and natural and synthetic prostaglandins on myometrial activity in early postpartum cows. *Theriogenology* 1989;32:537-543.

CHAPTER 2

A review on measurements of uterine contractility, with emphasis on spontaneous and drug-induced changes during the early postpartum period in cows

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To be submitted

Abstract

The measurement of early postpartum uterine activity in cows can offer information on uterine involution processes, and is therefore clinically relevant. An attempt has been made in this chapter to summarize the available knowledge on techniques, used for measuring uterine contractility.

A general review of the development of uterine activity measuring techniques, with detailed information on the recording, analyzing and evaluating of the data, is followed by a description of the most frequently applied methods in postpartum cows. The available reports on intrauterine pressure measurements taken during the bovine postpartum period are then summarized, starting with a description of spontaneous activity, and followed by an outline of several treatments, used to influence uterine contractility during this period.

Keywords: uterine contractility; postpartum cow; spontaneous activity, uterine treatment

1. Introduction

The uterus of the early postpartum dairy cow is of special interest in view of its role in a subsequent conception and pregnancy. As reproduction is an important factor in the economy of a cattle farm [1], the earlier the causes of reproductive disorders are recognized and eliminated, the better are the chances for improving reproductive performance and, indirectly, economic effectiveness [2].

The reproductive efficiency of a dairy herd is generally defined in terms of the calving interval. The length of the calving interval influences the milk yield, and affects the culling rate as a result of reproductive disorders [3,4]. The calving interval is prolonged, if, for example, the calving-to-conception interval increases due to an inappropriate uterine involution [4]. The latter can be associated with several dysfunctions of the organ, such as an improper uterine contractility during the puerperal phase [5-7]. Measurements of uterine contractility can provide essential information to clarify the pathophysiological conditions associated with abnormal uterine involution.

Although numerous reports are available on measurements of uterine activity in various species and during different reproductive stages, so far there has not been any summarizing paper presented, which outlines the development of uterine activity measurements and which concentrates on their use during the bovine early postpartum period. Therefore, the purpose of this review is:

a., to present an overview of the different data acquisition and analyzing techniques used to quantify uterine activity,

b., to summarize the results of measurements of spontaneous intrauterine pressure changes during the postpartum period in cows, and

c., to provide a short overview of the effects of different drug treatments on uterine activity during the postpartum period.

2. An overview of techniques used for measuring, analyzing and evaluating uterine activity

During the last century, the techniques for recording, analyzing and evaluating uterine activity underwent a major evolution. There was a special interest to develop such techniques for clinical studies in human pregnancy and labour; as a result of these efforts, conditions were created to apply several of the more accurate techniques also to veterinary research. By extending their use to on-farm situations, it will finally become possible to provide a scientific basis for drug treatment regimes under practical conditions.

2.1. Classification of techniques according to the recording circumstances

In order to quantify uterine mechanical activity, several methods have been developed, which can be applied under *in vitro*, *in situ* or *in vivo* circumstances. During *in vitro* measurements, the uterus, or pieces of uterine tissue, are studied after their removal from the animal's body. For *in situ* measurements, anaesthetized animals with an opened abdomen are used, while for *in vivo* measurements, recordings are made in conscious, intact animals [8]. The advantages of using *in vivo* methods, compared to using *in vitro* ones, are that they allow longer (longitudinal) observations to be made and that they provide more realistic results because measurements are performed within the physiological context of an intact animal [9]. On the other hand, with *in vivo* studies, numerous factors, which are difficult to control, may influence the results [8]. For example, several types of external disturbances [9], such as housing or feeding conditions but also internal metabolic and hormonal changes and even genetic background could cause interferences. Therefore, *in vivo* studies of myometrial activity require a rigorous control over such possible confounders, in conjunction with a careful analysis of the data [8].

2.1.1. In vitro techniques

Mechanical activity of the uterus can be measured *in vitro* either by applying *isotonic* or *isometric* techniques. Although the in vitro methods have numerous advantages, it should be noted here that the extrapolation of in vitro results to living animals should be handled carefully [8].

The shortening of the uterine muscle fibres in their unloaded, loaded and optimally loaded conditions is measured when an *isotonic* technique is applied [8,9]. Such measurements are simple, therefore, are often used [9]. Most frequently, the motility of smooth muscled organs are studied, based on the Magnus-Kehrer method [10]. Kehrer [1907] kept uterine strips in a nutritive solution and demonstrated the isotonic length changes of the myometrial tissue.

The principle of *isometric* measurements is based on the restraint of the muscle fibres from shortening during a contraction period, and on the measurement of the resultant tension changes in the muscle tissue. This can be achieved by using a calibrated isometric lever and a force transducer. The isometric lever is usually a torsion bar that can be calibrated and allows only minimal shortening (2-6%) of the uterine tissue during contraction. The force transducer converts the mechanical strain into electrical changes [8].

Other techniques, like the one described by Mosler [11], allow both *isotonic* and *isometric* measurements. One end of the completely excised uterus was closed and a polyethylene catheter was inserted into its other end, with the whole organ then being placed into a nutritive solution. The catheter was then connected to another container, filled also with a nutritive solution, thus allowing the contractions of the uterus to be recorded both as pressure and volume changes.

2.1.2. In situ techniques

The use of such techniques is not very common and their definition is sometimes unclear. Instead, in vivo techniques are often applied, where uterine contractility is measured in conscious patients even if in situ measurements are mentioned [12]. However, if we accept Finn's and Porter's definition [8] that *in situ*, intact or exteriorized organs of anaesthetized animals are investigated after the abdominal cavity was surgically opened, the visual inspection and recording of the direction of the uterine contractions on such an extraabdominally placed uterine horn [13-17], also belong to this category.

2.1.3. In vivo techniques

Evaluation of uterine mechanical activity with *in vivo* techniques, include the use of manual palpation, tocodynamometry (extra-abdominal tocography), strain gauge recording, intrauterine pressure (IUP) recording (intraluminal tocography) and ultrasonography (echography).

2.1.3.1. Manual palpation

Dickinson (1937) reported one of the first clinical studies, in which he discussed the limited diagnostic value of the bimanual, extra-abdominal palpation of the human uterus in the description of uterine contractility changes during the menstrual cycle [18]. Although this technique remained an important clinical tool to judge the occurrence of labour contractions, its scientific value for describing uterine mechanical activity is low, because it provides hardly any quantitative information. In large animals like the cow, transrectal manual palpation may provide information as to the presence or absence of myometrial tone [19-21].

2.1.3.2. Tocodynamometry

Tocodynamometry, or extra-abdominal tocography [22,23], measures myometrial activity using sensors, which are placed externally on the abdominal wall, to detect uterine pressure changes. This method already has some limitations for use in humans [22,23] but it is even less suitable for use in animals, because of their usually more solid abdominal wall [9] and different morphology (bicornuate type) of the uterus.

2.1.3.3. Strain gauge recording

Contractile force transducers, like strain gauges can be used to measure the muscle activity of various hollow organs, such as the uterus. The procedure of the assembling of strain gauges for use in unanesthetized animals has been described in details [24]. The principle of function of a strain gauge transducer is based on the change in electrical resistance in response to a mechanical deformation. A half Wheatstone-bridge circuit is often the main component of such systems [25]. Strain gauge transducers enable a direct recording of the muscle activity in different species if they are fixed on the outer surface of hollow organs, like uterine horns [25-27], cervix [26], or various parts of the gastrointestinal tract [28-30]. Strain gauges can only record unidirectional mechanical changes originating from muscle layers parallel to the sensor [24]. Therefore, the elicited electrical impulses depend on the position of the gauges. That means that for recording the activity changes of either the longitudinal or the circular layer of the uterus, placement of the different strain gauges should be accomplished in a different direction.

2.1.3.4. Intrauterine pressure recording

According to Laplace's Law, in a closed spheroid, pressure (P) is generated by the wall tension (T) based on the following formula: $P = (2w/R)T$, (w: wall thickness, R: radius) [31]. The principles of IUP recording [32,33], internal tocography [22], or intraluminal tocography [9], are in fact based on Pascal's Law, which implies that in a fluid filled, closed system, the pressure is equal at all points. However, in the postpartum uterus, the cervix might be temporarily open, thus allowing gases and/or fluid to escape from the uterine cavity. This can thus influence the accuracy of measurements.

Usually, intrauterine pressure (IUP) can be recorded by *closed* or *open systems*. Closed systems either operate with a closed tip catheter, or a balloon, while open systems always use an open tip catheter. The nature and application of these IUP systems will be described in more detail in the Section „The intrauterine pressure recording systems".

2.1.3.5. Ultrasonography

Since the late 1970s the use of transrectal ultrasonography has spread more intensively in the everyday bovine reproductive practice, initially describing and evaluating ovarian structures and morphological changes in the uterus [34] and focusing on early pregnancy diagnosis [35,36]. By utilizing the more and more advanced features of this diagnostic technique, ultrasonographic examination can supplement our knowledge, acquired by the use of other techniques to quantify myometrial contractility during the postpartum period [37].

3. Intrauterine pressure recording systems

3.1. Types

3.1.1. Closed systems

The first reports of intrauterine pressure recordings document the use of closed systems for signal acquisition. In closed systems either a rubber balloon, a microballoon , or a membrane is connected to the intrauterine end of a catheter (or a rubber tube), or a completely closed catheter is used [5,33,38-44].

When using any of the balloon types, once the balloon is in place at the measuring site, it is inflated with air or filled with a fluid (most frequently with saline solution /0.9% NaCl/ or water) [42], until the required basal pressure has been reached. Numerous modifications of the measuring systems were applied, especially in the human clinic. Such modification consisted of, for example, the introduction of a polyethylene „umbrella", which keeps the balloon inside the uterine cavity [33]. On the other hand, there can be several objections against the use of balloons. Because they vary in size, the volumes of contained fluid (or air) and the resultant basal (resting) pressures also vary, and hence the recordings by different research groups can not directly be compared [45]. Furthermore, measurements can usually only be taken at one site [46] and the recordings do not accurately reflect rapid pressure changes [38,44], because balloon systems suffer from inertia [46]. Finally, since balloons (like any intrauterine device), depending on their sizes, could exert irritative effects on the uterine wall [31], they can be expected to alter the uterine contractility pattern [45], for example by local production of prostaglandins [47,48]. But balloons do have obvious advantages as well. They can not malfunction because of a blocking of the catheter to which they are connected, and if the same balloon is used, the same internal volume remains valid for repeated measurements [8]. The use of fluid-filled microballoons (less than 1 ml capacity) were found to be even more accurate than open tip systems in the non-pregnant human uterus [31].

Figure 1 illustrates the position of a closed and an open catheter in the uterus simplex (human) during recording.

Fig. 1. The position of a balloon catheter and an open tip catheter in a human uterus according to Finn and Porter [8]

A combination of strain gauges as fixed to a metal bracket, which was sutured on the lateral site of the cow, and microballoons, which were imbedded in the mucosa beneath the myometrial layer of the pregnant uterus was introduced by Gillette and Holm in 1963 [49]. In this system, strain gauges served to transform the pressure changes, which were picked up by microballoons [49,50].

3.1.2. Open systems

Due to the inaccuracies and the irritative effects associated with the balloon techniques, the open catheter system was developed [51]. This system operates with a plastic (either vinyl or polyethylene) catheter. The internal end of the catheter is either open (open tip catheter, Fig. 2; [51-53]), or it is covered with a synthetic polyvinyl sponge (sponge-tip catheter, Fig. 3; [54]).

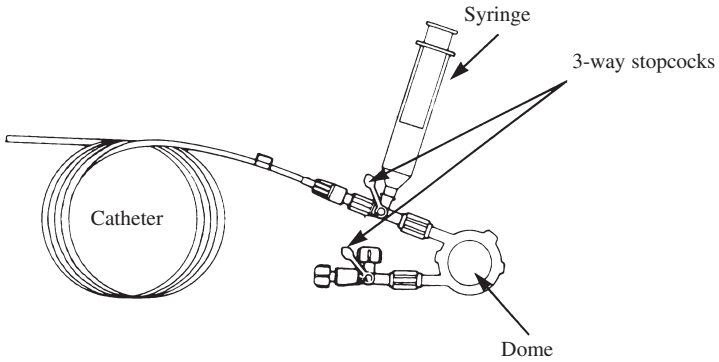

Fig. 2. A disposable open tip catheter with an attached syringe, two stopcocks and a dome (Ohmeda-Hewlett-Packard, Instruction Manual)

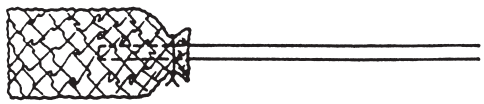

Fig. 3. A sponge-tip catheter according to Bengtsson [54]

In all these systems the catheter is filled with a (heparinized) saline solution as the pressure-transferring medium [38]. In open systems, a thin, flexible, plastic catheter is introduced through the vagina and cervix into the uterus. In cows, the internal end of the catheter is located in the uterine cavity - either in the uterine body, or in any of the uterine horns. The external end of the catheter is connected to a fluid filled pressure transducer [8], which is fixed externally to the animal's body, in cows either to the root of the tail or to the gluteal area [55]. If the catheter itself is merely inserted but not fixed to the uterine wall, it can only be used for a single recording session. For continuous or repeated recordings in the same animal, the catheter needs to be reinstalled during or before each new session. Alternatively, a surgical implantation of the catheter is necessary,

before the start of the experiment. In this latter case, the end of the catheter is fixed to a selected place on the uterine wall by performing a laparotomy [9]. Especially for measurements in the postpartum period, when the level of contractility can expected to be high and uterine size is diminishing rapidly, there is a risk that the previously transcervically installed will not remain in place when it is not properly fixed.

To ensure a proper functioning of these open tip systems, several conditions have to be met. It is important that the end of the catheter always remains free. Blockage can occur if fetal membranes are retained and cover the opening, or if the tip is attached too close to the endometrium, resulting in its partial obstruction. In order to prevent such blockage, a continuous, slow rate or a repeated flushing, with either a (heparinized) saline solution, or water, is necessary. As another means of overcoming blockage, some types of catheter are supplied with additional small holes, 5 to 10 cm along the internal end of the catheter. The so-called Bengtsson sponge-tip catheter as illustrated in Fig. 3; [54], offers a further modification to prevent obstruction of the internal end of the open tip catheter.

It should be noted that by using open tip catheters, some inaccuracies may occur, caused by local myometrial contractions, originating around the tip of the catheter. Finally, open systems enable absolute pressures to be measured, if they are properly calibrated and a manometer is available [9].

The open tip (also the sponge covered type) systems were proved to be much more reliable and they also gave more repeatable results than the closed (balloon) systems both in vivo and in vitro. Therefore, for accurate recordings, the open tip systems have been recommended [38,44].

3.1.3. Microtransducers

Continuing research, especially in cardiovascular studies, led to the development of the microtransducer catheters. Such a type was first introduced in 1970 [31]. A thorough comparison with an open tip system, both in vitro and with the non-pregnant human uterus in vivo, was reported in 1978 [56]. A frequently used example of such a microtransducer catheter is made of dacron, with an external diameter of about 2.3 mm and it contains a microtransducer at its tip [56]. The sensor surface is on the side of the tip of the catheter and consists of a silicon semiconductor, unbonded strain gauge, which acts as the pressure sensor (Fig. 4; Millar Instruments, Houston, TX; Instruction Manual No.501, 1980). Another type is the Konigsberg

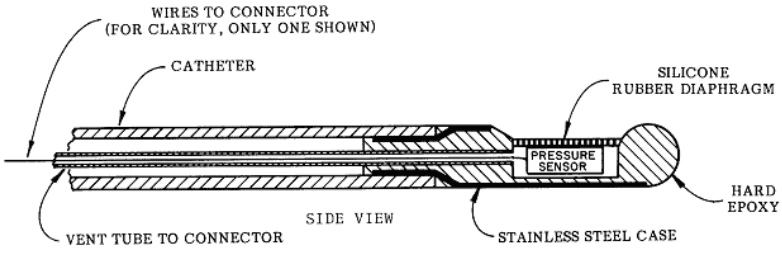

Fig. 4. The Millar catheter

microtransducer. This has a built in miniature pressure chamber with a metal membrane at the tip, and a miniature pressure transducer connected to this pressure chamber (Fig. 5; Konigsberg Instruments, Inc. Pasadena, CA; Implantable Pressure Transducer for Animal Research, Instruction Guide, 1992).

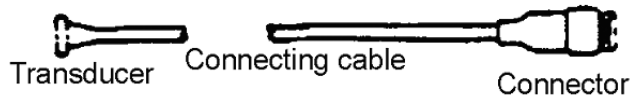

Fig. 5. The Konigsberg microtransducer

With both of these devices, the acquired pressure changes are transformed into electrical signals at the site of signal acquisition. Because external disturbances do not influence the data acquisition directly, a marked noise reduction in the signals can be achieved by using these devices. The reason for this is that with both types of sensors, the miniature pressure sensitive membranes are located at their internal uterine ends, and thus pressure transduction takes place immediately next to the signal acquisition sites. The connecting tube only contains electrical wires, which are not sensitive to pressure changes. This is also the reason why these systems react faster to pressure changes than do the open tip catheters [56,57]. These result in real-time pressure recordings, without any time delay, in contrast to those obtained using a balloon technique, in which the pressure curves are influenced by the measuring system itself [13,58].

3.2. Analysis

Several factors can have a significant influence on the characterization of myometrial activity when using an in vivo IUP recording technique. These include, for example, the accuracy of the measuring device itself, the regularity and variability of the contraction patterns, and the interpretation of the pressure tracings. Myometrial activity has been characterized by a number of different parameters. Some of these appeared to be of basic importance as they were used rather frequently, while others were only used occasionally [59]. The descriptive parameters of IUP recordings can also be classified into directly measured and indirectly, i.e. calculated or derived, parameters. However, we agree with Phillips and Calder (1987) who stated that the comparison of IUP results from various reports is hindered by confusion with respect to terminology and lack of a standardized use of measurement units [60]. Therefore, we will present in this section a list of the most often used parameters to characterize IUP recordings.

3.2.1. Parameters used during the evaluation of intrauterine pressure recordings

3.2.1.1. Basic parameters

The most frequently used parameters for the evaluation of the pressure recordings are frequency, amplitude and duration [9,60].

Contraction frequency (in a simplified form just termed as FREQ) represents the number of pressure cycles (contractions) occurring per unit of time [8]. Usually, it is expressed on an hourly time base [61] but a 1- [39], 10- [25,62] or 100-minute time base has also been used [49]; its official *SI* unit is Hz or mHz [60].

Contraction amplitude (often used in the simplified form amplitude /AMP/; also referred to as contraction intensity /I/, active pressure /AP/[8], or active tension /AT/) represents the difference between the peak of a pressure curve and the basal tone level (or resting pressure). Its unit is usually given as mmHg [8,63], but for the *SI*, kPa has to be used [60].

Basal tone (BT) [64], basal [9], or resting pressure (RP) [8], or resting tone (RT) is the pressure measured within the uterine cavity over the intervals when no uterine contraction is taking place. It thus represents the uterine pressure between contractions [31], Fig. 6; [8, 22]).

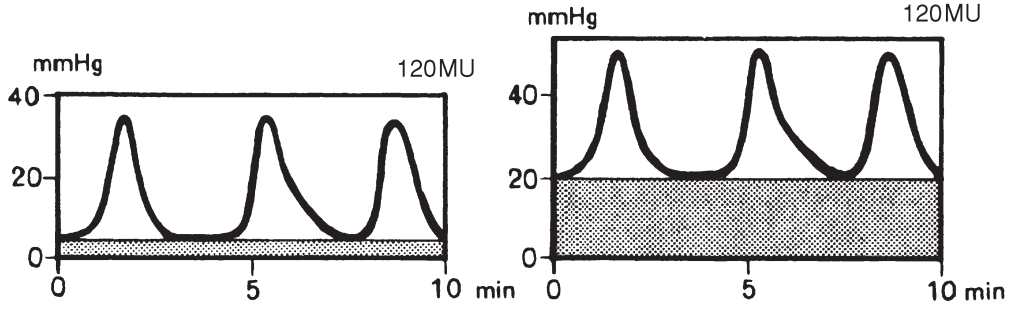

Fig. 6. Pressure waves with different basal tones according to Fischer [22]

Contraction duration (often used in the simplified form duration /DUR/) denotes the length of time that a pressure cycle curve (contraction) is above the basal tone, i.e. a contraction takes place, and is expressed either in minutes or in seconds.

3.2.1.2. Derived parameters

Because frequency, amplitude and duration, even taken together, do not completely describe myometrial contractility, a series of other parameters were introduced. The calculation of the area under pressure curves is the most obvious and simple way to quantify the pressure curves.

The average time interval between pressure cycles over a given time base is sometimes used as contraction interval (Int, [59]).

The area under the pressure curve (also used as area under the curve /AUC/) is a frequently used parameter, which means the surface area between the contraction curve line and the resting pressure; its value is usually expressed in mmHg x s.

The active pressure area (APA) is the sum of the AUC values within a given time period, i.e. it represents the area under pressure curves above the resting pressure during a time unit [8,63]. Its units are (pressure x time per unit time) - e.g. mmHg x s / 30 min, or kPA x s [65]. We used an alternative name for this parameter, the total area under the pressure curve (TAUC; mmHg x s in 1 h; see Chapter 4 of this thesis).

The tension-time index is another parameter, which is used as a measure to determine the effect of a drug. The active pressure area is calculated for a pre-determined time period before the administration of the drug, and again for an equal period after its administration. The change in the value due to the treatment, expressed as a percentage of the initial value, is the tension-time index [66].

The average active pressure is the average value of the amplitudes of all pressure cycles occurring within a selected time period. It can therefore be calculated by dividing the sum of amplitudes of all pressure curves with the number of contractions during that time period [63]. Its unit is mmHg.

A further parameter used to evaluate IUP recordings is the activity index [39]. This parameter is a product of the average amplitude (expressed in mm) and contraction frequency (expressed as number per minute), therefore its unit in conventional analysis is mm/min. However, the activity index has also been used in a different way, by representing the percentage of recording time occupied by uterine activity [25].

The Montevideo Unit (MU, MONT) was introduced in order to characterize uterine activity with just one parameter. It is the product of the average active pressure (average amplitude; mmHg) and the contraction frequency in 10 minutes periods [67].

The Alexandria Unit (AU, ALEX) is a modification of the Montevideo Unit and uses the product of the Montevideo Unit and the mean duration (minutes) of the pressure cycles over 10-minute periods (average amplitude x average duration x average frequency) [68]. Its application is especially advantageous if the duration of the pressure cycles varies.

The average rate of rise in pressure (AP/Tr) is defined as the average active pressure (AP) value, calculated from the amplitudes of 3 representative contraction cycles, divided by the time needed to reach the peak pressure (Tr or t) from the basal tone [63]. It gives information about the degree of coordination between smooth muscle cells within the uterine wall [69]. Some of the evaluating parameters are shown in Fig. 7.

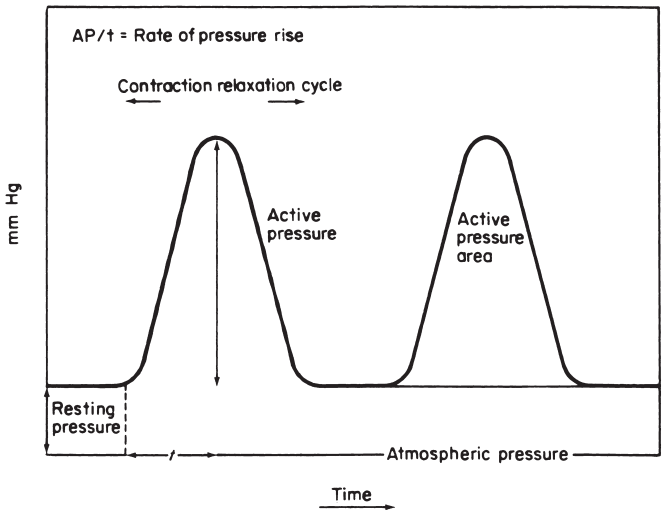

Fig. 7. Illustration of two intrauterine pressure curves with parameters for the evaluation of the records according to Finn and Porter [8]

Linear displacement analysis (LDA) was introduced to provide a measure of changes in intensity of uterine activity over time [70]. For the evaluation, first a certain recording session has to be divided into equally short time periods (9 or 15 minutes were initially used). Then for each of these short periods, the trace lengths are measured with a device usually used to determine distances on maps. Next, the lengths of the baseline, belonging to the same periods are subtracted. Finally, the results are averaged to get a number characteristic for that certain recording session. Several such recording sessions can then be compared by illustrating the changes in these averages or their percentage changes on a graph [70,71]. These percentage changes can be used to indicate the change in intensity of uterine activity over time. Although the exact physiological interpretation of this type of analysis is not clear, because the units are arbitrary, it can be used for testing the effects of drugs on uterine contractility, since it is simple, fast and rather accurate. LDA is certainly less laborious than the previously used planimetric determination of the area under the curve [72].

For the determination of the direction and the speed of propagation of uterine contraction waves, recordings at several uterine sites have to be performed simultaneously [58,73-75]. The propagation of uterine contraction waves can be in either a tubocervical or cervicotubal direction. For determination of the direction and the speed of uterine contraction waves propagation index and propagation time are the two most appropriate parameters [76]. The propagation index (%) is calculated as the percentage of contractions of the uterine body representing the end of a tubocervically propagated wave. The propagation time (seconds) is the mean time taken for the contraction waves to pass along the length of the uterus.
The different parameters that have been explained above are summarized in Table 1.

Table 1. Overview of the parameters used during the evaluation of intrauterine pressure recordings

Name of parameter	Possible synonyms	Abbre-viations	Units	Meanings; methods of calculation	Type of parameter	References
Contraction frequency		FREQ, C/10	n/h, n/min, n/10 min, n/100 min, Hz, mHz	the number of pressure cycles per unit of time	basic	[8,25,39,49, 60,62]
Contraction interval		Int.	s	the average time interval between contraction cycles	derived	[59]
Contraction amplitude	amplitude, contraction intensity, active pressure, active tension	AMP, I, AP, AT	mmHg, kPa	the difference between the peak of a pressure curve and the basal tone level	basic	[8,60,63]
Basal tone	resting pressure, basal pressure, resting tone, uterine tonus	BT, RP, RT	mmHg, kPa	the pressure in the uterine cavity over the intervals when no uterine contraction is taking place	basic	[8,9,22,3l,64]
Contraction duration	Duration	DUR	min, s	length of time when a pressure cycle curve is above the basal tone	basic	
Area under the pressure curve	area under the curve, contraction size	AUC	mmHg x s	the surface area between the contraction curve line and the resting pressure	derived	[25]
Active pressure area; Total area under the curve		APA, TAUC	mmHg x s / 30 min, or kPa x s; mmHg x s / h	the sum of the AUC values within a given time period; pressure x time per unit time	derived	[8,63,65, Chapter 4]
Total area under the intrauterine pressure curve			„planimeter unit”	the sum of all accepted areas under pressure curves during 10-min periods with or without adding the uterine tonus	derived	[77]
„Uterine work”			mm^2 / 10 min	the total area under recorded contraction curves (mm^2) for each 10-min of recording	derived	[25]
„Uterine activity”		UA, MONT	Montevideo Unit, mmHg / 10 min	average active pressure times contraction frequency per 10 minutes	derived	[67]
„Uterine activity”		UA, ALEX	Alexandria Unit, mmHg x min / 10 min	average amplitude times average duration times average frequency per 10-min	derived	[68]

Table 1. Overview of the parameters used during the evaluation of intrauterine pressure recordings (contd.)

Name of parameter	Possible synonyms	Abbre-viations	Units	Meanings; methods of calculation	Type of parameter	References
Average rate of rise in pressure		AP/T_r	mmHg / s	an average active pressure (AP) value, calculated for 3 characteristic contraction cycles of the amplitude of the cycle, divided by the time needed to reach the peak pressure (T_r) from the basal tone	derived	[63,69]
Activity index			mm / min; %	average amplitude times frequency (per minute); percentage of recording time occupied by uterine activity	derived	[25,39]
Linear displacement analysis		LDA	arbitrary units	indicate the change in intensity of uterine activity over time	derived	[70]
Tension-time index			%	the APA is calculated for a pre-determined time period before the administration of a drug, and again for an equal period thereafter; means the percentage change due to the treatment	derived	[66]
Propagation index			%	determination of the direction of uterine contraction waves; the percentage of contractions of the uterine body which form the end of a propagated tubocervical wave	derived	[25,76]
Propagation time			s	determination of the speed of uterine contraction waves; the mean time taken for propagated contraction waves to pass along the length of the uterus	derived	[25,76]

3.2.2. Recording and evaluating techniques (analogue vs. digital)

Uterine activity can be recorded and evaluated applying either an analogue (conventional) or a digital technique.

3.2.2.1. The analogue methods

With a conventional method, the amplitudes, frequencies and durations of the IUP changes, as previously recorded on paper bands, are directly measured with a ruler. To measure the active pressure area for a selected section of the contraction curve, it has to be cut out with scissors, and the paper shape weighed on a micro-scale. If the paper speed and the calibration of the recorder are known, the weight can be converted to IUP units expressed simply in mmHg or mmHg x min. Such analogue methods were very time consuming and are also less accurate than digital methods because every analysis had to be performed manually [59,78]. Moreover, paper recordings represent final data that can not be subjected to any kind of post-recording process, like filtering or amplification.

3.2.2.2. The digital methods

The area under pressure curves can be evaluated by digitizing the pressure changes on the paper recordings using a planimeter [42,72,79] or a computerized digitizer [80]. Digitalization of the data has also been accomplished by transferring the original magnetic tape recordings to a digital tape [59]. Later, a 1 Hz sampling frequency was applied for the direct acquisition of pressure changes into a computer as described in mares [81]. In more recent practice, the acquired signals are immediately transformed into digital form by using an analogue-digital (A-D) data converter. A validation of such a newly developed digital method is presented in Chapter 3 of this thesis.

Comparison of the results from analogue and digital evaluations of the same pressure curves, allows the following conclusions to be drawn: they were almost identical in determining amplitude and contraction interval, however, for duration, active pressure area and with LDA, they showed moderate or large differences [59]. It is important, that with the use of a properly designed computer program, the variability in results derived by different investigators can be markedly reduced.

3.2.3. Artefacts, noise

Although attempts have already been made at an early stage to automatically correct the slow rate changes (drift) in the basal tone that might sometimes occur [65], digital evaluation techniques still not allow always, to distinguish between real signals (caused by uterine mechanical activity) and noise [59]. Therefore, observation and notice of the events during the

IUP recording is still crucial, especially when recording takes place in vivo with conscious, non-restrained animals. By using these remarks during the evaluation phase, obvious artefacts can be excluded. Breathing, coughing, bellowing, straining, eructation, urination or defecation, increased intestinal motility, restlessness and movement are the most frequent causes of noise (artefacts) during IUP recordings. Such factors have already been reported in an early study by Jordan [6]. The concomitant abdominal pressure increases indirectly induce pressure rises within the uterine cavity. However, by careful observation of an animal during the recordings, and after some experience in the analysis of the IUP recordings, such artefacts can be distinguished from real contractility curves with considerable confidence [42,66]. Low-pass filters, e.g. with a 0.5 Hz ceiling can be used to avoid artefacts caused by circuit frequency fluctuation or the random movement of the animal [65]. Another approach to minimize the effects of artefacts is the use of differential pressure measurements. For this purpose, an additional pressure sensor is placed in the anterior part of the vagina, just caudal to the cervix [81,82], -or even better within the abdominal cavity by anchoring it to the external wall of the uterus [49,83]- to measure any rise in abdominal pressure that is not caused by uterine contractility. By continuous subtracting this pressure from the IUP, a more stable IUP pressure tracing can be obtained [82].

The IUP recording can also be influenced by the installation procedure of the measuring device. Thus a stabilization period is usually necessary for the uterine activity to return to its normal pattern; a period of approximately 30 minutes [42,66] is usually sufficient.

4. Uterine contractility in postpartum cows

Several methods have been used for measuring uterine contractility in the postpartum cow. Most of them involve the recording of either electromyographic or uterine pressure signals.

Electromyographic signals originate directly from the myometrial layer and are recorded by bipolar metal electrodes [84-87]. Although electromyography offers a wide range of possibilities to accurately characterize bovine uterine activity changes during the various phases of the reproductive cycle, pregnancy and peripartal period, including puerperium (see for example [84-91]), this chapter will focus only on results obtained with IUP recordings during the postpartum period.

Among the few reports, which describe changes in postpartum uterine activity in cows with normal or abnormal puerperium, a variety of approaches and durations of recordings exist. Some studies preferred the use of closed IUP measurement systems, such as rubber balloons, filled with air [5-7] or water [42], or water-filled microballoons [49], or other balloon types [66,80]. Other studies reported the use of open tip catheters in cows with fetal membrane retention [9,62]. Some researchers used strain gauge transducers in periparturient cows [25,76], however, this method requires previous surgical intervention, similarly to electromyography. The most accurate pressure recordings, where the effect of noise is minimal, are probably those obtained by using micro-transducers [74,87], but the risks of using these expensive devices under farm conditions have to be taken into account.

4.1. A chronological overview of the changes in IUP during the early postpartum phase

The main purpose of uterine contractions during the first few days of the postpartum period is to expel uterine contents [9]. The characteristics of contractions undergo major changes after calving. A summary of some reported observations on these changes is outlined below. However, differences between the various recording techniques that have been used in these studies makes the comparison of the data rather difficult.

4.1.1. Events of the first day postpartum with special emphasis on the first 8 hours

Although after expulsion of the fetus, the abdominal straining efforts disappear, frequent and strong uterine contractions remain [49,73], showing a high degree of regularity [25]. The physiological purpose of this activity is the dislodgement of cotyledons from their corresponding caruncles and the subsequent expulsion of the fetal membranes. In fact, this represents the final, post-calving stage of the parturition process.

The frequency of individual contractions at this time, has been reported to vary between 12 and 30 per hour and shows a decrease during the consecutive hours after expulsion of the calf [6,25,62,73,84]. The decline in frequency until 2 hours after calving was shown to be linear [25], with mean values ranging between 14 and 19 contractions per hour [5,25]. Amplitudes during the first two hours after normal calving have been found to vary between 20 and 40 mmHg [5,6,62]. However, if pressure sensitive microtransducers were implanted inside the myometrium itself, much higher pressure ranges were measured (100 to 200 mmHg; [87]). During this period, the duration of contractions varied between 30 and 150 seconds [5,62,84,87]. Most authors agree that during the first few postcalving hours, contractions are propagated in a tubocervical direction but apart from a few data, as summarized in Table 2, detailed quantitative results have not been provided.

Table 2. Characteristics in propagation waves of uterine contractions in postpartum cows without hormonal induction of calving

After calving at h	Propagation			Method	Type of calving	Fetal membranes	References
	direction	time (s)	index (%)				
1	tubocervical	14	ND	EMG	fetotomy	ND	[85]
8	tubocervical	60	ND				
2	tubocervical	52.7	94.8	strain gauges	normal	retained	[25]

ND: not discussed

The main function of the strong, tubocervically propagating contraction waves occurring during this period, appears to be the expulsion of the placenta [9,25]. However, the accurate description of the changes in uterine contractility during the shedding process of the placenta in cattle is still missing in the literature.

A possible connection between the occurrence of retained fetal membranes (RFM) and the intensity of uterine contractility is controversial. According to an early study, cows with retained fetal membranes showed a decreased uterine activity in recordings started 3 hours after calving [6], while neither Martin et al. (1981), nor Giama (1975) could find any significant differences at 1 and 6 hours (Table 3; [62]) or during the first 8 hours after calving [7] between cows that subsequently had or did not have RFM.

Table 3. Comparison of uterine contractility parameters in cows with (RFM) and without retention (NRFM) of the fetal membranes. Results are based upon 20-minute recordings. (Extracted in a modified form from the data of Martin et al., 1981; [62])

Parameter	Fetal membranes	Recordings postpartum at		
		1 h	6 h	48 h
FREQ / 10 min	RFM	2.64±0.89	2.08±0.34	2.21±0.75
	NRFM	2.64±0.35	1.68±0.84	1.00±0.96
AMP (mmHg)	RFM	20.61±4.20	17.16±0.22	16.39±7.06
	NRFM	22.53±5.01	19.35±11.51	7.37±7.20
DUR (min)	RFM	1.43±0.11	1.30±0.34	1.32±0.39
	NRFM	1.60±0.33	1.27±0.61	0.88±0.80

Mean ± SD values are presented.
FREQ / 10 min: Number of contractions per 10 minutes
AMP: amplitude of all contractions
DUR: duration of all contractions

In contrary, others reported about intensive and frequent myometrial activity in cows with RFM [5,9]. This was also strengthened by clinical experiences in practice [9], supporting the assumption that failing of uterine activity is not responsible for the retention [62,73] during the first days postpartum. Such cows still showed a regular myometrial pattern 7 to 8 hours after calving with separate uterine contractions, each lasting in average for 50 seconds [73]. Further studies also suggested a positive correlation between the presence of fetal membranes, and the intensity of uterine activity, reporting that contractility markedly decreased after shedding of the fetal membranes, within the first 8 hours postpartum [84,87].

Considerable activity still occurred at 24 hours after calving in cows with RFM showing a contraction frequency of 13.3 per hour, amplitudes of even up to 95 mmHg, and cycle length varying between 45 and 70 seconds [73].

4.1.2. Events during the second day postpartum

Uterine contractions become weaker on the second day, but they were still present and still appeared to be propagated in a tubocervical direction [73]. Maximal amplitudes of 50 to 80 mmHg, and contraction frequencies varying between 8.6 and 40 per hour, have been measured in RFM cows [9,73] with a significantly higher uterine activity at 48 hours in cows with RFM than in animals without RFM (Table 3; [62]). In agreement with these findings, Giama (1975; [7]) previously also reported a clear decrease in amplitude, frequency and duration of contractions but partly in contrast with the results observed by Martin et al. (1981; [62]), he could not find differences in terms of uterine contractility at this stage between cows with and without retention [7].

4.1.3. Further events beyond two days postpartum; the appearance of contractures

While the uterine contractility, observed during the first 8 hours after calving, did not depend on the fact whether a cow calved normally or had dystocia due to fetal oversize, amplitude decreased further on the third day, with a simultaneous slight increase in contraction frequency in cows previously having dystocia. On Day 4, these cows exhibited a further diminution of their uterotonic activity [7]. While some studies reported diminished and subsequently ceased uterine contractions from the third day onwards in cows with RFM [9], in other studies both the rhythmicity and the tubocervical direction of the contractions disappeared only by Days 4 or 5 in such cows, and the amplitude markedly decreased, to less than 20 mmHg [73]. A reappearance of individual contractions was reported from Day 19 until Day 27 [73].

Long-lasting, weak elevations (up to 20 mmHg) of the uterine basal pressure were especially characteristic on the 6th day postpartum [7]. From the 4th, 5th day onwards, only low frequency (0.2 to 0.5 contractions per hour) and low amplitude (20 to 50 mmHg) individual curves occurred, until the 12th and 13th day postpartum, when both amplitude and frequency increased again [87]. Such slowly rising changes in the basal uterine pressure (so called contractures) were first described in late pregnant ewes [92]. By definition, contractures last for at least 5 minutes and exceed basal uterine tone by at least 3.5 mmHg.

It can be **concluded** from the previous section that:

- data of the published studies are difficult to compare because of different methodologies, different recording protocols, different type of animals and different stages during the postpartum stage,
- only very few studies provide quantitative information on changes in uterine contractility during the postpartum period by using longitudinal recordings within the same animal,
- usually studies were performed under experimental conditions but not under on farm situations.

4.2. The effect of nursing and milking on early postpartum uterine contractility

The direct effect of milking or nursing on uterine contractility during the puerperal period is controversial. While Jordan (1952; [6]) could not observe any significant differences in uterine contractility patterns of the dam with the presence or absence of her calf, even if the calf was allowed to lick or suckle her mother, Venable and McDonald (1958; [5]) occasionally observed a positive effect of nursing, whereas other authors always reported increased uterine activity after milking [9,49,93] or nursing [9,49] or even only because of seeing their calves nearby [9]. The negative effect of calf removal on the release of oxytocin [94] most likely contributes to a decline of uterine contractility in postpartum cows.

5. Drugs influencing myometrial contractility during the early postpartum period

If a drug is affecting activity of myometrial cells, the effect can manifest itself either by a relaxation or a contraction.

5.1. Uterine relaxants

Despite several reports about the use of uterine relaxants in parturient animals, no data are available on their use during the early postpartum period. Such uterine relaxants or tocolytic agents can temporarily reduce uterine contractions. This could be beneficial during obstetrical aid in parturient animals with abnormal fetal presentations, fetal oversize or uterine displacements or torsion. It is often used during cesarean sections to exteriorize the pregnant uterine horn in ruminants. Among the few drugs exerting inhibitory effect on uterine contractility, mainly beta-mimetic drugs like **clenbuterol** and **isoxsuprine** are used [95,96]. However, clenbuterol exerted a longer tocolytic effect than isoxsuprine, as it was shown in pregnant and parturient ewes [95]. Clenbuterol temporarily suspended, and therefore prolonged delivery process in parturient cows [97-100], or gilts [99], but if this drug was applied during parturition in cows, no negative effects could be observed on subsequent puerperal events [74]. Further investigations in goats proved that both clenbuterol and nifedipine, a Ca-antagonist, prevented the increase in uterine contraction frequency and intensity as caused by a treatment with xylazin [101]. From experiments with late pregnant sheep after ovariectomy, it became obvious that clenbuterol exerted its uterine inhibitory effects even in the absence of ovarian steroids [102].

5.2. Uterine stimulants

The purpose of using uterine stimulants varies with the stage of the postpartum period. During the early puerperal phase, one of the most important pathological event is the retention of the fetal membranes. Subsequently, different forms of endometritis may occur. In order to facilitate the evacuating process of the early postpartum uterus, various uterine stimulant are routinely used in the veterinary practice [103-106]. However, the exact benefits of such treatments remain to be established.

The major groups of recently applied uterine stimulants are oxytocin, oxytocin-like drugs and prostaglandins. Various other drugs, such as oestrogens, ergot alkaloids or glucocorticoids have previously also been advocated to stimulate uterine contractility, but because of several objections and restrictions, their use became limited or even forbidden in cattle practice. Although in numerous studies different forms, dosages and application routes of these drugs on myometrial contractility have been tested, the majority of these investigations focuses on their effect during the oestrous cycle. Despite the large number of studies dealing with the effects of oxytocin, oxytocin-like drugs and prostaglandins on uterine involution processes and subsequent reproductive events, most of them did not quantify their effect on uterine contractility during this period. Therefore, we give here a brief overview on the uterotonic effect of such drugs applied during the bovine early postpartum period.

In general, **oxytocin** increases uterine activity in postpartum cows [74,104,107], independently from the route of administration and pretreatment [79,80]. Oxytocin (25 or 30 IU) induced significantly different uterotonic effects when applying in various forms (intramuscular, intravenous, subcutaneous or epidural), especially if treatment was performed during the first 8 hours postpartum. However, in cases of complete uterine atony, the most effective administration method appeared to be the intravenous one [104,108]. During the first hour after oxytocin treatment at 12 h postpartum, both mean FREQ and propagation index increased significantly, without any significant changes in the AUC and DUR values and the propagation time. The mean duration of the response to oxytocin was 2.3 h [79]; these findings are consistent with the findings to be presented in Chapter 5 of this thesis. Even if oxytocin was applied at a later stage of the puerperium, at 24 hours after parturition, mean FREQ, DUR and propagation index remained almost unchanged during the first hour after oxytocin treatment, as compared to a similar treatment at 12 h [79]. Treatments with oxytocin (30 IU) until the 2nd day always exerted a positive effect on uterine contractility [108]. However, if a large dosage of oxytocin (200 IU, im.) was applied 3 days after calving, uterine contractility still increased significantly, as was observed in beef cows [66]. The positive effect of oxytocin became less expressed in the 4th to 6th postpartum days [108].

Because the relative short effectiveness of an oxytocin treatment is most likely caused by the short half-life of this peptide [109], several types of oxytocin-analogues were developed. One of the most often used such drugs is **carbetocin** [110]. The advantage of a prolonged uterotonic effect in practical circumstances is obvious. Despite several reports of such a prolonged

uterotonic effect of carbetocin [103,104,111,112] postpartum, we could not demonstrate significant differences between carbetocin and oxytocin treatment if applied in a recommended dose and form at 14 to 16 h after parturition in cows without RFM (see Chapter 5 of this thesis).

In postpartum cows, the **prostaglandin $F_{2\alpha}$** analogue, fenprostalene did not increase uterotonic effects on any of the first 4 days, and no difference was found between cows with RFM and without [76]. There was also no cumulative effect after repeated treatments [76]. Natural $PGF_{2\alpha}$ did also have no uterotonic effect at 48 to 72 hours after calving, if applied in a luteolytic dosage (25 mg) [80], either given before or after an oxytocin (200 IU, im.) treatment [66]. If oxytocin (200 IU, im.) was applied at 48 h after calving after a pretreatment with natural $PGF_{2\alpha}$ (25 mg), uterine activity increased with more than 200% as compared to the initial value. This elevation was even more intensive (875%), when prostaglandin treatment followed the pretreatment with oxytocin 72 hours after calving [66]. The failing effect of exogenous $PGF_{2\alpha}$ on uterine activity in the postpartum cow raises doubt as to a possible therapeutic value of this compound during this period. Therefore, several authors did not recommend the use of $PGF_{2\alpha}$ as an uterotonicum [66,113]. Given the partly controversial results of prostaglandin treatments [106,114-116] during the postpartum period to support involution of the postpartum uterus, the use of this drug during the bovine puerperium needs further investigation.

6. Conclusions

The following main conclusions can be drawn from this chapter:

There is a high variety of techniques used for measuring and evaluating uterine contractility. Also methods for recording, analyzing and evaluating of the data in postpartum cows, underwent a major change during the past decades, but they still often involve the use of invasive methods to obtain recordings. By using non-invasive measurement techniques, which are based on exactly defined criteria during evaluation of the records, uterine activity can be studied more precisely, and recordings can be easier applicable even under farm circumstances. After summarizing published data on uterine contractility in postpartum cows, it appeared that only very little quantitative data are available. The same is true for data about treatments with uterotonic compounds, such as oxytocin and prostaglandins.

7. References

1. Louca A, Legates JE. Production losses in dairy cattle due to days open. *J Dairy Sci* 1968;51:573-583.
2. De Kruif A. Factors influencing the fertility of a cattle population. *J Reprod Fertil* 1978;54:507-518.
3. Risco CA, Archbald LF. Dairy Herd Reproductive Efficiency. In: Howard JL, Smith RA (eds): *Current Veterinary Therapy 4, Food Animal Practice.* Philadelphia: WB Saunders, 1999;604-606.
4. Tenhagen B-A, Heuwieser W. Comparison of a conventional reproductive management programme based on rectal palpation and uterine treatment of endometritis with a strategic prostaglandin $F_{2\alpha}$ programme. *J Vet Med A* 1999;46:167-176.
5. Venable JH, McDonald LE. Postparturient bovine uterine motility - normal and after experimentally produced retention of the fetal membranes. *Am J Vet Res* 1958;19:308-313.
6. Jordan WJ. The puerperium of the cow: a study of uterine motility. *J Comp Pathol Ther* 1952;62:54-68.
7. Giama I. Erfassung der postpartalen Uterusmotilität des Rindes und der motilitätssteigernden Wirkung eines Oxytozinpräparates. 1. Mitt.: Spontane Uterusmotilität im Frühpuerperium des Rindes nach normalen und gestörten Geburten. *Monatsh Veterinärmed* 1975;30:850-852.
8. Finn CA, Porter DG. Part 3: The Myometrium. In: Finn CA, Porter DG (eds): *Reproductive Biology Handbooks. The Uterus.* London: Elek Science, 1975;133-274.
9. Zerobin K, Spörri H. Motility of the bovine and porcine uterus and fallopian tube. *Adv Vet Sci Comp Med* 1972;16:303-354.
10. Kehrer E. Physiologische und pharmakologische Untersuchungen an den überlebenden und lebenden inneren Genitalien. *Arch Gynakol* 1907;81:160-210.
11. Mosler KH. Uber den Einfluss von Chinin auf die Dosierungskurve von Acethylcholin am isolierten Uterus der Ratte. *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol* 1961;242:12-16.
12. Fröhlich H. Steuermechanismen der Motilität des nichtgraviden Uterus in situ. *Wien Klin Wochenschr Suppl 24.* 1974;86:1-28.
13. Brinsfield TH, Hawk HW. Modification of the direction of uterine contractions by intra-uterine devices in the ewe. *J Reprod Fertil* 1969;18:535-537.
14. Lehrer AR, Schindler H. The fertility of ewes after implantation of dummy pressure transducers in the uterine wall. *J Reprod Fertil* 1974;24:109-110.
15. Crocker KP, Shelton JN. Influence of stage of cycle, progestagen treatment and dose of oestrogen on uterine motility in the ewe. *J Reprod Fertil* 1973;32:521-524.
16. Rexroad CE, Jr. Estradiol regulation of the frequency and site of origin of uterine contractions in ewes. *J Anim Sci* 1980;51:1139-1147.
17. Hawk HW. Hormonal control of changes in the direction of uterine contractions in the estrous ewe. *Biol Reprod* 1975;12:423-430.
18. Dickinson RL. The technic of timing human ovulation by palpable changes in ovary, tube, and uterus. *Am J Obstet Gynecol* 1937;33:1027-1033.
19. Morrow DA, Roberts SJ, McEntee K. A review of postpartum ovarian activity and involution of the uterus and cervix in cattle. *Cornell Vet* 1969;59:134-154.
20. Bostedt H. Chapter 2. Grundlagen für die Fortpflanzung. In: Bostedt H (ed): *Fruchtbarkeitsmanagement beim Rind.* 4th ed. Frankfurt am Main: DLG, 2003;9-68.
21. Noakes DE. Part Two: Pregnancy and Parturition; Chapter 3: Pregnancy and its Diagnosis. In: Noakes DE, Parkinson TJ, England GCW (eds): *Arthur's Veterinary Reproduction and Obstetrics.* 8th ed. London: WB Saunders, 2001;69-118..
22. Fischer WM. 3. Grundlagen und klinische Wertigkeit der Kardiotokographie. Physiologie der Uterusmotilität. In: Fischer WM (ed): *Kardiotokographie.* 2nd ed. Stuttgart: Georg Thieme Verlag, 1976;73-94.

23. Boden W. III. Zur Diagnostik funktioneller Dystokien. 6. Der Tokogrammbefund. 7. Die Analyse des Wehenschmerzes. In: Boden W (ed): *Die funktionellen Dystokien. Symptomatik, Diagnostik, Einteilung und Behandlung.* Stuttgart: Ferdinand Enke Verlag, 1969;18-36.
24. Bass P, Wiley JN. Contractile force transducer for recording muscle activity in unanesthetized animals. *J Appl Physiol* 1972;32:567-570.
25. Burton MJ, Dziuk HE, Fahning ML, Zemjanis R. Myometrial activity during natural and dexamethasone-induced parturition in the cow. *Am J Vet Res* 1987;48:37-44.
26. Toutain PL, Garcia-Villar R, Hanzen C, Ruckebusch Y. Electrical and mechanical activity of the cervix in the ewe during pregnancy and parturition. *J Reprod Fert* 1983;68:195-204.
27. Van der Weyden GC, Taverne MAM, Dieleman SJ, Wurth Y, Bevers MM, van Oord HA. Physiological aspects of pregnancy and parturition in dogs. *J Reprod Fertil Suppl* 1989;39:211-224.
28. Jacoby HI, Bass P, Bennett DR. In vivo extraluminal contractile force transducer for gastrointestinal muscle. *J Appl Physiol* 1963;18:658-665.
29. Ludwick JR, Bass P. Contractile and electrical activity of the extrahepatic biliary tract and duodenum. *Surg Gynecol Obstet* 1967;124:536-546.
30. Anderson JJ, Bolt RJ, Ullman BM, Bass P. Differential response to various stimulants in the body and antrum of the canine stomach. *Am J Dig Dis* 1968;13:147-156.
31. Csapo A. The diagnostic significance of the intrauterine pressure. Part I. General considerations and techniques. *Obstet Gynecol Surv* 1970;25:403-435.
32. Csapo A. The diagnostic significance of the intrauterine pressure. Part II. Clinical considerations and trials. *Obstet Gynecol Surv* 1970;25:515-543.
33. Csapo AI, Pinto-Dantas CR. The cyclic activity of the nonpregnant human uterus. A new method for recording intrauterine pressure. *Fertil Steril* 1966;17:34-38.
34. Pierson RA, Ginther OJ. Ultrasonic imaging of the ovaries and uterus in cattle. *Theriogenology* 1988;29:21-37.
35. Pieterse MC, Szenci O, Willemse AH, Bajcsy ÁC, Dieleman SJ, Taverne MAM. Early pregnancy diagnosis in cattle by means of linear-array real-time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. *Theriogenology* 1990;33:697-707.
36. Taverne MAM, Szenci O, Szétag J, Piros A. Pregnancy diagnosis in cows with linear real-time ultrasound scanning: a preliminary note. *Vet Q* 1985;7:264-270.
37. Pierson RA, Kastelic JP, Ginther OJ. Basic principles for transrectal ultrasonography in cattle and horses. *Theriogenology* 1988;29:3-19.
38. Braaksma JT, Janssens J, Eskes TKAB, Hein PR. Accurate pressure recording in the non-pregnant human uterus. A comparison of open and closed tip catheters. *Eur J Obstet Gynecol* 1971;6:195-206.
39. Hays RL, Van Demark NL. Spontaneous motility of the bovine uterus. *Am J Physiol* 1953;172:553-556.
40. Wilson L, Kurzrok K. Studies on the motility of the human uterus in vivo. A functional myometrial cycle. *Endocrinology* 1938;23:79-86.
41. Garrett WJ. Some observations on the human myometrial cycle. *J Physiol* 1956;132:553-558.
42. Döcke F. Untersuchungen zur Uteruskontraktilität beim Rind. *Arch Exper Veterinärmed Sonderdr* 1962;16:1205-1307.
43. Braaksma JT. Drukregistratie in de niet zwangere uterus in vivo -onderzoek van een methode- (Pressure recording in the non pregnant uterus in vivo). Vrije Universiteit te Amsterdam. 1970.
44. Braaksma JT, Janssens J, Eskes TKAB, Arp A, Hein PR. Accurate pressure recording in the non-pregnant human uterus. *Gynecol Invest* 1970;1:288-302.
45. Reynolds SRM. Chapter 4. Human Tocography: Nongravid Uterus. In: Reynolds SRM (ed): *Physiology of the Uterus.* New York: Hafner Publishing Company, 1965;34-41.
46. Posse N. The motility pattern of the non-pregnant uterus. *Acta Obstet Gynecol Scand Suppl2* 1958;37:1-124.
47. Cibils LA. Views and reviews: Contractility of the nonpregnant human uterus. *Obstet Gynecol* 1967;30:441-461.

48. Ramondt J, Verhoeff A, Garfield RE, Wallenburg HCS. Effects of estrogen treatment and inhibition of prostanoid synthesis on myometrial activity and gap junction formation in the oophorectomized ewe. *Eur J Obstet Gynecol Reprod Biol* 1994;54:63-69.
49. Gillette DD, Holm L. Prepartum to postpartum uterine and abdominal contractions in cows. *Am J Physiol* 1963;204:1115-1121.
50. Gillette DD. Placental influence on uterine activity in the cow. *Am J Physiol* 1966;211:1095-1098.
51. Hendricks CH. A new technique for the study of motility in the non-pregnant human uterus. *J Obstet Gynaecol Br Emp* 1964;71:712-715.
52. Eskes T, Braaksma J, Janssens J. Pressure recording in the non-pregnant human uterus. *Acta Physiol Pharmacol Neerl* 1969;15:402.
53. Johnson WL, Ek TW, Brewer LL. Motility of the human uterus before and after insertion of an intrauterine device. *Obstet Gynecol* 1966;28:526-527.
54. Bengtsson LP. The sponge-tipped catheter - A modification of the open end catheter for recording of myometrial activity in vivo. *J Reprod Fertil* 1968;16:115-118.
55. Bajcsy ÁC, van der Weijden GC, Doornenbal A, Breeveld-Dwarkasing VNA, de Jong RC, Szenci O, Taverne MAM. Validation of pressure measurements and electromyography of the bovine uterus during the early postpartum period. *Am J Vet Res* 2004; accepted for publication.
56. Åkerlund M, Bengtsson LP, Ulmsten U. Recording of myometrial activity in the non-pregnant human uterus by a micro-transducer catheter. *Acta Obstet Gynecol Scand* 1978;57:429-433.
57. Schmid G, Stolla R. Intrauterine Druckmessung beim Rind mittels Mikrotransducern. *Tierärztl Umsch* 1988;43:439-444.
58. Rodriguez-Marinez H, McKenna D, Weston PG, Whitmore HL, Gustafsson BK. Uterine motility in the cow during the estrous cycle. I. Spontaneous activity. *Theriogenology* 1987;27:337-348.
59. Braaksma JT, Veth AFL, Eskes TKAB, Stolte LAM: Digital evaluation of uterine contraction records. In: Josimovich JB (ed): *Uterine Contraction-Side Effects of Steroidal Contraceptives. Volume 1. Problems of human reproduction: A Wiley-Interscience series*. New York: John Wiley & Sons, 1973;9-18.
60. Phillips GF, Calder AA. Units for the evaluation of uterine contractility. *Br J Obstet Gynaecol* 1987;94:236-241.
61. Rawlings NC, Ward WR. Changes in steroid hormones in plasma and myometrium and uterine activity in ewes during late pregnancy and parturition. *J Reprod Fertil* 1976;48:355-360.
62. Martin LR, Williams WF, Russek E, Gross TS. Postpartum uterine motility measurements in dairy cows retaining their fetal membranes. *Theriogenology* 1981;15:513-524.
63. Csapo A, Sauvage J. The evolution of uterine activity during human pregnancy. *Acta Obstet Gynecol Scand* 1968;47:181-212.
64. Caldeyro-Barcia R, Alvarez H. Abnormal uterine action in labour. *J Obstet Gynaecol Br Emp* 1952;59:646-656.
65. Beck NFG, Carter MC, Jansen CAM, Joyce PL, Krane EJ, Nathanielsz PW, Steer P, Thomas AL. A method for the quantitative assessment of uterine activity in the pregnant sheep. *Proc Physiol Soc* 1977;9P-10P.
66. Eiler H, Hopkins FM, Armstrong-Backus CS, Lyke WA. Uterotonic effect of prostaglandin $F_{2\alpha}$ and oxytocin on the postpartum cow. *Am J Vet Res* 1984;45:1011-1014.
67. Caldeyro-Barcia R, Sica-Blanco Y, Poseiro JJ, Gonzáles Panizza V, Méndez-Bauer C, Fielitz C, Alvarez H, Pose SV, Hendricks CH. A quantitative study of the action of synthetic oxytocin on the pregnant human uterus. *J Pharmacol Exp Ther* 1957;121:18-31.
68. El-Sahwi S, Gaafar AA, Toppozada HK. A new unit for evaluation of uterine activity. *Am J Obstet Gynecol* 1967;98:900-903.
69. Coren RL, Csapo AI. The intra-amniotic pressure. *Am J Obstet Gynecol* 1963;85:470-483.
70. Callantine MR, O'Brien OP, Windsor BL, Brown RJ. Inhibition of uterine contractions in vivo in the unanaesthetized rabbit. *Nature* 1967;213:507-508.

71. Behrman SJ, Burchfield W. The intrauterine contraceptive device and myometrial activity. *Am J Obstet Gynecol* 1968;100:194-202.
72. Csapo AI, Takeda H. Effect of progesterone on the electric activity and intrauterine pressure of pregnant and parturient rabbits. *Am J Obstet Gynecol* 1965;91:221-231.
73. Zerobin K. Die Uterusbewegungen bei Kühen während der Geburt und der Nachgeburtsphase. *Schweiz Arch Tierheilkd* 1970;112:544-560.
74. Zerobin K. Die uterusmotorischen Ablaeufe waehrend Geburt und Puerperium beim Rind und deren Beeinflussbarkeit. In: *Proceedings* XI. Int Congr Dis Cattle 1980. Tel-Aviv.Vol.II:1157-1164.
75. Behrman SJ, Archie JT, O'Brien OP. Myometrial activity and the IUCD. II. Propagation waves. *Am J Obstet Gynecol* 1969;104:123-129.
76. Burton MJ, Herschler RC, Dziuk HE, Fahning ML, Zemjanis R. Effect of fenprostalene on postpartum myometrial activity in dairy cows with normal or delayed placental expulsion. *Br Vet J* 1987;143:549-554.
77. Stander RW. An approach to quantitative analysis of intrauterine pressure data. *Obstet Gynecol* 1966;27:110-115.
78. Braaksma JT, Veth AFL, Janssens J, Stolte LAM, Eskes TKAB, Hein PR, van der Weide H. A comparison of digital and nondigital analysis of contraction records obtained from the nonpregnant uterus in vivo. *Am J Obstet Gynecol* 1971;110:1075-1082.
79. Burton MJ, Dziuk HE, Fahning ML, Zemjanis R. Effects of oestradiol cypionate on spontaneous and oxytocin-stimulated postpartum myometrial activity in the cow. *Br Vet J* 1990;146:309-315.
80. Eiler H, Byrd WH, Hopkins FM. Uterokinetic activity of fenprostalene (a prostaglandin $F_{2\alpha}$ analog) in vivo and in vitro in the bovine. *Theriogenology* 1989;32:755-765.
81. Goddard PJ, Allen WE, Gerring EL. Genital tract pressures in mares. I. Normal pressures and the effect of physiological events. *Theriogenology* 1985;23:815-827.
82. Gutjahr S, Paccamonti DL, Pycock JF, Taverne MAM, Dieleman SJ, van der Weijden GC. Effect of dose and day of treatment on uterine response to oxytocin in mares. *Theriogenology* 2000;54:447-456.
83. Goddard PJ, Allen WE. The use of catheter-tipped pressure transducers for chronic measurement of genital tract pressures in the ewe. I. Implantation technique, catheter performance and data analysis. *Theriogenology* 1985;24:551-563.
84. Taverne MAM, van der Weyden GC, Fontijne P. Preliminary observations on myometrial electrical activity before, during and after parturition in the cow. In: Hoffmann B, Mason IL, Schmidt J (eds): *Calving Problems and Early Viability of the Calf. Volume 4*. The Hague: Martinus Nijhoff, 1979;297-311.
85. Hanzen C. Electrical activity of the bovine uterus prior to and post parturition. *Vet Res Commun* 1981;5:143-150.
86. Däberitz H, Wilhelm J, Eulenberger K, Richter A. Ergebnisse experimenteller Untersuchungen zur Erfassung der bioelektrischen Uterusaktivität im peripartalen Zeitraum und im Puerperium des Rindes. I. Mitteilung: Methode zur Erfassung, Aufzeichnung und Auswertung der bioelektrischen Aktivität des Uterus während der Geburt und in der Nachgeburtsperiode beim Rind. *Arch Exper Veterinärmed* 1984;38:676-686.
87. Kündig H, Thun R, Zerobin K, Bachmann B. Die Uterusmotorik des Rindes während Spätgravidität, Geburt und Puerperium. I. Die Spontanmotorik. *Schweiz Arch Tierheilkd* 1990;132:77-84.
88. Ruckebusch Y, Bayard F. Motility of the oviduct and uterus of the cow during the oestrous cycle. *J Reprod Fertil* 1975;43:23-32.
89. Gajewski Z, Faundez R. Characteristics and analysis of uterine electromyographic activity in pregnant cows. *Theriogenology* 1992;37:1133-1145.
90. Zerobin K. Die Uterusmotorik während der Brunst beim Rind. *Tierärztl Umsch* 1985;40:438-442.
91. Gajewski Z, Thun R, Faundez R, Boryczko Z. Uterine motility in the cow during puerperium. *Reprod Domest Anim* 1999;34:185-191.
92. Jansen CAM, Krane EJ, Thomas AL, Beck NFG, Lowe KC, Joyce P, Parr M, Nathanielsz PW. Continuous variability of fetal pO2 in the chronically catheterized fetal sheep. *Am J Obstet Gynecol* 1979;134:776-783.

93. VanDemark NL, Hays RL. The effect of oxytocin, adrenalin, breeding techniques and milking on uterine motility in the cow. *J Anim Sci* 1951;10:1083.
94. Tancin V, Kraetzl W-D, Schams D, Bruckmaier RM. The effects of conditioning to suckling, milking and of calf presence on the release of oxytocin in dairy cows. *Appl Anim Behav Sci* 2001;72:235-246.
95. Garcia-Villar R, Toutain P-L. Relative tocolytic effects of isoxsuprine and clenbuterol. An in vivo study in pregnant and parturient ewes. *Acta Vet Scand Suppl* 1991;87:211-213.
96. Taverne MAM, van der Weijden GC, Fontijne P, Dieleman SJ. Electrical activity of the myometrium and intra-uterine pressure changes around parturition in the cow and the influence of a single intramuscular injection of isoxsuprine. In: *Proceeedings* VIIIth Int Congr Anim Reprod AI 1976. Krakow.Vol.3:407-410.
97. Jonker FH, van der Weijden GC, Taverne MAM. Effect of clenbuterol administered during the expulsive stage of bovine parturition on uterine activity and the fetus. *Vet Rec* 1991;129:423-426.
98. Putnam MR. Initiation and control of parturition in the cow. *Compend Contin Educ* 1983;5:657-664.
99. Zerobin K, Kündig H. The control of myometrial functions during parturition with a β_2-mimetic compound, Planipart^{R}. *Theriogenology* 1980;14:21-35.
100. Arbeiter K, Thurnher M. Über die Wirkung des Sympathikomimetikums Planipart® (NAB 365) auf den Geburtsablauf beim Rind. *Tierärztl Umsch* 1977;8:423-427.
101. Perez R, Garcia M, Arias P, Gallardo M, Valenzuela S. Inhibition of xylazine induced uterine contractility by clenbuterol and nifedipine. *Res Vet Sci* 1997;63:73-76.
102. Al-Eknah MM, Noakes DE. The biphasic effect of clenbuterol hydrochloride on uterine activity of ovariectomized ewes. *J Vet Pharmacol Ther* 1988;11:109-111.
103. Sobiraj A, Hermülheim A, Herfen K, Schulz S. Einfluß verschiedener Uterotonika auf den Nachgeburtsabgang bei Rindern nach konservativen und operativen geburtshilflichen Eingriffen. *Tierärztl Umsch* 1998;53:392-399.
104. Eulenberger K, Wilhelm J, Schulz J, Gutjahr S, Wohanka K, Däberitz H. Uterotonika im Puerperium des Rindes. *Monatsh Veterinärmed* 1986;41:371-377.
105. Starke A, Fricke H-P, Elze K. Ein Behandlungsverfahren zur Stimulation der Uterusinvolution im Frühpuerperium des Rindes mittels Cloprostenol und Carbetocin. *Tierärztl Umsch* 1998;53:730-739.
106. Tian W, Noakes DE. Effects of four hormone treatments after calving on uterine and cervical involution and ovarian activity in cows. *Vet Rec* 1991;128:566-569.
107. Armstrong-Backus CS, Hopkins FM, Eiler H. The uterotonic effect of prostaglandin $F_{2\alpha}$ and oxytocin on the postpartum cow. In: *Abstracts* 64th Ann Meet Conf Res Workers Anim Dis 1983. Chicago. (93.abstract) 17.
108. Giama I, Elze K, Eulenberger K. Untersuchungen zur postpartalen Uterusmotilität des Rindes. 2. Mitt.: Uterusmotilität im Frühpuerperium des Rindes nach Oxytozinapplikation. *Monatsh Veterinärmed* 1976;31:940-942.
109. Wachs EA, Gorewit RC, Currie WB. Half-life, clearance and production rate for oxytocin in cattle during lactation and mammary involution. *Domest Anim Endocrinol* 1984;1:121-140.
110. Barth T, Slaninová J, Lebl M, Jošt K. Biological activities and protracted action of carba-analogues of deamino-oxytocin with O-methyltyrosine in position 2. *Collect Czech Chem Commun* 1980;45:3045-3050.
111. Kündig H, Thun R, Zerobin K. Die Uterusmotorik des Rindes während Spätgravidität, Geburt und Puerperium. II. Medikamentelle Beeinflussung. *Schweiz Arch Tierheilkd* 1990;132:515-524.
112. Bernhard A, Schulz J, Gutjahr S, Eulenberger K. Indikationen für die Anwendung eines Depotoxytozin-Präparates in der tierärztlichen Praxis. *Tierärztl Umsch* 1993;48:446-453.
113. Ko JCH, McKenna DJ, Whitmore HL, Chen CY, Gustafsson BK, Smith RP. Effects of estradiol cypionate and natural and synthetic prostaglandins on myometrial activity in early postpartum cows. *Theriogenology* 1989;32:537-543.
114. Benmrad M, Stevenson JS. Gonadotropin-releasing hormone and prostaglandin $F_{2\alpha}$ for postpartum dairy cows: estrous, ovulation, and fertility traits. *J Dairy Sci* 1986;69:800-811.

115. Burton NR, Lean IJ. Investigation by meta-analysis of the effect of prostaglandin $F_{2\alpha}$ administered post partum on the reproductive performance of dairy cattle. *Vet Rec* 1995;136:90-94.
116. Archbald LF, Tran T, Thomas PGA, Lyle SK. Apparent failure of prostaglandin $F_{2\alpha}$ to improve the reproductive efficiency of postpartum dairy cows that had experienced dystocia and/or retained fetal membranes. *Theriogenology* 1990;34:1025-1034.

CHAPTER 3

Validation of pressure measurements and electromyography of the bovine uterus during the early postpartum period

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Abstract

Objective–To select a method for future on-farm measurements of uterine contractility in postpartum dairy cows by comparing digitally recorded data from two, simultaneously used intrauterine pressure (IUP) devices with each other, and with quantified electromyographic signals, during the first 48 hours postpartum.

Animals–Five cows after induced parturition.

Procedure–Two electromyographic (EMG) electrodes were implanted on the surface of the pregnant uterine horn, and after a recovery period, parturition was induced with a $PGF_{2\alpha}$-analogue at Day 274. Simultaneously with the EMG recordings, IUP changes were recorded during 2-hour periods at 2, 6, 12, 18, 24, 36, and 48 h postpartum, using an open tip catheter and a pressure microtransducer, after their simultaneous transcervical insertion and fixation to a caruncle immediately after calving. Newly developed acquisition and analyzing softwares were used with a digital data-filtering capability for the IUP and EMG signals.

Results–The intrauterine fixation method of the two pressure measurement instruments was effective and allowed an easy, externally guided removal of the devices at 48 h after calving. There appeared to be a high correlation between the data obtained by the two pressure measuring systems. Good correlation was also found between pressure data obtained by the open tip catheter system and by electromyography. Even though the quantified IUP and EMG signals were highly comparable, synchronization could not always be observed when these signals were inspected visually.

Conclusions and Clinical Relevance–The open tip IUP catheter system is suitable for farm studies, allowing the recording of spontaneous and pharmacologically influenced uterine contractility in early puerperal dairy cows.

Keywords: intrauterine pressure; electromyography; uterine contractility; puerperium; cow

1. Introduction

In bovine practice, several uterotonic drugs are used during the puerperal period. Such treatments are still often based on tradition, or the practical experiences of veterinarians, rather than on the proper indication for treatment and the documented knowledge of their immediate and long-lasting effects on uterine functions.

There are several techniques available to characterize spontaneous uterine activity in vivo, and/or to test the effects of drugs on myometrial contractility. These include: measuring mechanical forces by means of strain gauges [1-3], recording intrauterine pressure changes (IUP) [4,5] or performing electromyography (EMG) [6-10].

IUP is recorded from inside the uterine lumen and pressure changes are assumed to reflect changes in the contractile activity of the uterine wall [11]. If IUP changes are recorded by a single device, only individual pressure cycles (spasmodic pattern) can be identified. However, if multiple pressure measuring devices, at different locations are used simultaneously, even the direction of the propagation waves (peristaltic pattern) can be described.

IUP measurements can be performed in two different ways, depending on the site where the pressure is transduced into an electrical signal. With one method, transduction takes place within the uterine cavity, at the site where the pressure is generated. This in situ transduction occurs both with catheters with built-in miniature pressure sensors (such as the Millar Micro Tip Catheter [Millar Instruments Inc, Houston, TX]) and with implantable microtransducers (such as the Konigsberg implantable pressure transducer [Konigsberg Instruments Inc, Pasadena, CA]), both of which are used mainly in cardiovascular studies. Pressure signals can also be transduced into electrical signals outside the body. In such systems the pressure is picked up by a fluid-filled catheter and is forwarded to an external transducer, which is fixed outside the body, either on the tail or on the gluteal area. Fluid filled catheter systems may have a completely free end – known as open tip catheters [12] – or their tip may be covered with a synthetic polyvinyl sponge [13]. In closed systems the catheter ends in a membrane or a fluid-filled balloon [12,14]. When using systems where the generated IUP is transduced inside the uterus, artefacts caused by movements of the catheters other than as a result of contraction, are minimal. In devices using fluid-filled catheters, the quality of pressure recording can be seriously affected if the animal moves its tail or frequently steps.

Because myometrial contractions may cause the displacement of the catheter within the uterine cavity, or even its complete expulsion, pressure catheters have to be fixed to the uterine wall to guarantee that the measurements are obtained from the same site of the uterus. This is especially the case for prolonged measurements, which are recorded over a period of several days. It has also been reported that, in the postpartum uterus, distinct local differences in contractile activity occur between the tip of the formerly pregnant horn and the more caudal parts of the uterus [15,16].

In the past, analogue data acquisition and analyzing techniques were used for IUP measurements in animals. As the signals were recorded on paper, the permanency of the records

was a major limitation of these studies. Paper recordings may be clear enough but may still be of poor quality because of an incorrect rate of amplification and filtering, or an inappropriate paper speed. The use of analogue to digital (A-D) conversion may overcome many of these drawbacks.

Another crucial part of the analysis is defining objective criteria to identify and quantify the IUP signals, which are associated with real myometrial contractility. The acquisition of digital data also offers obvious advantages for this purpose, especially if cheap, disposable measuring tools can be used instead of expensive instruments. A further advantage of using disposable measuring tools, when working under farm conditions, is that they are more appropriate to such conditions than the laboratory devices, such as microtransducers.

Electromyographic recordings are based on electric potential differences associated with contractions of smooth muscle cells within the uterine wall. Electromyography offers some advantages, one of which is, that the EMG electrodes remain in place for prolonged periods picking up electric events generated at the site of implantation; thus recordings from the same animal can be taken over several months [2,17]. However, the technique usually requires a surgical intervention [6,7,9,18], and this implies that it can not be used for on-farm studies.

Taking into account the above-mentioned requirements for in vivo studies of myometrial contractility, the present study was undertaken with the following aims:

– to evaluate and compare two different techniques for measuring IUP signals, in order to select one of them for possible on-farm use in postpartum dairy cows; and

– to develop a computer-assisted method for the analysis of uterine IUP and EMG signals while investigating to what extent quantified EMG signals from one electrode are correlated with quantified IUP signals recorded simultaneously within the same cow, during the first 48 hours after calving.

2. Materials and methods

2.1. Cows and treatment

A ventral midline laparotomy was performed on five pregnant Holstein Friesian dairy cows, (Cows A, B, C, D and E), two to three weeks before the expected parturition, with the animals in dorsal recumbency under general inhalation anesthesia. Two (manually prepared) bipolar silver electrodes, which were fixed into a silastic plate, were sutured about 30 cm apart onto the surface of the pregnant uterine horn, one near the tip (EMG1) and the other more caudally (EMG2). The insulated connecting cables were exteriorized at the left flank, from where they were tunneled subcutaneously - perforating the skin at the left paralumbal area, where they were then fixed. After the two EMG electrodes had been set in place, their cables exteriorized and the ventral midline abdominal wound closed, the cow was positioned on her right side for cannulation of the left circumflex artery. After the polyvinyl catheter had been inserted in the direction of the dorsal aorta, and been fixed to the proximal part of the circumflex artery, the

other end of the catheter was exteriorized at the same site as the EMG cables. Both EMG cables and the catheter were fixed to the skin dorsally to their skin perforation site, and placed in a plastic bag. More detailed descriptions of the surgical procedures, EMG electrodes and the recording equipment, have been previously published [6,19]. After surgery, each cow was housed individually in a pen, in which she had no possibility to turn around. Antibiotics (ampicilline [Praxavet Ampi-15 inj., Boehringer Ingelheim, Alkmaar, The Netherlands], 12 mg/kg, im., q 24 h) were given during the first 5 days after surgery. At 274 days of gestation (between 10 and 14 days after surgery), parturition was induced with a single synthetic prostaglandin $F_{2\alpha}$ analogue treatment (luprostiol [Prosolvin inj., Intervet, Boxmeer, The Netherlands], 15 mg, im.).

The use of the cows involved in this experiment, and the experimental protocol, were approved by the Committee for the Use of Experimental Animals of the Faculty of Veterinary Science of Utrecht University.

2.2. Experimental protocol and measurements of uterine activity

At 2, 6, 12, 18, 24, 36, and 48 hours postpartum, IUP and EMG changes were recorded continuously for two hours. For this purpose, both a thin polyethylene open tip catheter [Hewlett Packard (HP), Andower, MA] (inside diameter 1.4 mm, outside diameter 2.5 mm) with a few small holes on its intrauterine tip (IUP1), and a pressure sensitive head type microtransducer [Konigsberg Instruments Inc, Pasadena, CA] (IUP2) were transcervically inserted immediately after calving, i.e. at a time when the fetal membranes were still present. These two devices were then fixed together to the stalk of a caruncle in the previously pregnant uterine horn, using a system as illustrated in Fig. 1. This special fixation system consists of a piece of silicone tube (4 cm long, with an internal diameter of about 5 mm), and two different sizes of non-absorbable polyfilament suture materials Serafil, SERAG-Wiessner, Naila, Germany] (USP 3+4 and 8). The two pressure devices were

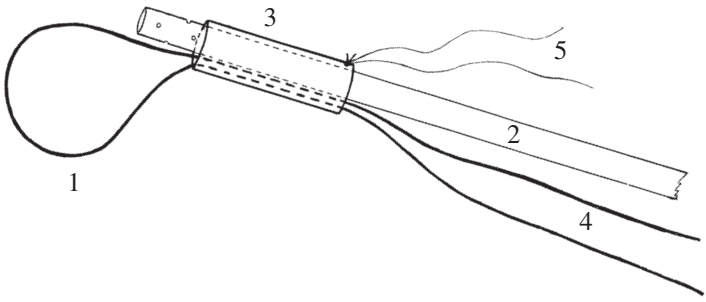

Fig. 1. Schematic illustration of a system to fix an open tip catheter to the stalk of a caruncle in the postpartum uterus of the cow (the „*virtual knot*").

For reasons of clarity, only this single open tip catheter is depicted, whereas for the present study also the Konigsberg microtransducer was fixed within the same silicone tube.

1: loop around a caruncle, 2: open tip catheter, 3: silicone tube, 4: thick suture material, 5: thin suture material

inserted into the silicone tube so that their free ends protruded from the tube approximately 2 cm. A loop was then formed with the thicker wire and passed through the tube so that it protruded at the same end as the catheters. This loop was used to fix the measuring devices to the stalk of a caruncle, from which the cotyledon had been manually detached. While, with one hand, the investigator held the two free ends of the thicker wire taut outside the cow, with his other hand inside the cow, he placed the loop over the caruncle and then narrowed the loop by pushing the silicone tube forwards until it reached the stalk of the caruncle. The thinner suture material had been previously attached to the opposite, outside end of the silicone tube. As described above, and as can be seen in a simplified form in Fig. 1, the IUP1 catheter is only held tightly inside the silicone tube due to the relative diameters of the components of the fixation system. This means that the IUP1 catheter can be pulled out of the tube if necessary. Once this catheter has been removed, the silicone tube only contains the thick suture material, resulting in a loosening of the fixation. The silicone tube can be removed by pulling on the attached thin suture material. Thereafter, only the thick loop remains around the caruncle. By pulling on one end of this loop, the entire suture material can be removed. Because every component of the fixation system can be removed from the outside, there is no need to intrude through the cervix during the removal process.

After fixing the measuring system, the electric cables of the Konigsberg microtransducer and the polyethylene catheter were exteriorised through the cervix and vagina, and fixed with adhesive tapes to a previously shaved area on the left gluteal region. At this site, the polyethylene catheter was connected to a disposable pressure transducer [Ohmeda Inc, Murray Hill, NJ]. In order to prevent obstruction, the open tip catheter was flushed with 10 ml saline (0.9% NaCl solution) at the beginning of each recording session. The electric cables of both IUP systems were connected to two independent analogue preamplifiers [Gould Inc, Cleveland, OH], from which signals were transmitted to the analogue-digital (A-D) converter [DAQCard™-1200, National Instruments Corp, Austin, TX] of the computer. The EMG signals were separately preamplified [Gould Inc., Cleveland, OH], and subsequently processed by the same A-D converter as the two IUP signals. The settings of the IUP and EMG preamplifiers were not changed during, or between the consecutive recording sessions with a cow.

LabVIEW™ 5.0 [National Instruments Corp, Austin, TX], a general purpose graphical programming system, was used to create specific data acquisition, and data analyzing programs. For data acquisition, a 40 Hz sampling frequency was used. During each recording session, accurate notes were made continuously on behavioural events such as body movements, urination, defaecation and vocalization.

2.3. IUP data analysis

IUP cycles, recorded with the open tip system, were analyzed in the following three main steps: In the *first* step, the largest amplitude in each recording session (n = 7) for each cow was determined by applying a post measurement Butterworth filter with order 2, and a 1.2 Hz high cut-off frequency, to the A-D converted signals. Averaging these maximum amplitudes defined

a mean maximum amplitude (MMA), and this cow-specific value was used for further analysis. As the *second* step, all pressure cycles were scanned using 10% of this previously calculated MMA value, and all cycles above this cut-off value were accepted on a preliminary basis. The duration, the final amplitude and the area under the curve (as described by the area between the 10% line and the curved line) were measured for each of these cycles (Fig. 2). Cycles that coincided with behavioural events, noted with continuous observation during the recording sessions, were eliminated. As the *third* step, a final selection of the pressure cycles, on the basis of their area under the curve was made, accepting only cycles, which were above the 10% level of the mean of the five largest areas from the first recording session (at 2 hours postpartum). This cut-off value was subsequently applied to all the recordings made with the same cow. This third step appeared crucial to eliminate minor pressure cycles, obviously representing noise around the baseline level. The characteristics of the remaining population of pressure cycles were then finally defined in terms of frequency (FREQ; per time unit), duration (DUR; s), amplitude (AMP; mmHg) and area (AUC; mmHg x s), for each 2-hour recording period.

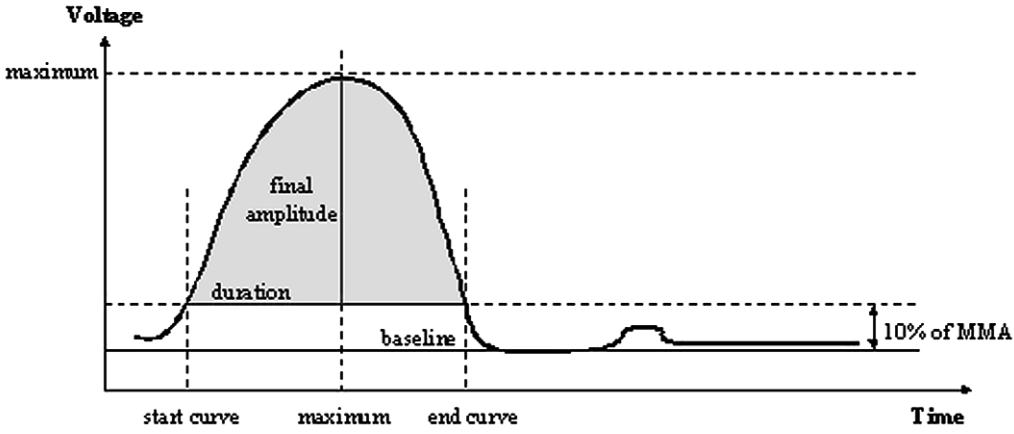

Fig. 2. Schematic illustration of the analysis of intrauterine pressure cycles.
MMA: mean maximum amplitude

The analysis of the IUP recordings with the Konigsberg implantable microtransducer followed the same three steps. However, in this case, the A-D converted signals were not filtered because the signals appeared to be much less affected by artefacts (Fig. 3).

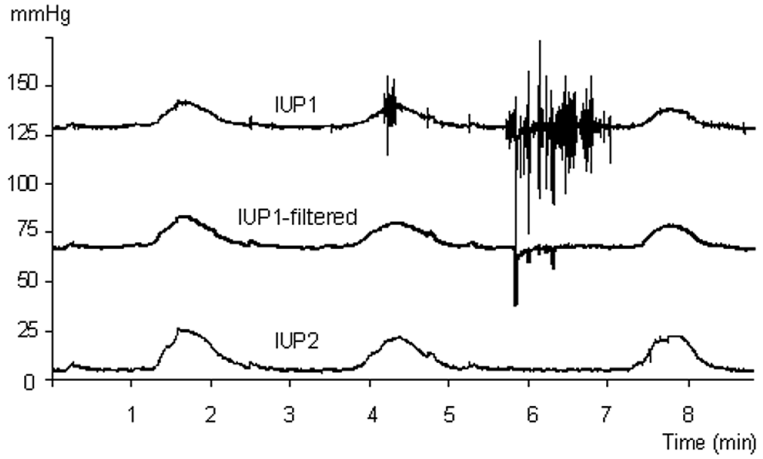

Fig. 3. Original and filtered IUP signals recorded simultaneously by means of the HP-Ohmeda disposable open tip catheter (IUP1) and the Konigsberg implantable microtransducer (IUP2) systems at 2 hours postpartum.

2.4. EMG data analysis

For the analysis of the EMG signals, the root mean square (RMS) values of 1-second intervals were calculated with the following equation:

$$RMS = \frac{\sqrt{x_1^{\,2} + x_2^{\,2} + \ldots x_i^{\,2}}}{n},$$

where $x_{i=1\ to\ n}$ represents the EMG value of the i^{th} data point within a 1 second time interval; as the sampling frequency for data acquisition was set to 40 Hz, n equals 40. This implies that every value represents the root mean square of one second of the original EMG data, recorded from each of the individual electrodes. Subsequently, the RMS values were subjected to a two-step filtering process to remove artefacts and noise around the baseline (Fig. 4). For every cow, each of the seven 2-hour recording periods was divided into 15-minute subperiods, and per each 15-minute subperiod, 900 RMS values (60 x 15) became available per electrode. Where the registered behavioural events coincided with synchronous, exceptionally large amplitude EMG signals from the two electrodes, RMS values equal to, or higher than, 1 mV were excluded (Fig. 4a-c). The remaining population still comprised many values close to the baseline, which obviously did not represent spike or burst

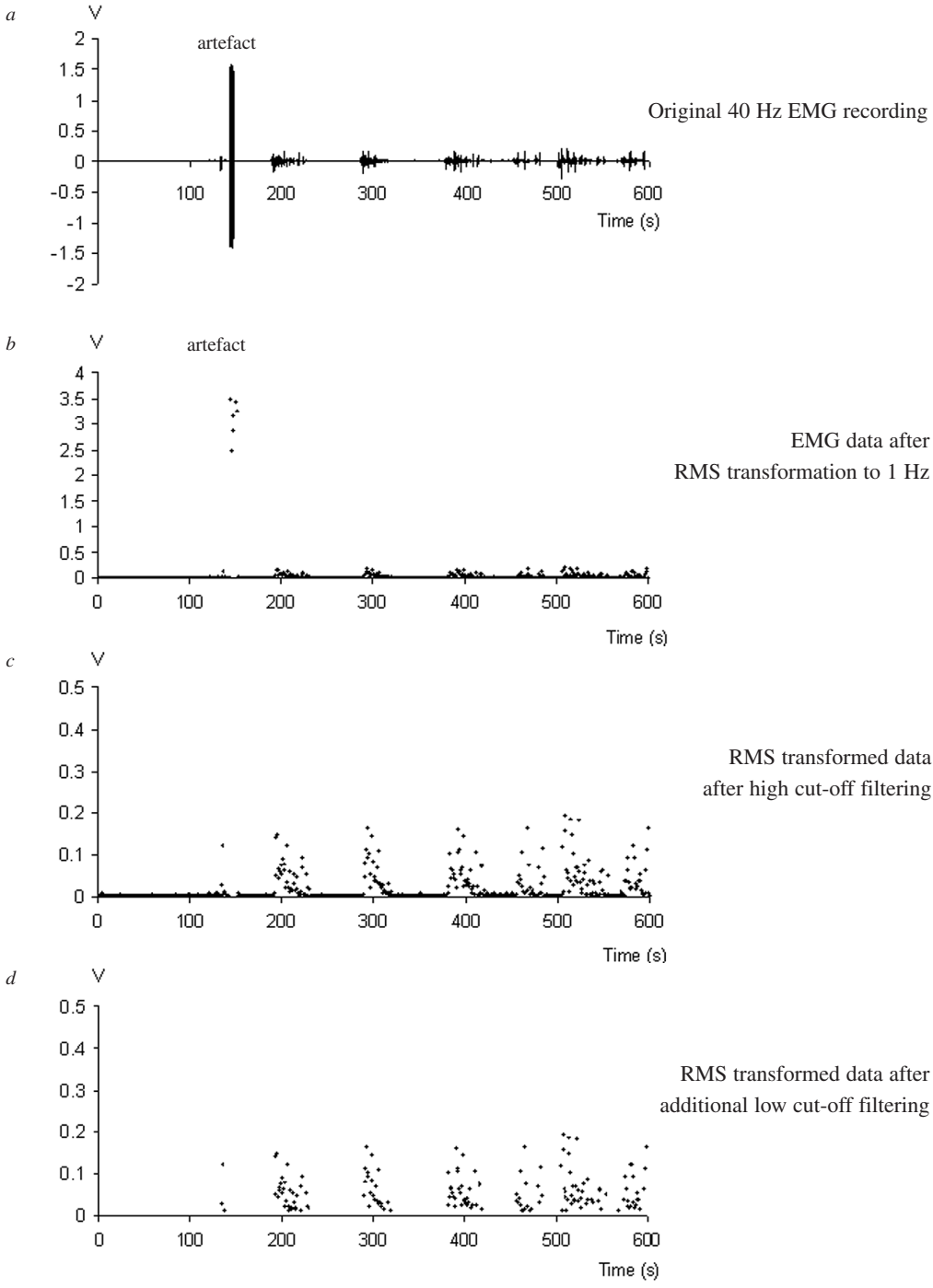

Fig. 4. Stepwise analysis of the uterine EMG signals.
EMG: Electromyography; RMS: Root Mean Square

activity originating from the myometrium. To eliminate these, as demonstrated in Fig. 4d, a cut-off value of 2.5% of the mean of the 100 largest RMS values from the entire first recording period (at 2 hours postpartum) was calculated for each electrode, in every cow, and this cut-off value was then applied to the other recording periods, made with the same electrode in the same cow. Following these steps, the remaining values were used to compare them with the results of the other electrode, and with the results of the pressure recordings obtained from the same cow.

2.5. Comparison of the two different IUP signals, the two separate EMG signals and the IUP and EMG signals

To make a within-cow comparison between the pressure data recorded by the IUP1 and the IUP2 systems, the sum of the areas of all the accepted pressure cycles were plotted against each other for each equivalent hour of the seven 2-hour recording sessions – yielding 14 TAUC (total area under the curve) values (7 x 2) for each cow and each IUP system. For the within-cow comparison of the two EMG signals, the sum of all the remaining RMS values for each 15-minute subperiod of recording was used, yielding 56 final values (7 recordings, each with 8 subperiods) for each cow and each EMG electrode. For the within-cow comparison between IUP and EMG data, the analyzed signals of both IUP devices were separately compared with those obtained from the more caudal EMG electrode (EMG2); this electrode was chosen because its position was closer to the ends of the IUP devices within the uterus. For each hour of recording, the TAUC values from each of the IUP devices were plotted against the sum of all the RMS values, obtained from the EMG2 electrode recording over that same hour, yielding 14 sets of values per comparison, per cow (7 x 2). Although the number of comparisons mentioned here, give the highest theoretically possible numbers, based on various reasons, these numbers are smaller for each comparison. Due to reposition of the IUP fixation system after shedding the fetal membranes in Cow C, the IUP data at 2 hours were not included in any analyses. Similarly, only those EMG data were evaluated, which had been recorded with unchanged EMG preamplifiers throughout the consecutive recording sessions. Therefore, recording sessions at 2 and 6 hours in Cow D and at 48 hours in Cow E were also excluded from all EMG analyses.

2.6. Statistical analysis

To obtain an initial rough description about the following comparisons: IUP1 vs. IUP2, EMG1 vs. EMG2, IUP1 vs. EMG2 and IUP2 vs. EMG2, correlation analyses were performed, using all the data i.e. without differentiating between recording times or the individual cows. As described above, the sum of the areas of the accepted pressure cycles were used for the IUP data, and the sum of the final RMS values were used for the EMG data for all of the analyses.

Two other statistical methods were also applied, one for the comparison of similar signal types (IUP1 vs. IUP2, and EMG1 vs. EMG2), and the other for the comparison of different signal types (IUP vs. EMG).

For the comparison of similar signal types, such as the IUP1 and the IUP2 signals, or the EMG1 and the EMG2 signals, the method of Altman and Bland [20] was applied. It is based on plotting the differences of two separate sets of measurements against their averages. Because the averages and the differences were significantly correlated at the original scale, both sets of data were log-transformed (ln) before doing this analysis [21]. The mean difference estimated on the log-scale, and the corresponding confidence interval, were then back-transformed to the original scale [STATISTICA, Version 6.1, StatSoft Inc, Tulsa, OK].

The different signal types, such as an IUP and an EMG signal, were compared using a General Linear Model (GLM) procedure. The GLM was used to analyse the relationship between the summarized RMS values for each hour (mV) from one EMG channel (EMG2), and the intrauterine pressure data for each hour as represented by the AUC (mmHg x s). Assuming that electromyography more directly reflects muscular activity than the pressure changes picked-up by any of the IUP-systems, the EMG2 signals were adopted as the standard for characterizing uterine contractility, and this statistical analysis aimed to test to what extent the results of the filtered IUP1 signals, or the unfiltered IUP2 signals, reflect this standard. For the sake of simplicity, and because the statistical tests for the two comparisons of IUP1 vs. EMG2 and IUP2 vs. EMG2 were identical, only the first comparison is given as an example. The effect of individual cows was included as independent variable in the following model [PROC GLM, SAS, Version 8.1, SAS Institute Inc, Cary, NC]:

$$IUP1 = \mu + Cow + EMG2(Cow) + \varepsilon.$$

In this equation, Cow represents the variation between individual cows in the IUP1 measurements, while EMG2(Cow) represents the cow-dependent relationship between EMG2 and IUP1. The correlation between EMG2 and IUP1 was calculated with the following formula:

$$r = \sqrt{\frac{SS_{EMG2(Cow)}}{SS_{EMG2(Cow)} + SS_{Error}}},$$

in which $SS_{EMG2(Cow)}$ represents the Type III sum of squares of the explanatory variable EMG2(Cow), and SS_{Error} represents the sum of squares of the error term for the expected value IUP1.

3. Results

3.1. Fixation of the catheter and the microtransducer

Due to excessive uterine contractions, one cow (Cow C) expelled the fetal membranes together with the IUP catheter and the microtransducer, but these measuring devices were successfully re-installed before the start of the next recording session (at 6 hours postpartum). In the other four animals the devices remained fixed until the end of the study. The applied special fixation system (the „*virtual knot*") could be thoroughly tested in the postpartum uterus,

both with and without placental retention, and was easily removed at the end of the study when it was no longer possible to insert the hand through the cervix. This new method of fixation of both the catheter and the microtransducer in the previously gravid uterine horn, was used effectively throughout the course of the study.

3.2. Comparison of the two IUP measuring systems

Original recordings from the IUP1 and the IUP2 systems are illustrated in Fig. 3. The proper digital filtering of the data remarkably improved the signal quality of the open tip system. To illustrate the types of data generated by the analysis, the descriptive data recorded by the IUP1 and IUP2 systems, for one of the cows that retained the fetal membranes throughout the course of the study, is presented in Table 1. Table 2a gives an hourly mean value of the total area under the curve, for each 2-hour recording session over the complete study period as measured by the two IUP systems, for all the cows.

Table 1. Mean frequency (FREQ), amplitude (AMP), duration (DUR) and area under the curve (AUC) in a cow with fetal membrane retention (Cow E), recorded by a disposable open tip catheter system (IUP1) and by a microtransducer system (IUP2).

Recording session (h):		2	6	12	18	24	36	48
FREQ (n/h)	IUP1	20.5	14.5	18.5	17.0	10.5	11.0	1.5
	IUP2	20.5	18.0	19.5	17.0	11.0	10.0	2.0
AMP (mmHg)	IUP1	25.2	20.8	21.7	19.5	17.7	12.0	10.0
	IUP2	25.4	31.0	19.1	25.7	27.3	18.1	12.1
DUR (s)	IUP1	52.8	56.2	56.2	68.1	79.6	66.9	92.2
	IUP2	56.2	48.7	48.0	49.8	58.0	47.0	46.2
AUC (mmHg x s)	IUP1	563.6	488.4	498.1	504.3	545.6	267.5	279.4
	IUP2	649.9	691.5	397.2	570.7	695.9	371.8	229.0

n/h: number of contractions per hour; mmHg: millimeter mercury; s: second; mmHg x s: millimeter mercury times seconds, a calculated unit for the area under the curves.

While FREQ represents the number of contractions during 1 hour, AMP, DUR and AUC values were obtained after averaging the individual data of all accepted pressure curves of a complete (2-hour) recording session.

In the initial comparison between the IUP1 and IUP2 systems, in which all the values were considered together, without differentiating between the cows or the recording sessions, a raw correlation coefficient, of r=0.617 (P<0.001) was obtained. The relatively low value of this correlation coefficient can be attributed to the lack of differentiation mentioned.

The more specific (Altman and Bland) analysis focused on the differences between the two simultaneous IUP measurements obtained on the same cow, during the same recording session.

Table 2a. Total area under the curve (TAUC) in early postpartum cows recorded by a disposable open tip catheter system (IUP1) and by a microtransducer system (IUP2).

Rec. session (h): Cow	System	2	6	12	18	24	36	48
A	IUP1	8501.0	11875.1	5716.6	7371.4	6236.3	4552.2	3726.2
	IUP2	6643.6	7945.5	6276.5	7380.1	6276.6	5651.8	2775.5
B	IUP1	9940.9	7244.6	5500.8	3337.3	3390.7	2389.2	1598.4
	IUP2	32383.5	21948.5	18328.9	15407.5	7600.4	3883.1	1134.9
C	IUP1		12005.7	6828.6	3052.7	3349.2	1921.9	1156.6
	IUP2		10486.9	4845.8	2486.2	3098.9	1249.8	551.5
D	IUP1	8993.3	1251.2	1565.7	1457.8	609.5	921.2	712.6
	IUP2	6770.9	2068.3	2775.6	1433.3	1004.0	871.2	0.0
E	IUP1	11553.5	7081.2	8295.7	8572.4	5729.3	2942.3	419.2
	IUP2	13322.8	12447.7	7745.6	9701.8	7655.0	3718.3	457.9

Data are expressed in mmHg x s, and they represent the mean TAUC values of 2 x 1-hour subperiods in each 2-hour recording session. TAUC values of each 1-hour subperiod were calculated by adding the individual AUC (area under the curve) values, which belonged to the accepted pressure cycles.

When Cow C dropped the fetal membranes before the recording session at 6 hours postpartum, the measuring devices were also expelled. Because from 6 hours onwards the new fixation site of the reposited IUP1 and IUP2 systems was obviously different from their original fixation site, pressure results of Cow C at 2 hours were not considered.

In Cow B, the IUP1 system seemed to show a markedly reduced sensitivity, because the TAUC values for most recordings were much lower than the ones, obtained with the IUP2 system (Fig. 7, Table 2a). This was probably due to a temporary obstruction of the open tip of the IUP1 system, and therefore the results from Cow B were excluded from this part of the analysis. In other cows, the IUP1 system did not show this reduced sensitivity. The resultant mean difference (without Cow B), measured on the log-scale, was 0.005, which, back-transformed to the original scale, resulted in a value of 1.005 (95% CI [-0.890; 1.134]). The correlation between the averages of, and the differences between the two intrauterine pressure measurement systems was not significant (r=0.040, P=0.785), which means that the reported differences are valid over the whole IUP measurement range.

3.3. Comparison of the two EMG electrodes

The initial comparison of the measurement from the two EMG electrodes, using the undifferentiated data, resulted in a raw correlation coefficient of r=0.600 (P<0.001). As already shown by the comparison of the two IUP systems, the neglect of the differences between individual cows and the effects of the different recording times would probably explain the value of this correlation coefficient (Table 2b).

Table 2b. Total root mean square (RMS) values of electromyographic activity in early postpartum cows recorded with the more cranial electrode (EMG1) and the more caudal electrode (EMG2).

Rec. session (h):		2	6	12	18	24	36	48
Cow	System							
A	EMG1	51.5	61.1	49.6	45.4	34.9	21.9	12.8
	EMG2	50.6	58.4	60.7	54.3	36.6	25.3	12.7
B	EMG1	27.7	16.6	17.8	12.8	9.9	6.7	4.2
	EMG2	62.7	50.0	81.1	63.0	56.2	27.7	15.8
C	EMG1	25.8	19.2	14.7	9.8	10.1	6.0	6.5
	EMG2	50.5	32.1	28.9	23.1	25.2	15.2	16.6
D	EMG1			9.0	13.0	5.6	6.2	6.2
	EMG2			11.9	15.4	8.9	6.2	8.9
E	EMG1	19.3	21.9	21.0	18.1	13.3	8.7	
	EMG2	15.7	16.6	15.2	13.9	11.1	6.4	

Data are expressed in mV and they represent the means of the 8 summarized RMS values of each 15-minute subperiods within every 2-hour recording session for an EMG electrode. Only those EMG data were evaluated, which had been recorded with unchanged EMG preamplifiers throughout the consecutive recording sessions. Therefore, recording sessions at 2 and 6 hours in Cow D and at 48 hours in Cow E were excluded from all EMG analyses.

The (Altman and Bland) analysis, including the data from all five cows, yielded a mean difference of -0.469 on the log-scale (ln); back transformation to the original scale resulted in a value of 0.626 (95% CI [0.564; 0.694]). The correlation between the averages and the differences of the two electromyographic recordings was not significant (r=0.111, P=0.076), proving that the calculated differences are valid over the whole EMG measurement range.

Although, using the derived data based on the 15-minute subperiods, there is a high similarity between the quantified signals from the two electrodes, there are also some differences. Inspection of the original EMG recordings (Figs. 5a and 6a), show beside exact synchronous also asynchronous pattern, thus partly explaining these differences.

3.4. Comparison of the two IUP signals with an EMG signal

Figures 5a and 5b show typical examples of simultaneous digitally recorded data from the IUP and EMG systems. Because of the good correlation between the quantified data from the two EMG-recordings, one electrode (EMG2) was selected for the comparison with the pressure data. Using the GLM procedure, in which calculations included a correction for the effect of the individual cows, the following results were obtained:

For the IUP1 vs. EMG2 comparison: r= 0.724, P<0.001; and

for the IUP2 vs. EMG2 comparison: r= 0.706, P<0.001.

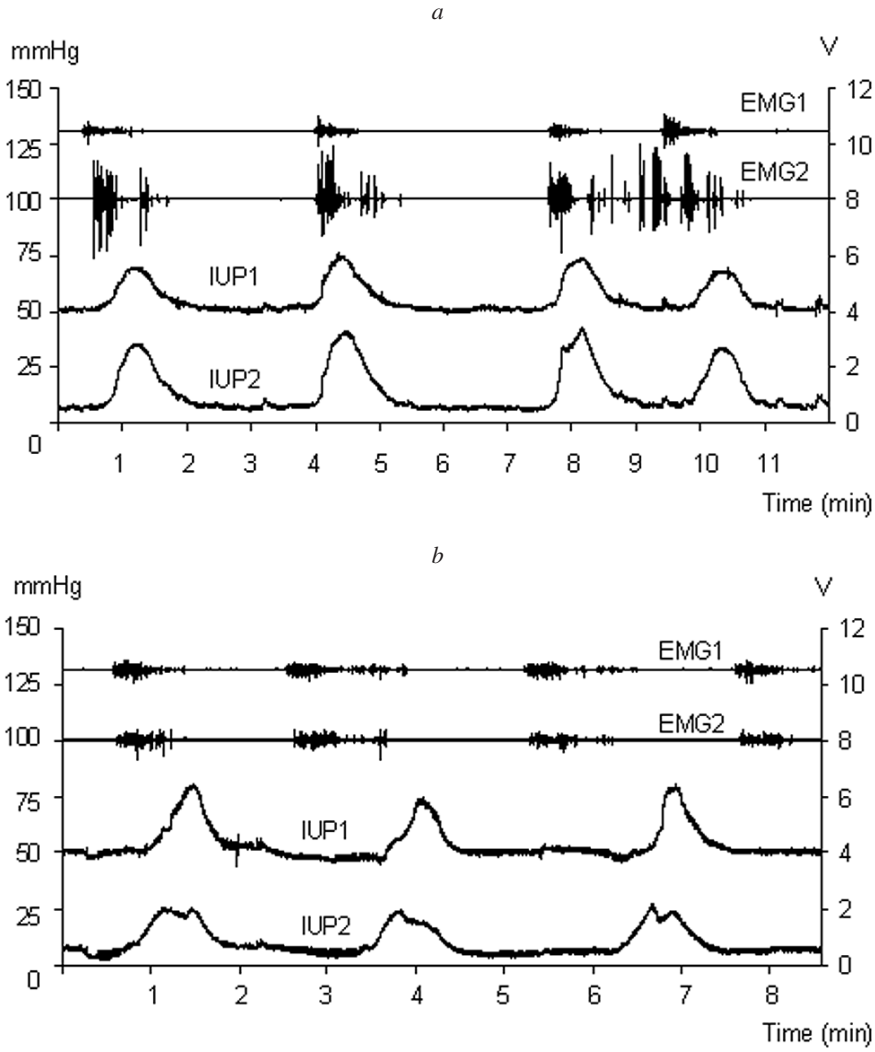

Fig. 5. Real time simultaneous electromyographic and pressure recordings at 2 hours postpartum *(a, b).*
EMG1: The more cranial EMG electrode; EMG2: The more caudal EMG electrode
IUP1: The HP-Ohmeda disposable open tip catheter system
IUP2: The Konigsberg implantable microtransducer system

3.5. Observations from the visual inspection of the IUP and EMG recordings

Even though a high correlation was calculated between the IUP and EMG signals, EMG-activity did not always coincide with the rising part of a pressure cycle - illustrating that the two

different signals are not obligatorily synchronized (Fig. 5b and 6a). Especially towards the end of the observation period, in some cows, the pattern of single individual electrical bursts accompanied by single pressure cycles, changed and prolonged periods, with more diffuse electrical activity and elevated pressure levels, became evident (Fig. 6b).

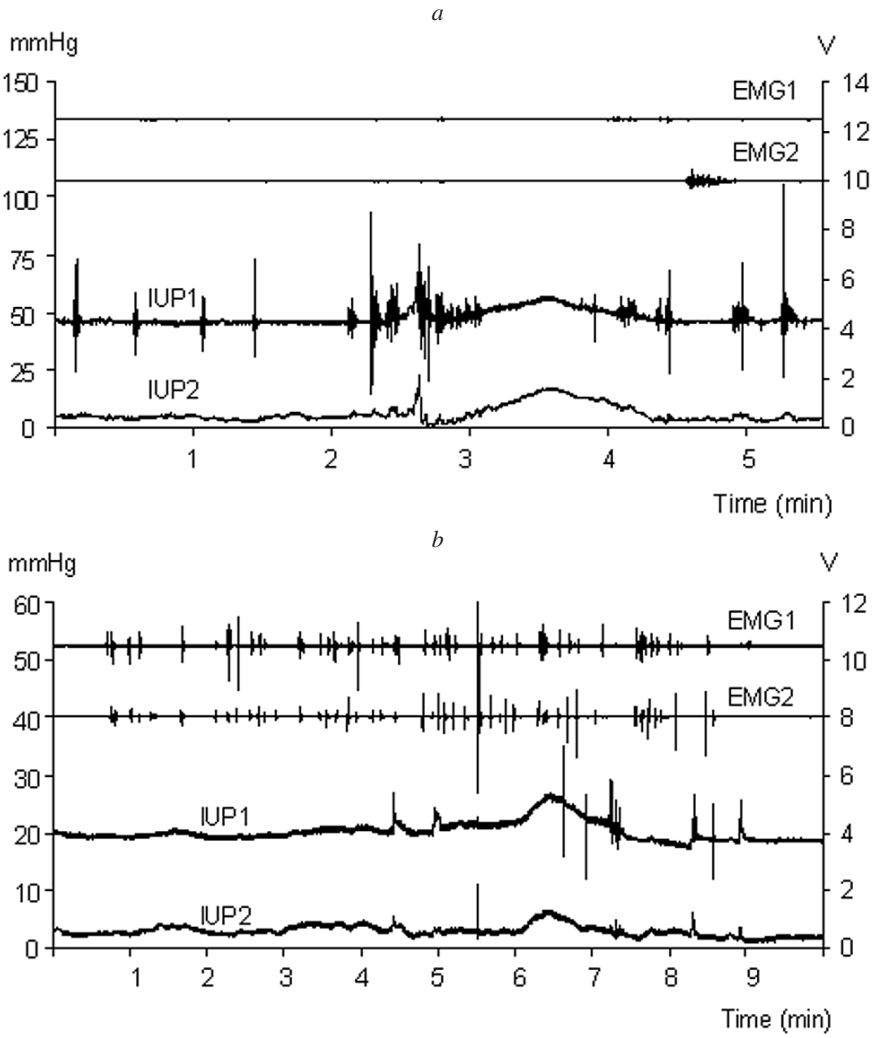

Fig. 6. Real time simultaneous electromyographic and pressure recordings at 6 *(a)* and 48 hours *(b)* postpartum.

EMG1: The more cranial EMG electrode; EMG2: The more caudal EMG electrode
IUP1: The HP-Ohmeda disposable open tip catheter system
IUP2: The Konigsberg implantable microtransducer system

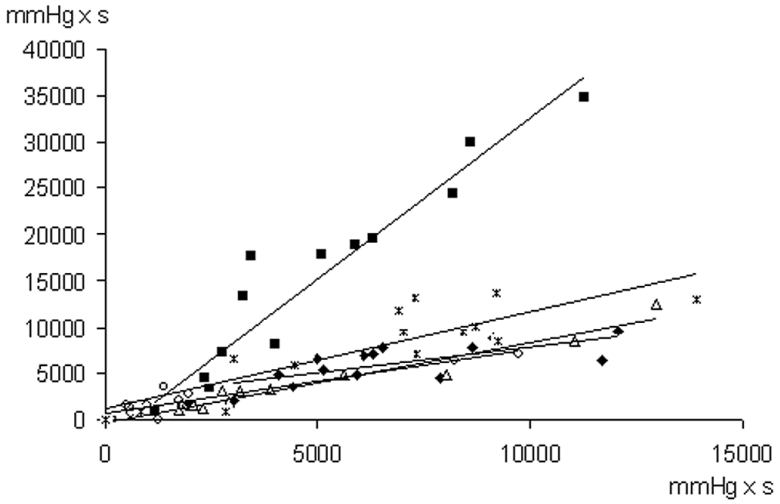

Fig. 7. Plots of the total area under the curve (TAUC) values, recorded by the IUP1 system (x-axis) and the IUP2 system (y-axis) in five postpartum cows.

◆: Cow A, ■: Cow B, Δ: Cow C, o: Cow D, *: Cow E

Each identical symbol represents a TAUC (total area under the curve) value of a 1-hour subperiod from the seven, 2-hour recording sessions of the same cow. The 1-hour TAUC values were calculated by adding the individual area under the curve (AUC) values, which belonged to the accepted pressure cycles during this subperiod.

4. Discussion

One of the main objectives of this study was to develop a reliable, non-invasive method for the fixation of the IUP measuring units during the early puerperal period in cows. Additionally, a digital data acquisition and analysis technique, allowing simultaneous IUP and EMG recordings, had to be established. Our results showed that we have met both of these objectives.

Based on the high correlation between the IUP1 and IUP2 data, and the significant correlations between both of these pressure signals and the electromyographic data, we conclude that the IUP signals are appropriate for characterizing the muscular activity of the uterus. This means that uterine mechanical activity in the early puerperal phase can be properly described using a disposable, low-cost catheter system, with the appropriate data acquisition and analysis technique.

This study has shown that the data obtained with the disposable open tip catheter system, when subjected to a post-measurement filtering process, can provide results, which are equally as good as those derived using the more expensive microtransducer; and since these measurements do not need surgical intervention, and because the intrauterine manipulation is restricted to the start of the observation period, this technique could be used „on-farm", in postpartum dairy cows.

Although numerous studies have described uterine mechanical and electrical activity in different species, including in human, especially during the gestational period and during parturition [8,10,17,19,22-32], scant attention has been paid to quantifying early puerperal uterine contractility. This is especially true in the case of cows [1,3,5,14,33-35].

4.1. Fixation of the catheter and the microtransducer

There is a certain risk that non-fixed IUP measuring units could be expelled by propagated uterine contractions during the early postpartum days. This risk is even higher in animals with fetal membrane retention, as the membranes themselves can also move backwards and forwards. In addition, fetal membrane retention is often combined with increased uterine activity [7,14]; this was confirmed by three cows (Cows A, B and E) in our study.

It has been reported that different parts of the uterus may show different levels of contractility [15,16,36]. If successive IUP signals are picked up from different locations within the uterine cavity, results become difficult to interpret. Therefore, the permanent intrauterine fixation of the measuring device was an important outcome of the procedure, followed in the present study, ensuring that data from repeated measurements would be comparable.

This study also demonstrated that, irrespective of whether a cow suffered from retained fetal membranes or not, the final removal of the measuring device could easily be accomplished 48 hours after calving, without the need for intrauterine or intravaginal manual assistance. Avoiding manual intrusion could be crucial, as with an advancing puerperium, cervical diameter diminishes [37] and closure proceeds rapidly, thereby restricting access. Especially in the case of cows that have already expelled their fetal membranes, manual enlargement of the cervix would be painful and could damage the organ. A further advantage of our fixation technique is that the risk of any iatrogenic effects is reduced because no repeated intrauterine manipulations are necessary.

4.2. Comparison of the two IUP measuring systems

In recording and decoding IUP measurements, it is important to distinguish between real uterine activity and artefacts [22]. Analogue IUP recordings are especially problematic during analysis, because the elimination of artefacts can not be standardized [38]. The use of digital data reduces the subjective influence introduced by the operator, and allows a greater repeatability of the results. Digitally stored data has the further advantage that it can be used at any later stage and that it is suitable for any type of further transformation and calculation.

The IUP signals, with their typical low frequency components, contained a lot more artefacts, usually in the high frequency domain, when using the disposable pressure recording system. Although Butterworth filtering of the data recorded by this system resulted in IUP curves that were very similar to those derived using the microtransducer, the degree of similarity, or the differences between the results as measured by the two devices, can be the

consequence of many factors.

One such factor, which becomes very obvious when a cow changes her position, is caused by the differences in the physical properties of the two IUP systems. The microtransducer incorporates a closed, gas-filled chamber that has been set to a fixed internal pressure and validated at a known temperature, during manufacture. The disposable system on the other hand, has an open-end catheter that is filled with fluid, which transmits pressure changes to the extracorporally placed transducer, and which has to be calibrated against the open air, before starting the measurements. The transduction in the microtransducer system takes place at the level of the measuring chamber, whereas in the disposable system, the transducer is usually attached at a distance of 1 to 1.5 meters from the site of pressure generation, usually to the gluteal area or to the root of the tail. This means that whenever a cow stands up, or lies down, a difference occurs in the measured pressure, because the relative position of the intrauterine measuring point changes in relation to the external position of the transducer. In a standing cow, there is always an unknown height difference between the internally fixed, open end of the fluid-filled catheter, and the externally fixed transducer. This height difference represents a negative pressure difference, which is reflected in the IUP curve. The height difference obviously changes when the cow lies down. The resulting pressure increase consists of two components. The first component is caused by the increased intra-abdominal pressure, due to the compression of the abdominal cavity in the recumbent cow. This component is equally present in recordings made with the microtransducer. The second component of the pressure rise results from the change in height between the level of the transducer fixed at the back of the cow, and the level of the open end of the catheter within the uterine lumen. This height difference is less when the cow is recumbent than when it is standing, and thus results in the base level of the pressure curve being elevated. One way to overcome the effects of intra-abdominal pressure changes on the IUP recording is, to simultaneously record the intra-abdominal pressure, using a separate measuring unit placed into the cranial vagina, and to correct the IUP curves with this data. This approach has been recommended for the measurements of IUP in cyclic mares [39].

Another factor that can lead to differences in the results from the two IUP measuring systems, is the changeability of the tissue and uterine contents in the area immediately surrounding the tips of the catheters. In the case of the disposable open tip catheter, the small holes on its internal end can become blocked by mucous or fetal membranes, or they can be covered by the stalk of the caruncle. In such cases pressure transmission is obviously hampered. This was most probably the case with the results for Cow B in this study, which appeared less sensitive (Fig. 7, Table 2a). The tip of any of the catheters may be permanently located in a fluid phase, or in an area where gas/air accumulation occurs. It is, therefore very difficult to distinguish between ideal and suboptimal measuring conditions within the uterine lumen. To reduce the risk of obstruction of the open tip catheter, flushing with saline is recommended [40], either repeatedly or at a low, continuous rate. In this study, 10 ml of saline was used to flush the open tip catheter at the start of each 2-hour recording session. Since this flushing procedure does not markedly increase the large amount of fluid already present within the uterine cavity,

and is quick and simple, its application under farm circumstances can be easily performed and is recommended if the open tip catheters are used. Another technique reduces this problem, using a small sponge attached to the internal end of the open tip catheter [13]. The sponge avoids the obstruction of the catheter, by preventing small endometrial fragments from entering into the lumen of the catheter, or fetal membranes from completely covering the small openings of the device.

4.3. Comparison of the two EMG electrodes

It turned out from this study, that the two EMG signals from the same uterus are highly comparable, but not identical. Despite the conformity, a part of the difference between the signals from the two electrodes is reflected by the absence of exact synchrony between the two EMG recordings, which were obtained simultaneously (Fig. 6a). This lack of a perfect synchrony can be attributed to two main factors. Firstly, the locations of the two electrodes were different; one was consequently sutured near the tip of the gravid uterine horn (EMG1), while the other was fixed more caudally (EMG2). It has previously been observed in cows [36] and mares [41] during the oestrus cycle, that there can be different EMG patterns at the tip, and at the more caudal part of the uterine horn. This could explain part of the lack of correspondence of the two EMG signals. Secondly, it could be caused by the different degree of implantation of the electrodes at the two sites. During the postoperative phase, due to connective tissue formation, the electrode pins might be subject to different degrees of contact with the surrounding muscle layer into which they were implanted. The more such tissue there is around an electrode tip, the greater the electrical isolation of the silver pins, and the weaker the resulting outgoing signals. This could be an explanation, why one electrode (EMG1) especially in Cow B was less sensitive over the whole observation period (Table 2b).

4.4. Comparison of the two IUP signals with an EMG signal

In agreement with findings in late pregnant sheep [2,42-44], rhesus monkey [45] and according to our expectations, good correlations were found in this study, between simultaneous IUP and EMG measurements of the postpartum bovine uterus. The increase in mechanical activity, as measured by the rise of intrauterine pressure, can obviously be explained by the coincident rise in EMG activity in a certain population of the neighbouring uterine smooth muscle cells. Typically, this increase in electrical activity starts a few seconds before the onset of a pressure curve, and is only present during the rising phase of the cycle; this has been reported previously in parturient sheep [24] and has now been visualized in our study in early postpartum cows (Fig. 5a). However, it is sometimes difficult to distinguish, which electrical burst, and which IUP cycle are functionally coupled (Fig. 5b and 6a). There might be at least two reasons for this. On the one hand, the electrical and mechanical signals are not sourced from

exactly the same location in the uterus; on the other hand, the patterns of electrical and mechanical activity (see below) change with advancing puerperium.

Despite our efforts to standardize the surgical and non-surgical techniques, the locations of the surgically implanted EMG electrodes, and the intrauterine fixation sites of the transcervically inserted pressure-recording devices obviously varied from cow to cow. Such locational differences can, by themselves, cause a smaller or larger asynchrony between the recordings of the electrical and mechanical activity of the uterus.

In addition to the single pressure waves, which are typical for the immediate postpartum phase (Fig. 5a), we also observed that, sometimes, prolonged periods of uterine EMG activity and elevated IUP occur from 36 hours postpartum onwards (Fig. 6b). Similar slowly rising changes in the basal uterine pressure (so-called contractures) were previously observed in late pregnant ewes. These contractures were defined as pressure increases lasting for at least 5 minutes and exceeding basal uterine tone by at least 3.5 mmHg [46]. Similarly, as reported by others [42,47], we also found that bursts of EMG activity were not consistently accompanied by a prolonged increase of IUP. The reason for this could be that, even if myoelectrical activity is measured in certain locations of the uterus, the whole organ does not contract, and thus the IUP will not increase. Beside an intrinsic control, which is associated with the occurrence of these contractures, as reported in pregnant sheep [30], oxytocin, prostaglandins and catecholamines may also affect this appearance [32]. Therefore, the characteristics and regulation of contractures in the postpartum cow need further investigation.

In conclusion, because the quantified signals from both the microtransducer system, and from the EMG electrodes, correlated well with those from the disposable IUP catheter system, this latter device is considered to be suitable for investigating spontaneous uterine activity in early puerperal cows under on-farm conditions. It will also allow the testing of various uterotonic drugs for their effect on uterine mechanical contractility.

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5. References

1. Gillette DD, Holm L. Prepartum to postpartum uterine and abdominal contractions in cows. *Am J Physiol* 1963;204:1115-1121.
2. Toutain PL, Garcia-Villar R, Hanzen C, Ruckebusch Y. Electrical and mechanical activity of the cervix in the ewe during pregnancy and parturition. *J Reprod Fertil* 1983;68:195-204.
3. Burton MJ, Dziuk HE, Fahning ML, Zemjanis R. Myometrial activity during natural and dexamethasone-induced parturition in the cow. *Am J Vet Res* 1987;48:37-44.
4. Hays RL, Van Demark NL. Spontaneous motility of the bovine uterus. *Am J Physiol* 1953;172:553-556.
5. Giama I. Erfassung der postpartalen Uterusmotilität des Rindes und der motilitätssteigernden Wirkung eines Oxytozinpräparates. 1. Mitt.: Spontane Uterusmotilität im Frühpuerperium des Rindes nach normalen und gestörten Geburten. *Monatsh Veterinärmed* 1975;30:850-852.
6. Naaktgeboren C, van der Weyden GC, Klopper PJ, Kroon CH, Schoof AG, Taverne MAM. Electrophysiological observations of uterine motility during the oestrous cycle in sheep. *J Reprod Fertil* 1973;35:511-518.
7. Taverne MAM, van der Weyden GC, Fontijne P. Preliminary observations on myometrial electrical activity before, during and after parturition in the cow. In: Hoffmann B, Mason IL, Schmidt J (eds): *Calving Problems and Early Viability of the Calf. Volume 4.* The Hague: Martinus Nijhoff, 1979;297-311.
8. Taverne MAM, Naaktgeboren C, van der Weyden GC. Myometrial activity and expulsion of fetuses. *Anim Reprod Sci* 1979;2:117-131.
9. Hanzen C. Electrical activity of the bovine uterus prior to and post parturition. *Vet Res Commun* 1981;5:143-150.
10. Taverne MAM, Scheerboom JEM. Myometrial electrical activity during pregnancy and parturition in the pygmy goat. *Res Vet Sci* 1985;38:120-123.
11. Finn CA, Porter DG. Part 3: The Myometrium. In: Finn CA, Porter DG (eds): *Reproductive Biology Handbooks. Volume 1. The Uterus.* London: Elek Science, 1975;133-274.
12. Braaksma JT, Janssens J, Eskes TKAB, Hein PR. Accurate pressure recording in the non-pregnant human uterus. A comparison of open and closed tip catheters. *Eur J Obstet Gynecol* 1971;6:195-206.
13. Bengtsson LP. The sponge-tipped catheter - A modification of the open end catheter for recording of myometrial activity in vivo. *J Reprod Fertil* 1968;16:115-118.
14. Venable JH, McDonald LE. Postparturient bovine uterine motility - normal and after experimentally produced retention of the fetal membranes. *Am J Vet Res* 1958;19:308-313.
15. Zerobin K. Die Uterusbewegungen bei Kühen während der Geburt und der Nachgeburtsphase. *Schweiz Arch Tierheilkd* 1970;112:544-560.
16. Taverne MAM. Uterine motility in the post partum female. In: *Proceedings* Xth Int Congr Anim Reprod AI 1984. Illinois.Vol.IV:XI-1-8.
17. van der Weyden GC, Taverne MAM, Dieleman SJ, Fontijne P. Myometrial electrical activity throughout the entire course of pregnancy in the ewe. *Europ J Obstet Gynecol Reprod Biol* 1981;11:347-354.
18. Ruckebusch Y. L'Electromyographie globale des muscles lisses a partir d'electrodes chroniques intraparietales souples. *Rev Méd Vét* 1973;124:1407-1434.
19. Janszen BPM, Knijn H, van der Weyden GC, Bevers MM, Dieleman SJ, Taverne MAM. Flumethason-induced calving is preceded by a period of myometrial inhibition during luteolysis. *Biol Reprod* 1990;43:466-471.
20. Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. *Statistician* 1983;32:307-317.

21. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;347:307-310.
22. Csapo A. The diagnostic significance of the intrauterine pressure. Part I. General considerations and techniques. *Obstet Gynecol Surv* 1970;25:403-435.
23. Seitchik J, Chatkoff ML. Intrauterine pressure wave form characteristics in hypocontractile labor before and after oxytocin administration. *Am J Obstet Gynecol* 1975;123:426-434.
24. Krishnamurti CR, Kitts DD, Kitts WD, Tompkins JG. Myoelectrical changes in the uterus of the sheep around parturition. *J Reprod Fertil* 1982;64:59-67.
25. Tromans PM, Beazley JM. Application of a real-time microcomputer monitoring system: surveillance of induced labour by uterine activity quantitation. *Br J Obstet Gynaecol* 1983;90:40-48.
26. Randall GCB, Taverne MAM, Challis JRG, Kendall JZ, Tsang BK. Interrelationships between endocrine changes in peripheral and uterine-venous blood and uterine activity at parturition in the pig. *Anim Reprod Sci* 1986;11:283-294.
27. Jonker H, Taverne MAM, van der Weyden GC. Cardiotocography in cows: a method for monitoring calves during delivery. *Theriogenology* 1989;31:425-436.
28. Nathanielsz PW. The regulation of the switch from myometrial contractures to contractions in late pregnancy: studies in the pregnant sheep and monkey. In: Gluckman PD, Johnston BM, Nathanielsz PW (eds): *Research in Perinatal Medicine (VIII). Advances in fetal physiology: Reviews in Honor of G.C. Liggins.* Ithaca, New York: Perinatology Press, 1989;409-420.
29. Kündig H, Thun R, Zerobin K, Bachmann B. Die Uterusmotorik des Rindes während Spätgravidität, Geburt und Puerperium. I. Die Spontanmotorik. *Schweiz Arch Tierheilkd* 1990;132:77-84.
30. Lye SJ. Evidence for an intrinsic control of myometrial contractile periodicity in sheep during pregnancy. *J Reprod Fertil* 1992;96:337-345.
31. Buhimschi C, Boyle MB, Saade GR, Garfield RE. Uterine activity during pregnancy and labor assessed by simultaneous recordings from the myometrium and abdominal surface in the rat. *Am J Obstet Gynecol* 1998;178:811-822.
32. Taverne MAM, de Schwartz NCM, Kankofer M, Bevers MM, van Oord HA, Schams D, Gutjahr S, van der Weijden GC. Uterine responses to exogenous oxytocin before and after pre-partum luteolysis in the cow. *Reprod Domest Anim* 2001;36:267-272.
33. Zerobin K. Die uterusmotorischen Ablaeufe waehrend Geburt und Puerperium beim Rind und deren Beeinflussbarkeit. In: *Proceedings* XI. Int Congr Dis Cattle 1980. Tel-Aviv.Vol.II:1157-1164.
34. Däberitz H, Wilhelm J, Eulenberger K, Richter A. Ergebnisse experimenteller Untersuchungen zur Erfassung der bioelektrischen Uterusaktivität im peripartalen Zeitraum und im Puerperium des Rindes. I. Mitteilung: Methode zur Erfassung, Aufzeichnung und Auswertung der bioelektrischen Aktivität des Uterus während der Geburt und in der Nachgeburtsperiode beim Rind. *Arch Exper Veterinärmed* 1984;38:676-686.
35. Gajewski Z, Thun R, Faundez R, Boryczko Z. Uterine motility in the cow during puerperium. *Reprod Domest Anim* 1999;34:185-191.
36. Ruckebusch Y, Bayard F. Motility of the oviduct and uterus of the cow during the oestrous cycle. *J Reprod Fertil* 1975;43:23-32.
37. Bekana M, Ekman T, Kindahl H. Ultrasonography of the bovine postpartum uterus with retained fetal membranes. *J Vet Med A* 1994;41:653-662.
38. Braaksma JT, Veth AFL, Eskes TKAB, Stolte LAM: Digital evaluation of uterine contraction records. In: Josimovich JB (ed): *Uterine Contraction-Side Effects of Steroidal Contraceptives. Volume 1. Problems of human reproduction: A Wiley-Interscience series.* New York: John Wiley & Sons, 1973;9-18.
39. Gutjahr S, Paccamonti DL, Pycock JF, Taverne MAM, Dieleman SJ, van der Weijden GC. Effect of dose and day of treatment on uterine response to oxytocin in mares. *Theriogenology* 2000;54:447-456.

40. Langendijk P, Bouwman EG, Soede NM, Taverne MAM, Kemp B. Myometrial activity around estrus in sows: spontaneous activity and effects of estrogens, cloprostenol, seminal plasma and clenbuterol. *Theriogenology* 2002;57:1563-1577.
41. Troedsson MHT, Wiström AOG, Liu IKM, Ing M, Pascoe J, Thurmond M. Registration of myometrial activity using multiple site electromyography in cyclic mares. *J Reprod Fertil* 1993;99:299-306.
42. Nathanielsz PW, Bailey A, Poore ER, Thorburn GD, Harding R. The relationship between myometrial activity and sleep state and breathing in fetal sheep throughout the last third of gestation. *Am J Obstet Gynecol* 1980;138:653-659.
43. Harding R, Poore ER, Bailey A, Thorburn G, Jansen CAM, Nathanielsz PW. Electromyographic activity of the nonpregnant and pregnant sheep uterus. *Am J Obstet Gynecol* 1982;142:448-457.
44. Lye SJ, Challis JRG. Paracrine and endocrine control of myometrial activity. In: Gluckman PD, Johnston BM, Nathanielsz PW (eds): *Research in Perinatal Medicine (VIII). Advances in fetal physiology: Reviews in Honor of G.C. Liggins.* Ithaca, New York: Perinatology Press, 1989;361-375.
45. Hsu HW, Figueroa JP, Honnebier MBOM, Wentworth R, Nathanielsz PW. Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation. *Am J Obstet Gynecol* 1989;161:467-473.
46. Jansen CAM, Krane EJ, Thomas AL, Beck NFG, Lowe KC, Joyce P, Parr M, Nathanielsz PW. Continuous variability of fetal pO2 in the chronically catheterized fetal sheep. *Am J Obstet Gynecol* 1979;134:776-783.
47. Nathanielsz PW, Poore ER, Brodie A, Taylor NF, Pimentel G, Figuerora JP, Frank D. Update on the molecular events of myometrial activity during pregnancy. Chapter 4. In: Nathanielsz PW, Parer JR (eds): *Research in Perinatal Medicine*. Ithaca, New York: Perinatology Press, 1984;87-111.

CHAPTER 4

Characteristics of bovine early puerperal uterine contractility recorded under farm conditions

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Abstract

A non-invasive, digital technique was used to measure and quantify intrauterine pressure (IUP) changes in early postpartum dairy cows kept under farm conditions in order to document physiological changes in uterine contractility after uncomplicated calvings. In addition, possible relationships between characteristics of uterine contractility and blood ionized calcium (Ca^{2+}) - concentrations were investigated. Recordings of uterine contractility were made by using a transcervically inserted open tip catheter in 12 healthy cows during their first 48 hours after calving. The IUP recording technique appeared easily applicable under farm conditions. Although mean frequency (FREQ), amplitude (AMP) and area under the curve (AUC) of the myometrial contractions significantly decreased due to time, untreated early postpartum cows showed a high variability in characteristics of uterine contractility. There was no correlation between blood Ca^{2+}-concentrations and any of the contractility parameters.

Keywords: early puerperium; dairy cows; farm conditions; uterine contractions; ionized calcium

1. Introduction

Immediately after calving uterine involution starts, preparing the genital tract for a subsequent conception. Puerperal disorders in this early period may cause an extension of complete uterine involution and may lead to a delayed resumption of ovarian activity, causing prolonged calving to conception intervals and increasing production costs at the farm [1].

Uterine smooth muscle activity plays an important role in expulsion of uterine contents, clearing of its cavity and in the reduction of uterine size. These processes, however, can be perturbed, resulting in puerperal disorders, such as retained fetal membranes (RFM), or endometritis. It is still not clear whether retention of the fetal membranes is the result of a decreased uterine muscle activity. Some reports have even found an increased level of uterine muscle activity during the first days postpartum in cows with RFM [2,3]. Uterine activity is decreased in cows with severe hypocalcemia as it was shown in a preliminary study [4] where hypocalcemia was experimentally induced. In milk fever also RFM more often occurs than in cows not suffering from milk fever [5,6].

In order to improve the efficacy of puerperal uterine involution in dairy cows, first the spontaneous physiological changes in uterine contractility should be known. One possible way of characterizing uterine contractility is to measure changes in intrauterine pressure (IUP). IUP is usually obtained from the uterine lumen and it reflects internal pressure changes caused by the contractile activity of the uterine muscular tissue. It is supposed to give a good estimation about uterine mechanical activity [7,8]. In principal, there are two different ways to measure intrauterine pressure changes. Pressure obtained at a certain location within the uterine cavity can be transformed into an electrical signal either in situ or outside the body. In situ transformation can be reached by using either a catheter with built in miniature pressure sensors (such as the Millar catheter), or an implantable microtransducer (such as the Konigsberg transducer). When pressure signals are transformed into electrical signals outside the body, pressure waves picked up with either a fluid-filled catheter or a fluid-filled balloon, are first forwarded to an external transducer, which is fixed outside the body. In systems where pressure changes are transformed into electrical signals at the place of pressure generation, the effect of noise is reduced, while in systems that use fluid-filled catheters between the data acquisition point and the transducer, more artefacts can be expected especially by movements of the animal. Our previous investigations showed that by using post measurement digital filtering, this noise can be effectively reduced and filtered signals became nearly similar to those obtained through an on site signal transforming microtransducer technique [7].

More descriptions are available in human obstetrics dealing with IUP measurements and analysis; among them Finn and Porter [8], Csapo [9] and Fischer et al. [10] reviewed basic methodological knowledge of IUP recordings, while Braaksma et al. [11] compared different recording techniques. Several studies have also been performed in cows [2,12-18], but they provide hardly any quantitative information about physiological changes in intrauterine pressure during the early normal puerperium.

The aim of the present study was to record and quantify intrauterine pressure changes in early postpartum dairy cows kept under commercial farm conditions using a validated, non-invasive technique for measuring and analyzing IUP changes [7]. Uterine contractility was recorded in 12 healthy cows during the first 48 hours after uncomplicated calvings. In addition, possible relationships between characteristics of uterine contractility and blood Ca^{2+}-concentrations were investigated.

2. Materials and methods

2.1. Cows

Twelve Holstein Friesian cows that had expelled the fetal membranes within 12 h postpartum were included in the study. Six of these cows had one previous calving, 4 cows had two and 2 cows had three earlier parturitions. Although light assistance (one or two persons pulling) had been applied to each cow, all calvings were uncomplicated and resulted in deliveries of healthy calves. The cows were kept in a free stall housing system where unrestrained calvings were allowed. The calves were separated from their dams after they have sucked within 4 to 6 hours after parturition. The cows were then placed into another building next to the calving barn. In their new place, where also the IUP measurements were performed, the cows were kept non-restrained in small groups of 4 to 5 cows. Animals were restrained twice daily for milking and for the time of the measurements according to the protocol, but they were allowed to move freely between these events.

2.2. Recording equipment, experimental protocol

At 12-14 h after calving[1], a thin polyethylene, open tip catheter (Hewlett-Packard, Andover, MA) was inserted transcervically into the previous pregnant uterine horn and it was fixed in a non-invasive way to the stalk of a caruncle according to a method, described elsewhere [7]. The type of internal fixation that was used enabled an easy removal of the catheter by the end of the study. The external end of the catheter was connected to a disposable pressure transducer (Ohmeda Inc., Murray Hill, NJ), which had been previously fixed with adhesive tapes to the shaved skin of the gluteal area. After connection, the catheter was filled with a standard amount of saline (10 ml 0.9% NaCl solution). The catheter was then flushed with the same amount of saline before every session.

[1] *Although a catheter was inserted between 12 and 14 hours after calving, and subsequent IUP recording sessions were started at 12-hour intervals, for an easier understanding these sessions were labeled in the text and in the graphs as 12, 24, 36 or 48 hours after calving.*

Specific, operator-made programs were developed for IUP digital data acquisition and analysis, using the software LabVIEW® 5.0 (National Instruments Corp., Austin, TX). The amplified, analogue electrical signals were converted into digital signals using an analogue-digital converter. All the instruments used for measuring IUP, like the preamplifier (GP 471 DC preamplifier unit, Schwarzer GmbH Medical Diagnostic Equipment, Munich, Germany), the A-D converter (PCMCIA DAQCard™-1200, National Instruments Corp., Austin, TX) and the computer (Compaq Armada V300 notebook computer, Compaq Inc., The Netherlands), were relatively small in size, allowing their placement on a trolley with inflated rubber wheels.

After a 30-minute adaptation period, uninterrupted IUP recordings were performed for 60 minutes. The sampling frequency of the digital recordings was set to 4 Hz. Similar recording sessions were repeated three times at 12 h intervals. Before the first recording and at the end of each session, a blood sample was obtained anaerobically via heparinized syringes (S 4500 calcium titrated heparin solution; Radiometer, Copenhagen, Denmark) from either the coccygeal artery or vein for determination of the Ca^{2+}-concentration, using a portable blood gas and electrolyte analyzer (ABL™ 77, Radiometer, Copenhagen, Denmark). Identical values after measurements in arterial and venous blood Ca^{2+}-concentrations [19] allowed us to neglect the origin of the blood during sampling of the tail vessels. Details of the blood withdrawal and handling of the samples were described previously [19,20]. The experimental protocol is illustrated in Fig. 1.

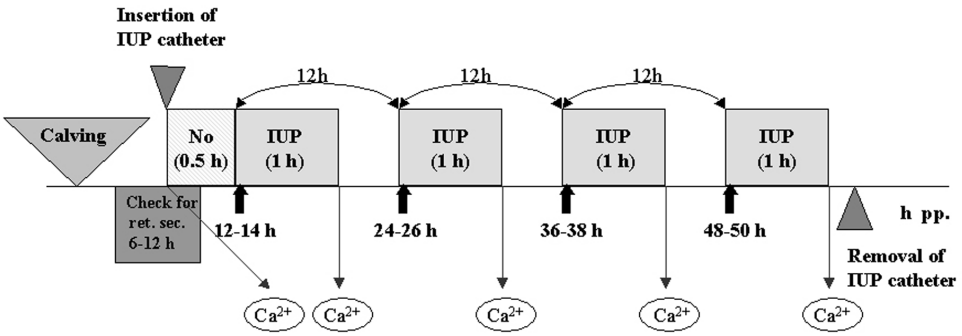

Fig. 1. Experimental protocol for measurements of intrauterine pressure (IUP) and blood ionized calcium (Ca^{2+})-concentration in early puerperal cows.

No = no recording (adaptation period), ret. sec. = retentio secundinarum, retained fetal membranes (RFM), pp. = postpartum

2.3. Data analysis

Standardized criteria for accepting IUP curves as real uterine contractions have been described in detail elsewhere [7], and can be summarized as follows. The analysis of the recordings consisted of three main steps. For each cow *first* the pressure cycle with the largest amplitude was selected from every recording session (n = 4) from the analogue-digital converted filtered signals. Then these amplitudes were averaged resulting in the mean maximum amplitude (MMA), a basic value

for further analysis that was specific for each cow. Based on this value, every IUP peak was manually scanned in the *second step*, using the 10% of the MMA. All pressure cycles above that threshold level were preliminary accepted and measured for their starting and ending point, as well as their highest amplitude. The program calculated amplitude, duration and area under pressure curves immediately. Remarks about any events such as defecation or urination as obvious artefacts coinciding with pressure changes, were used to skip pressure cycles even if they met the above-mentioned criteria. During the *third step* a final selection from the identified pressure waves was made on the basis of the size of area under the pressure curves. Only those pressure cycles were included in the final statistical analysis that exceeded the 10% level of the mean of the five largest areas from the first recording session at 12 h postpartum (pp). This value was subsequently used for all the recordings of the same cow. According to a previous calibration, amplitude (AMP) was finally expressed as relative pressure in mmHg, duration (DUR) in seconds and area under the curve (AUC) in mmHg x seconds. Contraction frequency (FREQ) was expressed as number per hour.

For statistical analysis, a repeated measures analysis of covariance (ANCOVA) procedure was used to evaluate the time effect in uterine contractility. Any of the IUP parameters (FREQ, AMP, DUR, AUC) were used as dependent variable, and blood Ca^{2+}-concentration as changing covariate in the ANCOVA procedures. Contrast analysis was applied to test significance of selected time differences in any IUP parameters as a component of the ANCOVA procedure. Additionally, regression analysis was performed to investigate the relationships between different IUP parameters (FREQ, AMP, DUR, AUC) and blood Ca^{2+}-concentration at each time point. Apart from their association with IUP parameters, changes in blood Ca^{2+}-concentrations were separately tested with repeated measures analysis of variance (ANOVA) as well. All data were initially tested for normality using the Kolmogorov-Smirnov test. [21].

3. Results

Representative examples of IUP recordings are shown in Fig. 2a (at 12 h postpartum).

The statistical analysis of the recordings gave the following results:

Despite the high individual variability in FREQ, overall time dependent changes proved to be highly significant ($P<0.001$). Mean FREQ was 8.9/h at 12 h postpartum, where individual cows had values between 6 and 11/h. Thereafter, mean contraction frequency decreased to 1.8/h at 48 h. The largest drop in mean values occurred between 12 and 24 h after parturition. FREQ decreased by 46% of the initial mean value. In addition, contrast analysis revealed significant differences ($P<0.001$, or $P<0.01$) when FREQ at 12 h was compared with the other three time points, but after 24 h no significant differences occurred between subsequent time points from 24 h after parturition onwards (Fig. 3a).

Significant time dependent changes ($P<0.001$) in mean AMP occurred with typical initial individual mean values ranging up to 40 mmHg at 12 h. Changes in mean AMP showed a similar pattern as FREQ changes, with highest initial mean values at 12 h postpartum (19.6 mmHg) and

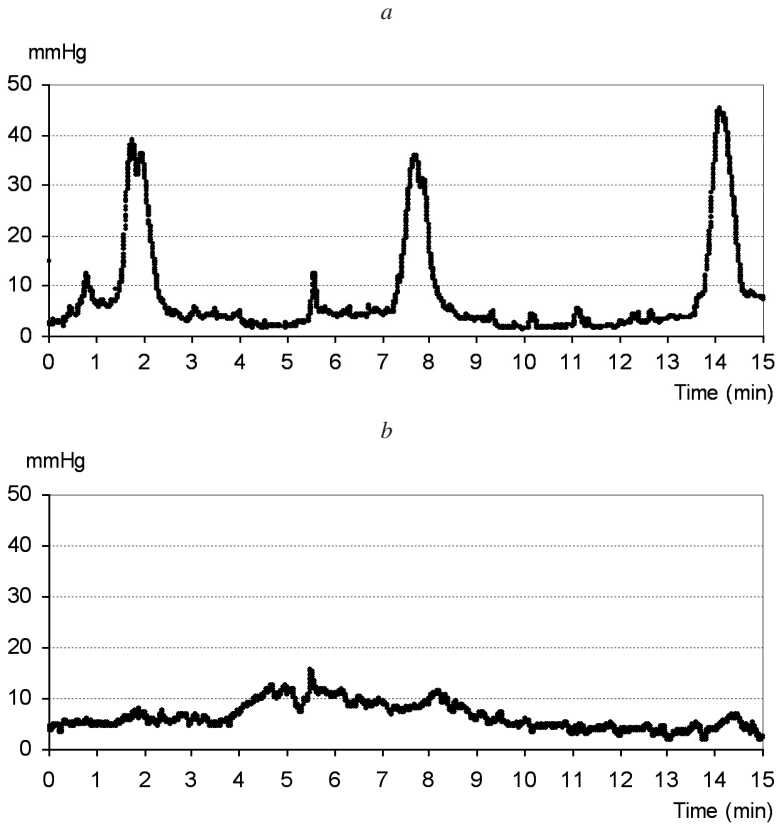

Fig. 2. (*a*) Representative example of a 15-min intrauterine pressure (IUP) recording, containing three individual contractions (at 12 hours postpartum). (*b*) A so-called contracture at 36 hours postpartum.

a reduction of the AMP-s to one sixth of the starting values by 48 h postpartum (3.2 mmHg). The most marked drop in this parameter also occurred between 12 and 24 hours (by 42 %). Contrast analysis resulted in significant changes between values at any time points, except that between 36 and 48 h after calving (Fig. 3b).

Mean DUR of the pressure curves varied between 67.9 and 102.5 seconds, with the lowest value at 48 h pp, and the highest one at 36 h (Table 1). Changes in DUR appeared to be non significant in any test applied. Instead of these typical individual pressure curves, long lasting elevations (≥2.5 min) from the baseline developed at 36 h pp in three cows and at 48 h pp in a fourth cow. These elongations of the increased pressure tone are also called contractures in accordance with previous studies in ruminants [22, 23]. However, they were not present in the remaining eight cows. Contractures were included in the analysis if they met the standardized criteria for accepting IUP curves as described earlier. Fig. 2b illustrates a typical contracture at 36 h postpartum.

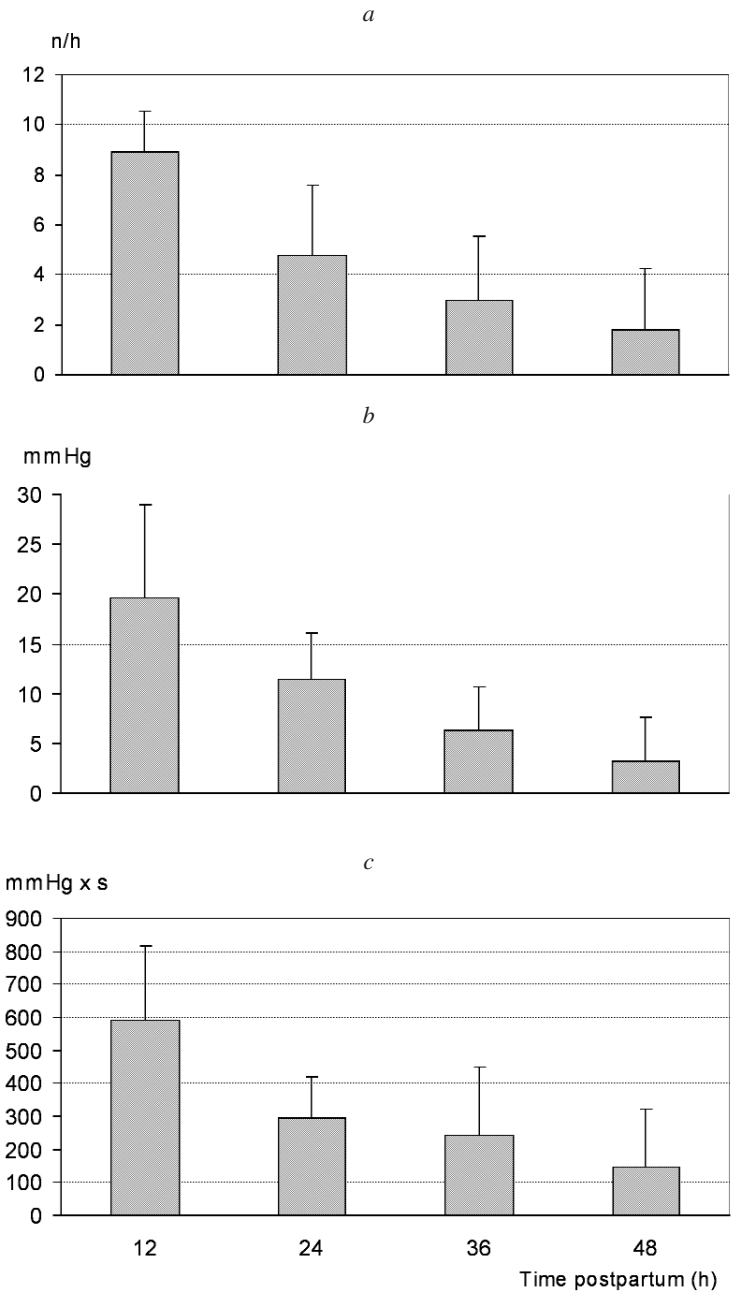

Fig. 3. (*a*) Changes in mean (+SD) contraction frequency (FREQ), (*b*) relative amplitude (AMP) and (*c*) area under the curve (AUC) during the early postpartum period.

Table 1. Mean (±SD) durations (DUR; in s) of intrauterine pressure (IUP) waves and their ranges during the early puerperium of untreated cows.

	12 h	24 h	36 h	48 h
Mean	89.8	72.9	102.5	67.9
±SD	32.0	33.3	97.2	83.4
Range	53.0-169.3	17.9-137.9	0-313.0	0-217.5

Mean values of AUC were found to change significantly over time (P=0.001). Contrast analysis for the ANCOVA showed significant differences at 24, 36 and 48 h, although only with the initial values (P<0.01, or P<0.05). Mean AUC decreased to the half of the initial values already by 24 h postpartum and the lowest mean value was found during the last recording session at 48 h postpartum (Fig. 3c).

Mean ionized calcium concentrations ranged between 0.94 and 1.05 mmol/l. They gradually decreased until 36 h, and then an elevation occurred. Analysis of variance showed a highly significant time dependent change in this parameter (P=0.003; Fig. 4). Characteristics of uterine activity, based on each of the parameters reported above, did not show significant relationships to blood Ca^{2+}-levels in cows at any of the 4 recording times (Table 2).

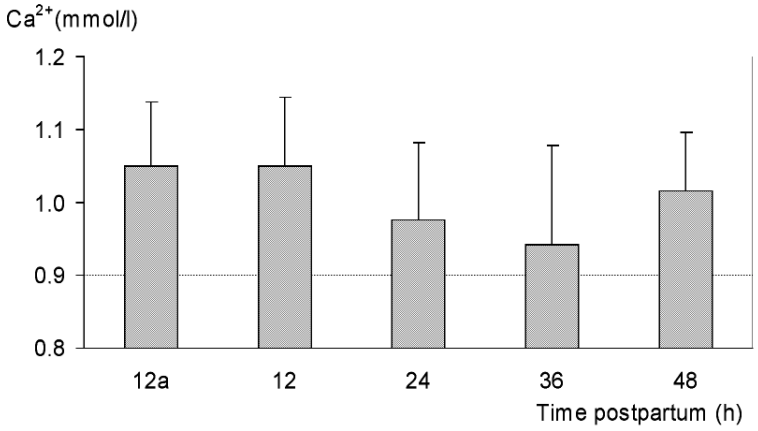

Fig. 4. Changes in mean (+SD) blood ionized calcium (Ca^{2+})-concentrations during the early postpartum period.

12a: Blood sample was taken before the beginning of the intrauterine pressure (IUP) recording.

Table 2. Relationships (R^2) between intrauterine pressure (IUP) parameters and blood Ca^{2+}-concentrations in untreated cows during the early puerperal phase.

	12 h	24 h	36 h	48 h
FREQ	0.239	0.009	0.042	0.023
AMP	0.102	0.231	0.046	0.002
DUR	0.214	0.034	0.088	0.017
AUC	0.004	0.069	0.134	0.031

FREQ: frequency of contractions (number per hour); AMP: amplitude of the contraction curves as relative pressure (mmHg); DUR: duration of the contraction curves (s); AUC: area under pressure curves (mmHg x s).

4. Discussion

A recently evaluated non-invasive IUP measuring and analyzing technique [7] was successfully used in dairy cows kept under farm conditions. Characteristics of IUP of 12 untreated healthy cows were quantified during the first 48 hours after uncomplicated calvings and showed a continuous decrease of FREQ and AMP during the first 48 hours postpartum. This decrease was most pertinent between 12 and 24 hours. Early postpartum cows showed remarkably high individual differences in their IUP parameters. Although FREQ, AMP, DUR and AUC as basic parameters for analyzing IUP curves were also used previously [2,13,15,24,25], these studies applied different recording and analysis methods as presented here. In the present study uterine activity was measured by a non-invasive method, using only one single open-end catheter, fixed inside the uterine lumen. No significant correlations were found between blood Ca^{2+}-concentration and any of the IUP parameters in this study.

Our results described uterine mechanical activity in untreated cows during the first two days after calving. We only performed recordings in cows that dropped their fetal membranes at latest by 12 hours following the delivery of a vital calf. The mean contraction FREQ of 8.9 per hour as found in this study between 12 and 14 hours after calving, is different from previous data [2], which showed a higher uterine contraction FREQ, with values ranging between 10 and 15 per hour at 10-15 hours after calving, and from the findings of Jordan [14], who reported on average 12 contractions per hour at 12 hours postpartum. In the present study, mean (±SD) AMP turned out to be 19.6 mmHg (±9.3 mmHg) 12-14 hours after parturition, which was in agreement with amplitudes given in the above reports. Partly similar results were published by Giama [18], who found the longest uterine contractions with the highest amplitudes during the first 8 hours of the puerperium. Others [15], only gave a general description of uterine contractility during the early puerperium, reporting that contraction frequencies ranged between 1 and 12 per hour and amplitudes up to 120 mmHg between Days 1 and 4 postpartum.

The slightly lower contraction FREQ we found in untreated farmed cows compared to the results, reported in the above mentioned previous studies is difficult to explain, but might be

attributed to differences in recording and/or analyzing techniques, or by the differences based on zootechnical or animal-related factors. However, age appeared not to be an important factor in affecting uterine activity [26].

We also found that contraction FREQ continuously decreased during the first 48 hours after parturition. A marked fall in mean contraction FREQ as expressed by a 46% decrease during the first 12-hour interval represented the most obvious changes in uterine contractility between 12 and 24 hours. Mean AMP showed similar pattern as mean contraction FREQ, with significant time-dependent changes from the highest initial mean values at 12 hours and the largest drop between 12 and 24 hours. It has been shown before that soon after expulsion of the fetal membranes uterine activity quickly decreases. The declining pattern then continued during the subsequent days in NRFM cows [15,27], similarly to what we observed in the present study. In addition, and also in agreement with our findings, Venable and McDonald [2] described the continuous decline in contraction FREQ and AMP with the progression of the puerperium; observing a fall in FREQ to less than one contraction per hour by 42 hours, and the complete disappearing of the regular uterine motility after 48 hours.

Several factors could explain why uterine contractility rapidly decreases during the early puerperium. One interpretation can be to postulate a reduced secretion of oxytocin and/or a reduced sensitivity for this hormone in this early postcalving period. Elevated plasma oxytocin levels have been measured during milking and parturition [28] and during the oestrous cycle [29], but Schams [28] reported very low levels of oxytocin during the first 4 days after calving. Besides, although detailed studies are available about the occurrence and role of uterine oxytocin receptors (OTR) throughout the gestation period [30-32], we only found one study where receptors were measured until the first day postpartum, showing a marked decline as compared to the values at parturition [31]. No data are available about the concentration of OTR in the later stages of the puerperium. So although the uterus might become less sensitive to oxytocin during the early puerperium, it is very unlikely that the marked decline of uterine contractility between 12 and 24 hours postpartum and its consequently low activity during the subsequent period until 48 hours observed in our study could be explained by a decrease of circulating oxytocin levels. Since milking was always completed at least one hour prior to the start of the IUP recording session, a possible effect of milking on uterine contractility could not be observed.

Prostaglandins are potent stimulators of uterine contractility. As peripheral Prostaglandin $F_{2\alpha}$-metabolite concentration used to be an indicator of uterine $PGF_{2\alpha}$ synthesis [33-35], and puerperal plasma Prostaglandin $F_{2\alpha}$-metabolite levels are generally elevated for several days after normal calving [36], one would expect uterine contractility to remain at a high level for several days as well. It is possible that the number of prostaglandin-receptors decreases drastically after expelling the calf, but we could not find any published data on this topic in puerperal cows. A decrease in prostaglandin receptors during this period could also be an argument for the rapid decline in uterine contractility due to time, as we demonstrated in this study.

Due to prepartum luteolysis, progesterone level rapidly declines at calving and remains low during the early postpartum days. So, although an inhibiting effect of progesterone on myometrial activity is well known, it is very unlikely that this hormone plays any role in decreasing contractility during the first days after calving.

Alternatively, nitric oxide (NO), an active inhibitor of the myometrium [37] that originates from an increased iNOS (the inducible isoform of nitric oxide synthase) expression, could be a good candidate for causing the decrease in uterine activity during the first days after calving. In a preliminary study [38], applying real-time PCR on repeatedly taken myometrial biopsies, an elevation of iNOS expression occurred only on Day 6 but not on Day 3 after calving. So at this stage it is difficult to explain the early myometrial inhibition, as found in the present study, by this rather late increase of iNOS expression.

Milk fever has been associated with impaired uterine activity in early postpartum cows [5,14,39]. Experimentally induced hypocalcemia with Na_2EDTA in parturient cows [4], parturient and postpartum sows [40] and ewes [41] led to a significant reduction or even a cessation of uterine contractility. If plasma Ca^{2+}-concentrations were experimentally lowered to 0.45 mmol/l in a cow, a rapid reduction of FREQ and AMP occurred in the gravid uterine horn and clinical signs of milk fever developed [4]. So the maintenance of normocalcemia appears crucial for a proper uterine mechanical activity. However, it has not been confirmed to what extent a minor degree of hypocalcemia may influence postpartum uterine contractility. It has been demonstrated before, that blood Ca^{2+}-concentrations in normal healthy cows decline immediately after calving and start to increase again from 24 to 36 hours onwards [42,43]. This was confirmed in the present study (Fig. 4). Six out of our twelve cows showed slight hypocalcemia throughout the entire course according to a classification as described by Kvart et al. [44]. Mean levels of blood Ca^{2+} in our study ranged within a slightly hypocalcemic range (0.80 to 1.05 mmol/l), while severe hypocalcemia (<0.50 mmol/l) could not be measured in any of the cows. This might explain why we did not find any significant correlations between blood Ca^{2+}-concentrations and the measured IUP parameters. We, therefore, suppose that a slight hypocalcemia that developed during the first 48 h after calving was not responsible for the reduction of uterine activity. We also postulate that individual animals probably have different set points as to the amount of available calcium-ions and calcium-ion channels needed for proper contractility. Until that low level is reached, uterine contractility is not influenced. Only if severe hypocalcemic episodes are present in the early puerperium, they might become important as causal factors in reducing uterine activity, thereby contributing to a delayed uterine involution [45].

During the recordings made at 36 and 48 h, we registered so-called contractures in 4 of the 12 cows, which finding was in agreement with Venable and McDonald [2], who also observed low AMP pressure increases of 3-4 minute DUR on the second day of puerperium. This different type of myometrial activity was first defined in pregnant sheep as a slow and steady rise of uterine pressure that lasted at least 5 minutes and reached at least 3.5 mmHg pressure increase above the basal tone [22]. Contractures were later also registered as episodes of EMG-activity in other pregnant domestic species, like cows [23], goats [46], or pigs [47], but similar regular activity periods also occurred in the non-pregnant uterus of sheep [48]. Since contractures showed a low frequency rate in pregnant cows (13.6±0.9 per day), and they also did not appear to be equally distributed over the day, contractures are preferably to be registered by long lasting continuous recordings [23]. The presence of these so-called contractures,

observed only in one third of our postpartum animals, as illustrated in Fig. 2b, also attributes to the great variability in IUP parameters, especially in DUR (Table 1) and AUC (Fig. 3c). The remarkable variation of myometrial activity between animals, independent of the type of the IUP measuring system, has also been described in pregnant and parturient sheep [49].

In summary, the digital IUP recording technique applied under farm conditions appeared to be an accurate tool for measuring temporal changes in uterine contractility during the first two days after calving. Although intensity and frequency of uterine contractions significantly decreased due to time, untreated early postpartum cows showed a high variability in characteristics of uterine contractility. Blood ionized calcium levels did not correlate with any of the IUP parameters during this early puerperal stage.

Acknowledgements

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5. References

1. Risco CA, Archbald LF. Dairy Herd Reproductive Efficiency. In: Howard JL, Smith RA (eds): *Current Veterinary Therapy 4, Food Animal Practice.* Philadelphia: WB Saunders, 1999;604-606.
2. Venable JH, McDonald LE. Postparturient bovine uterine motility - normal and after experimentally produced retention of the fetal membranes. *Am J Vet Res* 1958;19:308-313.
3. Taverne MAM, van der Weyden GC, Fontijne P. Preliminary observations on myometrial electrical activity before, during and after parturition in the cow. In: Hoffmann B, Mason IL, Schmidt J (eds): *Calving Problems and Early Viability of the Calf. Volume 4.* The Hague: Martinus Nijhoff, 1979;297-311.
4. Al-Eknah MM, Noakes DE. A preliminary study on the effect of induced hypocalcaemia and nifedipine on uterine activity in the parturient cow. *J Vet Pharmacol Ther* 1989;12:237-239.
5. Pelissier CL. Herd breeding problems and their consequences. *J Dairy Sci* 1972;55:385-391.
6. Muller LD, Owens MJ. Factors associated with the incidence of retained placentas. *J Dairy Sci* 1974;57:725-728.
7. Bajcsy ÁC, van der Weijden GC, Doornenbal A, Breeveld-Dwarkasing VNA, de Jong RC, Szenci O, Taverne MAM. Validation of pressure measurements and electromyography of the bovine uterus during the early postpartum period. *Am J Vet Res* 2004; accepted for publication.
8. Finn CA, Porter DG. Part 3: The Myometrium. In: Finn CA, Porter DG (eds): *Reproductive Biology Handbooks. Volume 1. The Uterus.* London: Elek Science, 1975;133-274.
9. Csapo A. The diagnostic significance of the intrauterine pressure. Part I. General considerations and techniques. *Obstet Gynecol Surv* 1970;25:403-435.
10. Fischer WM. 3. Grundlagen und klinische Wertigkeit der Kardiotokographie. Physiologie der Uterusmotilität. In: Fischer WM (ed): *Kardiotokographie*. 2nd ed, Stuttgart: Georg Thieme Verlag, 1976;73-94.
11. Braaksma JT, Janssens J, Eskes TKAB, Hein PR. Accurate pressure recording in the non-pregnant human uterus. A comparison of open and closed tip catheters. *Eur J Obstet Gynecol* 1971;6:195-206.
12. Zerobin K. Die Uterusbewegungen bei Kühen während der Geburt und der Nachgeburtsphase. *Schweiz Arch Tierheilkd* 1970;112:544-560.
13. Gillette DD, Holm L. Prepartum to postpartum uterine and abdominal contractions in cows. *Am J Physiol* 1963;204:1115-1121.
14. Jordan WJ. The puerperium of the cow: a study of uterine motility. *J Comp Pathol Ther* 1952;62:54-68.
15. Kündig H, Thun R, Zerobin K, Bachmann B. Die Uterusmotorik des Rindes während Spätgravidität, Geburt und Puerperium. I. Die Spontanmotorik. *Schweiz Arch Tierheilkd* 1990;132:77-84.
16. Eiler H, Byrd WH, Hopkins FM. Uterokinetic activity of fenprostalene (a prostaglandin $F_{2\alpha}$ analog) in vivo and in vitro in the bovine. *Theriogenology* 1989;32:755-765.
17. Eiler H, Hopkins FM, Armstrong-Backus CS, Lyke WA. Uterotonic effect of prostaglandin $F_{2\alpha}$ and oxytocin on the postpartum cow. *Am J Vet Res* 1984;45:1011-1014.
18. Giama I. Erfassung der postpartalen Uterusmotilität des Rindes und der motilitätssteigernden Wirkung eines Oxytozinpräparates. 1. Mitt.: Spontane Uterusmotilität im Frühpuerperium des Rindes nach normalen und gestörten Geburten. *Monatsh Veterinärmed* 1975;30:850-852.
19. Bajcsy ÁC, Bartyik J, Szenci O. Comparison of blood ionized calcium and acid-base variables in samples obtained from different sampling sites in dairy cows. *J Vet Med A* 1999;46:255-259.
20. Szenci O, Brydl E, Bajcsy ÁC. Effect of storage on measurement of ionized calcium and acid-base variables in equine, bovine, ovine and canine venous blood. *J Am Vet Med Assoc* 1991;199:1167-1169.
21. STATISTICA (data analysis software system), Version 6.1. StatSoft Inc. Tulsa, OK, USA, 1984-2004; www.statsoft.com

22. Jansen CAM, Krane EJ, Thomas AL, Beck NFG, Lowe KC, Joyce P, Parr M, Nathanielsz PW. Continuous variability of fetal pO2 in the chronically catheterized fetal sheep. *Am J Obstet Gynecol* 1979;134:776-783.
23. Taverne MAM, Breeveld-Dwarkasing VNA, van Dissel-Emiliani FMF, Bevers MM, de Jong R, van der Weijden GC. Between prepartum luteolysis and onset of expulsion. *Domest Anim Endocrinol* 2002;23:329-337.
24. Zerobin K, Spörri H. Motility of the bovine and porcine uterus and fallopian tube. *Adv Vet Sci Comp Med* 1972;16:303-354.
25. Giama I, Elze K, Eulenberger K. Untersuchungen zur postpartalen Uterusmotilität des Rindes. 2. Mitt.: Uterusmotilität im Frühpuerperium des Rindes nach Oxytozinapplikation. *Monatsh Veterinärmed* 1976;31:940-942.
26. Burton MJ, Dziuk HE, Fahning ML, Zemjanis R. Myometrial activity during natural and dexamethasone-induced parturition in the cow. *Am J Vet Res* 1987;48:37-44.
27. Gajewski Z, Thun R, Faundez R, Boryczko Z. Uterine motility in the cow during puerperium. *Reprod Domest Anim* 1999;34:185-191.
28. Schams D, Schmidt-Polex B, Kruse V. Oxytocin determination by radioimmunoassay in cattle. I. Method and preliminary physiological data. *Acta Endocrinol (Copenh)* 1979;92:258-270.
29. Schams D. Oxytocin determination by radioimmunoassay. III. Improvement to subpicogram sensitivity and application to blood levels in cyclic cattle. *Acta Endocrinol (Copenh)* 1983;103:180-183.
30. Fuchs A-R, Helmer H, Chang SM, Fields MJ. Concentration of oxytocin receptors in the placenta and fetal membranes of cows during pregnancy and labour. *J Reprod Fertil* 1992;96:775-783.
31. Fuchs A-R, Helmer H, Behrens O, Liu H-C, Antonian L, Chang SM, Fields MJ. Oxytocin and bovine parturition: a steep rise in endometrial oxytocin receptors precedes onset of labor. *Biol Reprod* 1992;47:937-944.
32. Ivell R, Fuchs A-R, Bathgate R, Tillmann G, Kimura T. Regulation of the oxytocin receptor in bovine reproductive tissues and the role of steroids. *Reprod Domest Anim* 2000;35:134-141.
33. Kindahl H, Granström E, Edqvist LE, Neely D, Hughes J, Stabenfeldt G. The advantages of measuring a prostaglandin $F_{2\alpha}$ metabolite in peripheral blood in studies of the physiological role of prostaglandin release during luteolysis in domestic animals. In: *Proceedings* VIIIth Int Congr Anim Reprod AI 1976. Krakow. Vol. III:145-148.
34. Kindahl H, Edqvist L-E, Bane A, Granström E. Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ during the normal oestrous cycle and early pregnancy in heifers. *Acta Endocrinol (Copenh)* 1976;82:134-149.
35. Kindahl H, Edqvist L-E, Granström E, Bane A. The release of prostaglandin $F_{2\alpha}$ as reflected by 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ in the peripheral circulation during normal luteolysis in heifers. *Prostaglandins* 1976;11:871-878.
36. Lindell J-O, Kindahl H, Jansson L, Edqvist L-E. Post-partum release of prostaglandin $F_{2\alpha}$ and uterine involution in the cow. *Theriogenology* 1982;17:237-245.
37. Yallampalli C, Garfield RE, Byam-Smith M. Nitrit oxide inhibits contractility during pregnancy but not during delivery. *Endocrinology* 1993;133:1899-1902.
38. De Jong R. Characterisation of uterine contractility of the postpartum cow and the role of nitric oxide in its regulation. MSc Thesis, Faculty of Veterinary Medicine, Utrecht University: Utrecht, The Netherlands, 2002.
39. Martin LR, Williams WF, Russek E, Gross TS. Postpartum uterine motility measurements in dairy cows retaining their fetal membranes. *Theriogenology* 1981;15:513-524.
40. Ayliffe TR, Noakes DE, Robalo Silva J. The effect of experimental induced hypocalcaemia on uterine activity in the sow during parturition and post-partum. *Theriogenology* 1984;21:803-822.
41. Robalo Silva J, Noakes DE. The effect of experimentally induced hypocalcaemia on uterine activity at parturition in the ewe. *Theriogenology* 1984;21:607-623.

42. Szenci O, Chew BP, Bajcsy ÁC, Szabó P, Brydl E. Total and ionized calcium in parturient dairy cows and their calves. *J. Dairy Sci* 1994;77:1100-1105.
43. Blum JW, Ramberg CFJ, Johnson KG, Kronfeld DS. Calcium (ionized and total), magnesium, phosphorus, and glucose in plasma from parturient cows. *Am J Vet Res* 1972;33:51-56.
44. Kvart C, Björsell KA, Larsson L. Parturient paresis in the cow. Serum ionized calcium concentrations before and after treatment with different calcium solutions – classification of different degrees of hypo- and hypercalcemia. *Acta Vet Scand* 1982;23:184-196.
45. Kamgarpour R, Daniel RCW, Fenwick DC, McGuigan K, Murphy G. Post partum subclinical hypocalcaemia and effects on ovarian function and uterine involution in a dairy herd. *Vet J* 1999;158:59-67.
46. Taverne MAM, Scheerboom JEM. Myometrial electrical activity during pregnancy and parturition in the pygmy goat. *Res Vet Sci* 1985;38:120-123.
47. Taverne MAM, Naaktgeboren C, Elsaesser F, Forsling ML, van der Weijden GC, Ellendorff F, Smidt D. Myometrial electrical activity and plasma concentrations of progesterone, estrogens and oxytocin during late pregnancy and parturition in the miniature pig. *Biol Reprod* 1979;21:1125-1134.
48. Garcia-Villar R, Toutain PL, Schams D, Ruckebusch Y. Are regular activity episodes of the genital tract controlled by pulsatile releases of oxytocin? *Biol Reprod* 1983;29:1183-1188.
49. Nathanielsz PW, Bailey A, Poore ER, Thorburn GD, Harding R. The relationship between myometrial activity and sleep state and breathing in fetal sheep throughout the last third of gestation. *Am J Obstet Gynecol* 1980;138:653-659.

CHAPTER 5

The effect of a single oxytocin or carbetocin treatment on uterine contractility in early postpartum dairy cows

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Abstract

The uterotonic characteristics and effectiveness of a single treatment with either oxytocin or carbetocin were quantified in early postpartum dairy cows after normal, uncomplicated calvings. Both the short-term (within 4 h), and the long-term effects (between 12 and 36 h) of the two treatments were compared.

Between 14 and 16 h after parturition, 27 multiparous Holstein-Friesian dairy cows, without fetal membrane retention, were selected and divided into three groups. The first group (n = 9) was administered 50 IU oxytocin intramuscularly, the second group (n = 10) received 0.35 mg carbetocin, while animals of the third group (n = 8), serving as a control, were administered 5 ml saline solution. A transcervically introduced open tip catheter system was used for the non-invasive acquisition of the intrauterine pressure (IUP) recording. After digitalization, the signals were analyzed, using a specially adapted graphical software program.

A significant short-term effect was found both in the oxytocin and carbetocin treated groups from the analysis of the contraction frequencies (FREQ) and of the total area under the curve (TAUC). After significant peaking during the 1st post-treatment hour, the values of the parameters for these two groups remained higher during the 2nd h, returning to the initial levels again during the 3rd h and reaching the level of the control group by the 12th h. Mean amplitude (AMP), duration (DUR) and area under the curve (AUC) of pressure cycles were not significantly affected by any of the treatments. Although mean FREQ and TAUC significantly declined from the initial values to 12, 24 and 36 h in all groups, mean AMP and AUC in the oxytocin and carbetocin treated groups, and mean DUR only in the carbetocin treated group to 12 and 36 h, the long-term analysis revealed no significant treatment differences for any IUP parameters.

Because treatment with either oxytocin, or carbetocin elicited similar uterotonic effects in healthy, early postpartum cows, it cannot be expected, that using carbetocin in preference to oxytocin, will result in a more beneficial clinical effect on uterine involution during this period.

Keywords: intrauterine pressure; oxytocin; carbetocin; puerperium; cow

1. Introduction

During the bovine puerperal stage, involution of the uterus has an important role in a cow to becoming pregnant again. During involution, beside the morphological changes of the endometrium, the size of the uterus also markedly decreases. The greatest size reduction in normal cows occurs during the first few days after parturition [1]. Regression processes accelerate between Days 10 and 14 [2], resulting in that by Day 25, uterine diameter almost reaches its final size [2,3]. This can be judged from the size measured after involution is completed. However, the time of complete involution can vary within wide ranges; 25 to 50 days after parturition in cows [1,3-6].

Uterine muscle activity is important in the process of involution. A contractile uterus is advantageous in removing excessive fluid and debris from the uterine lumen early postpartum. Our previous observations on cows without fetal membrane retention showed us, that while there was a high variability among individual animals, uterine contractions generally diminished rapidly, with a major decline between 12 and 24 h after calving, and with very little spontaneous contractility left at 48 h [7].

Treatment protocols, in which uterotonic drugs are applied during the puerperal phase, in order to increase uterine contractility, aim to accelerate the process of involution. The uterotonic drugs, used in bovine practice during the postpartum period, include natural prostaglandin $F_{2\alpha}$ or its synthetic analogues [8-12], parasympathomimetics (e.g. carbachol) [11], calcium borogluconate [9], ß-sympatholytics (e.g. carazolol) [9], or ergot alkaloids (e.g. ergometrin) [9-11]. The therapeutic use of prostaglandins, in order to evacuate the puerperal uterus, is questionable. Although some results suggested a beneficial effect from using endogenous uterine $PGF_{2\alpha}$ synthesis [13] or exogenous prostaglandin treatment [14] for shortening the puerperal period by promoting uterine contractility, several other studies failed to show an effect on myometrial activity [8,12,15,16]. No such contradictory results have been reported for oxytocin (OT) preparations, which are, therefore used in preference, although very few studies have quantified their uterotonic effects when given shortly (within 12 h) after calving [17,18]. OT preparations can be used as a single treatment or as repeated applications. They are mainly administered as intramuscular injections, but intravenous, subcutaneous, epidural, intravaginal treatment forms have also been reported [8-11,17,19-21]. Although OT induced an uterotonic activity increase during the early puerperal phase in cows [8,17,18,22,23], it can be expected that the increase will be short lasting as the half-life of OT has been reported to be short in cows (mean rapid $T_{1/2}$ = 3.87 ± 0.1 min; and mean slow $T_{1/2}$ = 25.53 ± 1 min) [24].

The biological effect of OT and its analogues depends on two factors: on how quickly it is removed from the circulation by excretion and through metabolism, and on whether there are enough specific receptors available, which are capable of binding the drug. Numerous attempts have been made to achieve a longer uterotonic effect by using oxytocin. A continuous, slow-rate intravenous drip infusion, as is often used in humans, is difficult to perform with cows under farm conditions. The increased plasma oestrogen levels in the cow around calving could enhance the effect of oxytocin in a very early postpartum uterus, possibly by stimulating the

synthesis of endometrial OT-receptors, but not affecting or even prohibiting that of myometrial OT-receptors, as it was shown in mid-pregnant, parturient and non-pregnant cows [25,26]. Because the effects of oestrogens, with respect to expression of oestrogen receptors in the uterine wall, are still unclear, and because of their human food safety implications, the possibility as to enhance uterotonic activity by external oestrogen treatment, should be rejected.

An alternative way to produce a prolonged uterotonic effect is to use OT-analogues with a longer biological activity, such as carbetocin (CB; [9-11]), which appeared to have a prolonged uterotonic activity in swine [27] and cows [20]. The short plasma half-life of the native OT molecule can be partly explained by its chemical structure. The circulating hormone is sensitive to the effects of aminopeptidase and disulfidase enzymes; the aminopeptidase can cleave the C-N bond at position 1-2 of the molecule, while the disulfidase affects the S-S bridge at position 1-6 [28]. It has been shown that desamination of the N-terminus and replacement of the sulphur bridge with a CH_2S group [28], protects the structure of the molecule and results in a prolonged oxytocin effect in rats [29-31]. The OT-analogue carbetocin (CB; 1-deamino-1-monocarba-[2-O-methyltyrosine]-OT) also produced uterotonic activity in rats [32], in sows during oestrus [27,33] and in parturient cows [34], although its uterotonic activity, measured in vivo or in vitro, was 10 [34], more than 25 [32] or even 30 [35] times weaker than that of OT. The prolonged uterotonic effect of CB is associated with a biphasic half-life [33]. Side effects, such as uterine tetany or tachyprophylaxis, did not occur in vivo or in vitro after CB treatment in swine [27], but did more often occur with the use of higher dose rates of OT in cows [34].

The effectiveness of any of these drugs depends on the existence and receptivity of membrane receptors, which are more or less specific for each drug. The presence of a disulphide bridge was shown not to be a prerequisite for the interaction of OT, with its specific receptors, to occur [30]. This has also been reported for CB, which showed similar membrane receptor affinity in vitro to that shown by OT [35]. Up until recently, there has only been limited information available on the changes in oxytocin receptor (OTR) concentrations during the postpartum phase in cows. Peak levels of myometrial and intercaruncular endometrial oxytocin receptor concentrations were reached shortly before the onset of labor, and these declined rapidly during active labor and the first day postpartum, which was the last day under study [26]. No further data are available on the changes in OTR concentrations during the following period of the bovine puerperium.

The aim of the present study was to evaluate to what extent a single treatment with either OT or CB, 14 to 16 h after normal parturition, influences uterine contractility on Days 1 and 2 postpartum in dairy cows. Therefore, this field study addressed the following questions:

1. What is the short-term intrauterine pressure response to a single treatment with OT or CB, when injected intramuscularly between 14 and 16 h postpartum?

2. Do the long-term (12-36 h post-treatment) characteristics of uterine contractility, induced by OT or CB, given between 14 and 16 h postpartum, differ from that recorded in untreated control animals?

3. Are there any differences between the OT and CB induced contractility characteristics in terms of their short-term and long-term effects?

2. Materials and methods

2.1. Cows and treatment

Twenty seven Holstein-Friesian dairy cows, kept at a large-scale dairy cattle farm in Hungary, were used in this study, during the autumn of 2001. All the cows had shed their fetal membranes within 12 h of normal calving. If necessary, light assistance only, the aid of one or two persons, was provided during calving. Cows with lacerations or ruptures of the soft birth canal were excluded from the study. For two to three weeks preceding the expected calving, the cows were housed in a calving barn with two compartments. The cows remained in the straw yard part of the calving barn until obvious signs of the onset of parturition appeared. They were then moved to the neighbouring compartment, which consisted of six equal calving boxes with places for 4 to 5 cows in each, where the deliveries took place. Once the expulsion of the fetus had been completed, nursing was allowed and aided if it was necessary. Within 12 h after calving, the cows were driven into another building, where they were bound and kept together for the next 36-38 h (that is 48-50 h after calving), until all the measurements were completed. Milking and feeding took place twice daily, but not in conjunction with the measurements.

The postpartum multiparous cows were randomly assigned into three groups and each cow was given a single intramuscular treatment at 14-16 h after parturition. Cows in the first group were treated with oxytocin (OT Group; n = 9; 5 ml Oxytocine® NCP injectable solution [Newco Pharma Inc, Clifton, NJ, /10 IU oxytocin synth./ml/; Lot: 02-2001/1837]); those in the second group with carbetocin (CB Group; n = 10; 5 ml Hypophysin [Veyx-Pharma GmbH, Schwarzenborn, Germany, /0.07 mg carbetocin/ml/; Batch Nr. 01A18]); and those in the third, with a saline solution (CON Group [control]; n = 8; 5 ml 0.9% NaCl solution).

2.2. Recording equipment and experimental protocol

Between 12 and 14 h after calving, a thin polyethylene, open tip catheter (Hewlett-Packard, Andover, MA) was inserted transcervically into the previously pregnant uterine horn and was fixed to the stalk of a caruncle, using a non-invasive internal fixation method as previously described [36]. The external end of the catheter was connected to a disposable pressure transducer (Ohmeda Inc, Murray Hill, NJ), which had been fixed with adhesive tapes to the shaved skin of the gluteal area. After connection, the catheter was filled and flushed with a standard amount of saline solution (10 ml 0.9% NaCl solution). The analogue signals were amplified using a preamplifier (GP 471 DC preamplifier unit, Schwarzer GmbH Medical Diagnostic Equipment, Munich, Germany) and digitalized using an analogue-digital (A-D) converter (PCMCIA DAQCard™-1200, National Instruments Corp, Austin, TX), which was inserted into the recording computer (Compaq Armada V300 notebook computer, Compaq Inc, The Netherlands). For the data acquisition and analysis of the IUP signals, specific tailor-made programs were developed using LabVIEW® 5.0 (National Instruments Corp, Austin, TX) [36]. Sampling frequency for the acquisition of the digital data was set to 4 Hz.

Following a 30-min adaptation period after insertion of the catheter, IUP was uninterruptedly recorded over the next 13 h. The cows were treated after the first hour of this recording session. After the completion of this 13-h session, two additional, 1 h long recording sessions were performed, one starting at 24 h and the other at 36 h after treatment.

In order to prevent the obstruction of the inside tip of the catheter, repeated flushing was performed with the same amount of saline as used immediately after insertion (10 ml): at the end of every third hour during the first, 12-h continuous post-treatment recording session and prior to the start of the additional 1-h recordings at 24 and 36 h after treatment.

2.3. Data analysis

The recordings were analyzed in two parts, focusing on the short-term and the long-term effects of the treatments separately. For the evaluation of the short-term effects of each drug, the IUP characteristics for the four consecutive hours, immediately post-treatment, were compared with those for the 1-h pre-treatment period. For the evaluation of the long-term effects, the IUP characteristics for the 12th post-treatment hour and those for the 1-h recordings during the 24th and 36th post-treatment hours were compared with those for the 1-h pre-treatment period (Fig. 1). From the 13-h uninterrupted IUP recordings of the first session, the 5-11 h post-treatment period was not used for the analysis.

The IUP curves were analyzed using the method, previously described [36], with minor modifications. The analysis can be summarized in three main steps. As the *first step*, for each cow, using the A-D converted filtered signals, the pressure cycle with the largest amplitude was selected from each of the pre-treatment and the first three consecutive post-treatment 1-h recordings (n = 4). These amplitudes were then averaged to give the mean maximum amplitude (MMA) for each cow. In the *second step*, 10% of the MMA was used as a low threshold cut-off value during the scanning of every IUP peak; all pressure cycles above this level were accepted on a preliminary basis. The starting and ending point of each individual pressure cycle was measured at this threshold level, with the computer program automatically calculating the amplitude, duration and area under the curve. Remarks, noted during the recording sessions about obvious artefacts and other events such as defecation or urination, which coincided with pressure changes, were used to eliminate pressure cycles even if they met the above-mentioned criteria. During the *third step*, a final selection was made from the remaining pressure cycles, based on the magnitude of the area under the curve. A cut-off magnitude was calculated, using a value of 10% of the mean of the five largest areas from the pre-treatment 1-h recording. Only those pressure cycles were included in the final statistical analysis, which exceeded this value; that was then used for analyzing the pre- and all the post-treatment recordings for the same cow.

A calibration of the recording instrument was performed before starting the measurements with each cow; amplitude (AMP) was expressed as relative pressure in mmHg, duration (DUR) in seconds and the area under the curve (AUC) in mmHg x s. Contraction frequency (FREQ) was defined as a number per hour. A further descriptive parameter of uterine mechanical

activity, the total area under the curve (TAUC; mmHg x s) was also defined, representing the sum of all accepted areas under pressure curves within a one-hour recording period. Each of the above measured and calculated parameters (see Table 1), were used for the statistical analysis of the short- and the long-term effects, in accordance with the protocol outlined in Fig. 1.

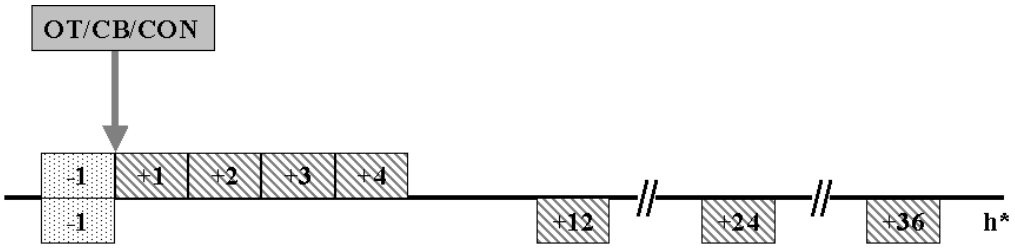


Fig. 1. Evaluation protocol for postpartum cows treated with either 50 IU oxytocin (OT), 0.35 mg carbetocin (CB), or 5 ml saline (CON) intramuscularly.

The upper part illustrates the protocol for the short-term evaluation, the lower part, the one for the long-term evaluation of the effect of treatment. Dotted boxes represent the 1-h IUP recordings immediately before treatment at 14-16 h after calving (-1 h), while oblique striped boxes represent IUP recordings indicating the passed hours related to the moment of treatment as scale units of the horizontal axis.

*h: treatment related time in 1-h periods

For the statistical analysis, first all data were tested for normality using the Kolmogorov-Smirnov test. A repeated measures analysis of variance (RM-ANOVA) procedure was then performed on each IUP parameter (FREQ, AMP, AUC, DUR, TAUC) for each treatment group, to check the time-dependent changes. In these analyses, the IUP parameters were used as the dependent variable and time as the repeated measures factor. Where the RM-ANOVA resulted in a significant time effect for any parameter, a Dunnett-test was applied to compare the mean values from each post-treatment recording session to the pre-treatment control period. To determine if there were any differences in the effects produced by the two treatments (OT or CB), a one-way ANOVA was performed for each time point. If this analysis indicated a significant treatment effect at a certain time point, a Tukey-test was applied to compare the mean values from the two treatment groups and the control group for that time [37].

3. Results

Figures 2a and 2b present typical examples of IUP recordings around the single intramuscular treatment at 14-16 h postpartum, with either 50 IU OT or 0.35 mg CB, showing their effect on IUP changes.

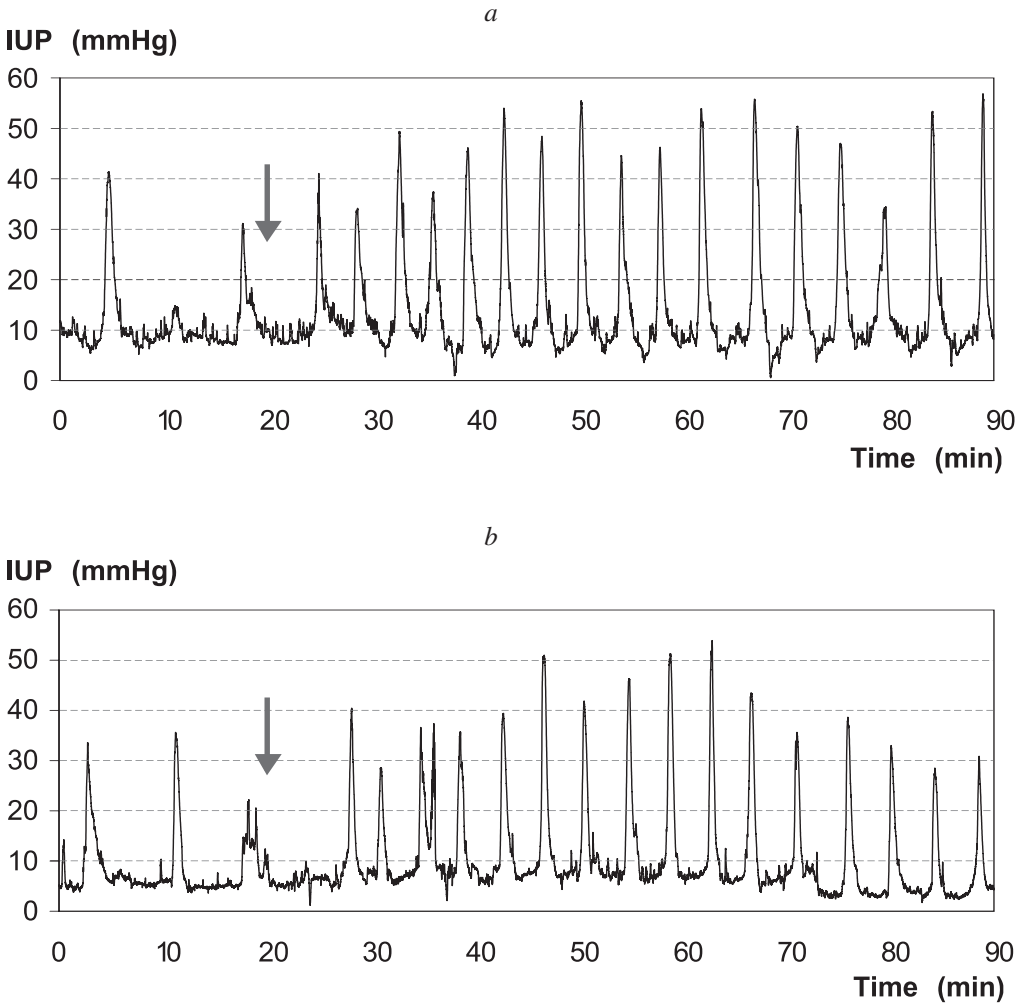


Fig. 2. A 90-min recording showing the effect of a single im. injection of 50 IU of oxytocin (arrow; *a*) or of a single im. injection of 0.35 mg of carbetocin (arrow; *b*) on uterine contractility.

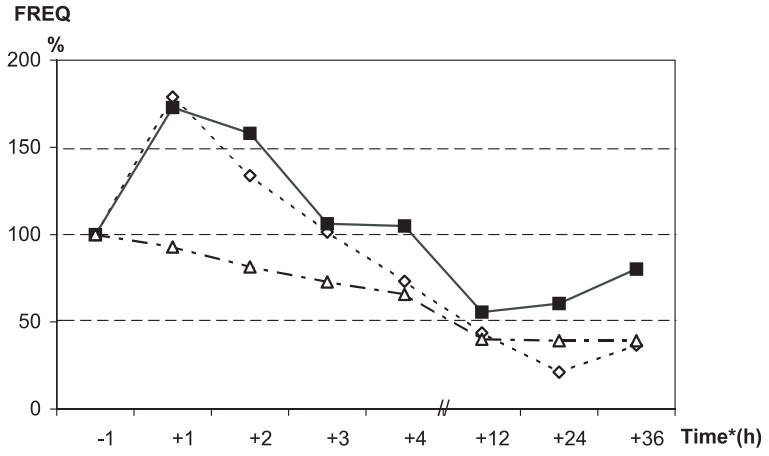
Table 1 summarizes the mean values (±SEM) of the IUP parameters for each 1-h IUP recording, in each experimental group - for the statistical analysis of both the short- and the long-term effects.

Table 1. Mean (± SEM) values of contraction frequency (FREQ), amplitude (AMP), duration (DUR), mean and total area under the curve (AUC, TAUC) in postpartum cows treated with oxytocin (50 IU), carbetocin (0.35 mg) or saline (5 ml) intramuscularly

			Long-term evaluation							
			Short-term evaluation							
		Time*(h)	-1	+1	+2	+3	+4	+12	+24	+36
FREQ (n/h)										
	Oxytocin	**Mean**	**7.9**	**14.1**	**10.6**	**8.0**	**5.8**	**3.4**	**1.7**	**2.9**
		SEM	*0.6*	*0.6*	*0.6*	*0.9*	*0.9*	*1.2*	*0.6*	*0.8*
	Carbetocin	**Mean**	**8.1**	**14.0**	**12.8**	**8.6**	**8.5**	**4.5**	**4.9**	**6.5**
		SEM	*0.7*	*0.7*	*0.8*	*0.7*	*0.9*	*1.5*	*1.3*	*1.6*
	Saline	**Mean**	**8.8**	**8.1**	**7.1**	**6.4**	**5.8**	**3.5**	**3.4**	**3.4**
		SEM	*1.2*	*1.5*	*0.8*	*1.1*	*0.8*	*1.3*	*1.0*	*1.0*
AMP (mmHg)										
	Oxytocin	**Mean**	**21.7**	**26.6**	**23.9**	**23.2**	**19.1**	**8.7**	**10.2**	**11.6**
		SEM	*2.3*	*3.5*	*3.3*	*2.7*	*1.5*	*2.4*	*2.7*	*1.7*
	Carbetocin	**Mean**	**23.1**	**24.2**	**21.5**	**20.2**	**18.7**	**10.2**	**12.4**	**12.0**
		SEM	*4.0*	*3.4*	*3.7*	*4.1*	*2.9*	*3.2*	*2.2*	*2.3*
	Saline	**Mean**	**19.5**	**18.0**	**18.0**	**15.8**	**16.5**	**10.0**	**11.1**	**8.8**
		SEM	*4.6*	*2.3*	*2.5*	*3.7*	*2.1*	*3.6*	*2.7*	*2.1*
DUR (s)										
	Oxytocin	**Mean**	**66.5**	**66.7**	**65.1**	**60.9**	**59.8**	**33.1**	**41.7**	**50.6**
		SEM	*4.9*	*3.5*	*3.3*	*4.0*	*5.0*	*9.1*	*19.4*	*8.6*
	Carbetocin	**Mean**	**63.4**	**73.7**	**65.4**	**60.9**	**60.8**	**27.0**	**54.4**	**41.5**
		SEM	*5.2*	*5.0*	*4.2*	*2.7*	*4.6*	*8.1*	*7.4*	*7.7*
	Saline	**Mean**	**68.8**	**69.4**	**66.9**	**61.2**	**59.6**	**44.7**	**37.3**	**83.2**
		SEM	*14.5*	*9.1*	*4.6*	*11.0*	*5.4*	*12.4*	*9.1*	*38.8*
AUC (mmHg x s)										
	Oxytocin	**Mean**	**620.3**	**746.2**	**627.1**	**618.5**	**442.1**	**168.8**	**226.9**	**202.8**
		SEM	*81.6*	*123.6*	*94.6*	*90.1*	*53.2*	*51.9*	*106.0*	*34.0*
	Carbetocin	**Mean**	**659.5**	**749.9**	**573.3**	**519.8**	**462.0**	**187.2**	**242.7**	**230.2**
		SEM	*131.1*	*97.3*	*109.9*	*110.4*	*53.6*	*69.1*	*52.3*	*55.6*
	Saline	**Mean**	**562.0**	**516.0**	**494.7**	**467.4**	**403.4**	**239.2**	**187.6**	**264.8**
		SEM	*152.6*	*54.5*	*60.9*	*128.6*	*55.3*	*85.8*	*42.8*	*97.7*
TAUC (mmHg x s)										
	Oxytocin	**Mean**	**4829.3**	**10440.5**	**6481.2**	**4885.1**	**2562.2**	**697.2**	**547.0**	**715.7**
		SEM	*765.1*	*1726.0*	*1010.8*	*985.0*	*560.6*	*195.3*	*229.3*	*255.8*
	Carbetocin	**Mean**	**5052.9**	**10553.8**	**7467.6**	**4834.2**	**4190.1**	**1149.7**	**1161.9**	**1617.6**
		SEM	*1035.4*	*1489.4*	*1514.9*	*1212.6*	*686.6*	*499.4*	*346.0*	*428.1*
	Saline	**Mean**	**4454.9**	**4152.8**	**3558.3**	**3636.2**	**2358.1**	**1325.8**	**691.9**	**744.4**
		SEM	*1043.8*	*826.9*	*593.1*	*1304.9*	*561.9*	*630.1*	*198.1*	*213.5*

Time*(h): 1-h periods labeled relative to the moment of treatment.

a



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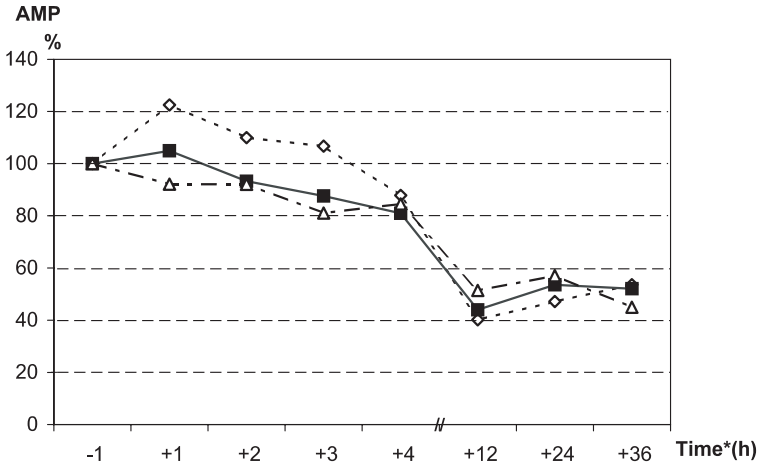


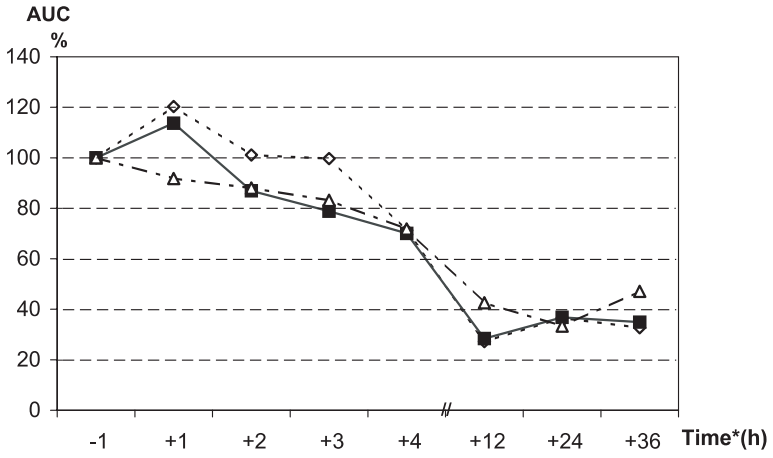
Fig. 3 a,b. Effect of a treatment either with oxytocin (50 IU, im.), carbetocin (0.35 mg, im.), or saline (5 ml, im.), as illustrated by the relative changes in mean contraction frequency (FREQ; *a*) and amplitude (AMP; *b*) of pressure cycles, using the pretreatment values (at -1 h) as reference.

◇: Oxytocin; ■: Carbetocin; ▵: Control

Time*(h): 1-h periods as related to the moment of treatment either with oxytocin, carbetocin or saline. Treatment was given between -1 and +1, at time 0.

Figure 3a-d illustrate the changes in the FREQ, AMP, AUC and TAUC values for the two treated groups and the control group, over time. For reason of clarity, the changes are expressed as overall mean percentages of the initial values as obtained for each cow during the 1-h recording session before treatment.

c



d

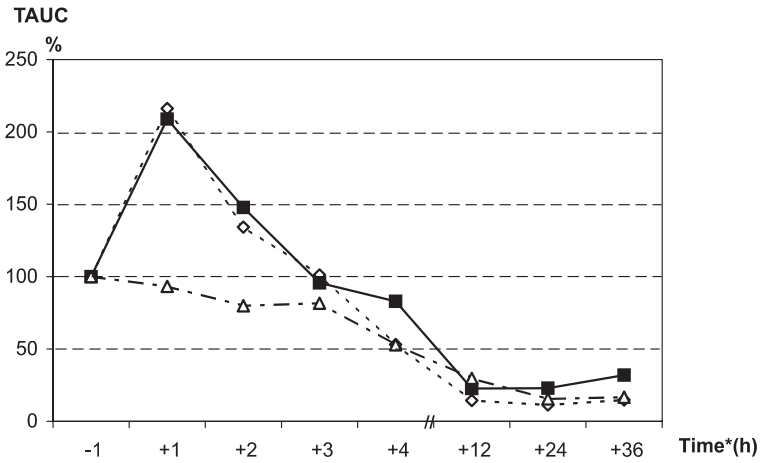


Fig. 3 c,d. Effect of a treatment either with oxytocin (50 IU, im.), carbetocin (0.35 mg, im.), or saline (5 ml, im.), as illustrated by the relative changes in mean area under the curve (AUC; *c*) and total area under the curve (TAUC; *d*) of pressure cycles, using the pretreatment values (at -1 h) as reference.

◇: Oxytocin; ■: Carbetocin; ▵: Control

Time*(h): 1-h periods as related to the moment of treatment either with oxytocin, carbetocin or saline. Treatment was given between -1 and +1, at time 0.

3.1. Results of the short-term analysis

In the first hour after treatment, the mean FREQ increased significantly both in the OT and in the CB group, compared to their own initial value and to the 1st post-treatment hour value of the control group ($P<0.001$). Although this increase was followed by a decline both in the OT and the CB group from the second hour onwards, the values were still significantly higher during that second hour than they were initially (OT: $P<0.05$, CB: $P<0.001$), and they were also still significantly higher than those of the controls (OT: $P<0.01$, CB: $P<0.001$). During the third hour, the mean FREQ for both treated groups decreased nearly to their initial levels. The values may have shown a trend but were not higher than those for the control group, and they declined further during the fourth hour. While the mean FREQ of the CB group remained higher than that of the OT group, this difference between the two was found not to be significant ($P = 0.078$) when applying the Tukey-test.

Although the mean AMP for both treated groups increased in the first hour post-treatment and then decreased from then on, these changes were statistically not significant. The values for the OT group remained above the initial value for the first three post-treatment hours, those for the CB group, for only the first hour. These group differences were also not found to be significant at any time points.

The mean DUR for all three groups showed a similar pattern, decreasing slightly but not significantly over time. Neither a significant time effect, nor a treatment effect could be found with this parameter.

Changes in the mean AUC results were very similar to those from the mean AMP values, and except for a significant overall time effect in the CB group (overall $P<0.05$, but no differences with the Dunnett-test), no further statistically significant effects could be attributed to time and treatment.

Similarly to the changes in the mean FREQ values, those for the mean TAUC showed a significant effect due to both treatments during the first post-treatment hour when comparing their mean values with those of the controls ($P<0.05$), and also when their markedly higher values were compared with the initial ones of the pre-treatment hour ($P<0.001$). Although the mean TAUC values of the treated groups during the 2nd hour were still higher than the initial ones and also than those of the control group, the differences could not be attributed to the treatments with any statistical significance. Again, there was no statistically significant difference between the effects from the two treatments at any time points in this parameter.

3.2. Results of the long-term analysis

Comparison of the mean FREQ values for the periods at +12, +24 and +36 h, with the initial values at -1 h, showed a drop to about 50% of the initial values in both treated groups and returned near to the level of the controls by the 12th h after treatment. The absolute values underlying these decreases (Table 1) at 12, 24 and 36 h after treatment were found to be

statistically significant (OT: $P<0.001$, CB: $P<0.05$ [but *NS* at 36 h], CON: $P<0.01$ [but $P<0.05$ at 12 h]). Although the CB group showed higher mean values during this period than the other two groups, the difference was not statistically significant at $P<0.05$.

From 12 h after treatment onwards, the mean AMP values ranged between 40 and 60% of the pre-treatment levels for all three groups. A significant time-dependent decline in the absolute values for this parameter was only found within the OT and CB groups (OT: $P<0.01$ [but $P<0.05$ at 36 h], CB: $P<0.01$ [but $P<0.001$ at 12 h]), while there was no significant difference between the three groups at any time points in the long-term evaluation.

Except for a significantly lower mean DUR in the CB group during the 12th and 36th post-treatment hour ($P<0.001$ and $P<0.05$), no further significant time or treatment effects were found.

Although AUC was significantly lower only in the OT and CB groups from the 12 h after treatment onwards when compared to the initial means ($P<0.001$ [but OT: $P<0.01$ at 24 h]), a significant treatment difference between the groups at any time points, could not be proved.

Mean values of TAUC, during the 12th, 24th and 36th post-treatment hour, were significantly lower than the mean values prior to treatment ($P<0.001$ after OT and CB treatment, and $P<0.01$ for CON [but $P<0.05$ at 12 h]), however, a significant treatment difference could not be found.

4. Discussion

The uterotonic effects caused by the administration of a single intramuscular treatment of either oxytocin (50 IU), or its long-acting analogue, carbetocin (0.35 mg), were compared in this field study on early postpartum dairy cows, after normal parturition. It turned out that both drugs significantly increased the FREQ and TAUC values of the pressure cycles, mainly during the first post-treatment hour. The uterotonic effects of the two drugs were almost equal, and a prolonged uterotonic effect, resulting from the CB treatment, could not be proved.

Eulenberger et al. [11] reported, that while the effect of OT, administered either intravenously or intramuscularly, ceased within 2 h, CB caused a prolonged effect, lasting 2 to 8 h if these treatments had been used during the first 5-6 days after parturition. We, therefore, evaluated the myometrial effects of OT and CB on both a short- and a long-term basis, comparing results from recordings made in the first four post-treatment hours with the initial (pre-treatment) values, as well as results from the 12, 24 and 36-h post-treatment recordings with the initial values.

Inspection of the IUP recordings (Fig. 2a and 2b) showed a fast response to both OT and CB, which suggests, that at the time of treatment (between 14 and 16 h after calving), the uterus was still sensitive to these drugs. The uterotonic effect of both drugs started within approximately ten minutes after treatment, and lasted over the next two hours. During the third hour, uterine activity returned to its initial levels for each treatment group, and by the twelfth hour, had dropped to the level of the control group, at around 50% of the own initial levels. The statistical analyses of the results from the short- and the long-term evaluations showed that there was no significant difference in uterine activity, caused by a single intramuscular treatment either with OT or CB. The analyses also showed that there

was no evidence of a prolonged uterotonic effect from using the CB treatment, even though the decline of the FREQ (Fig. 3a) and TAUC (Fig. 3d) values after such a treatment was slower than that after the OT treatment. In this latter respect, our findings disagree with previous reports [11,20]. The nearly identical immediate effects of the two tested drugs are possibly due to a similarity in the membrane receptors' affinity towards either OT or CB. Although we did not find any data to support this hypothesis for cows, it would not be unexpected as CB is an oxytocin-analogue, and it has been shown to bind to the uterine receptors in experiments with isolated rat myometrial strips [35].

The biological effect of uterotonic drugs depends on several factors. The molecular structure of a drug, its dosage and the method of administration determine, amongst others (see below), the intensity, the rapidity of onset, and the duration of a specific, uteromotoric response. However, for the manifestation of such a response, the availability of sufficient specific, drug-sensitive receptors in the myometrium and the endometrium, as well as a sufficient number of gap junctions between the myometrial cells, are crucial.

Until recently, very little data had been published, describing the changes in OTR concentrations of the uterine myometrial and endometrial tissue in cows, during the early postpartum period [26,38], and they indicate a rapid decline in OTR concentrations during the first day after parturition. Assuming that this decline continues during the subsequent days, it could be expected that the uterotonic effects, caused by the treatments, would weaken and eventually disappear, even though the time-span, during which this would occur, has not been exactly defined. It is also important that a sufficient number of gap junctions remain between the myometrial cells during the puerperal phase. It is known in several other species that at the termination of pregnancy, oestrogen facilitates the establishment of these intercellular structures. Gap junctions enable ion transport between neighbouring smooth muscle cells, so coordinated muscle contractions and propagation waves can develop [39]. The reduced uterine contractility may explain our previous findings with untreated postpartum cows after uncomplicated calvings [7], where a steady decline of the measured IUP parameters, together with a high individual variability, was described.

The main effect of both the OT and CB treatment appeared to increase the frequency of myometrial contractions and this was also mirrored by an increase of the TAUC values, for the calculation of which, FREQ is one of the two components. AMP, DUR, and AUC values of the pressure cycles were less affected by the two drugs. So these results demonstrate that the applied external uterotonic treatments do not affect the type of the pressure cycles but they do positively influence the occurrence rate of these cycles. These findings suggest that in healthy cows at this early stage of the puerperium, when uterine contractility is still present, – even if it can be characterized with a high level of inter-animal variability, – it can be enhanced. This also implies that all required components, such as specific receptors, gap junctions and ion channels, are still present in sufficient (unoccupied) numbers at 14-16 h postpartum to allow the induction of additional myometrial contractility. It is probably because of a self-defense mechanism of the organ against extreme intra-luminal pressure and a possibly associated mechanical damage and pain perception that AMP does not increase in an uncontrolled manner. Far fewer of those complications would result from an increased frequency of contractions. Yet, a frequency

increase will finally lead to a markedly higher total amount of work being performed by the uterus, as it can be well characterized by our new descriptive parameter, the TAUC value.

There is evidence available to show that OT acts directly on the myometrium and also indirectly, through the local endometrial release of prostaglandin $F_{2\alpha}$ [40]. However, there is no information available on the early postpartum changes in uterine prostaglandin receptor concentrations. It is known from in vitro studies on ovine endometrium [41] and also from in vivo studies with cyclic cows at the end of the luteal phase [42] that increased OT release, together with an increase in OTR concentrations, cause the release of prostaglandin $F_{2\alpha}$ from the endometrium. The occurrence of similar processes can also be expected at the termination of pregnancy, however, uterine prostaglandin release seems to depend on circulating blood oxytocin concentration. While prostaglandin release could be measured with higher doses of oxytocin (10 and 100 IU) in cows at D 250 of pregnancy [43], such release did not occur with lower, physiological doses (0.8, 1.6 or 3.2 IU) either in late pregnant cows, or after preterm induced luteolysis [44].

The duration of the effects from a drug depends on the structure of its molecule, which basically also determines its biological half-life. It was, therefore, quite surprising that in our study the effects of the two different drugs lasted equally long, because a more prolonged half-life of CB had been anticipated. Failure to achieve a prolonged uterotonic effect with CB was therefore, probably due to other factors, such as the administered dose. This could be true even though we used the recommended (high) dose for this product. The clearance rate of CB in early postpartum cows also still needs to be determined.

Since in this study, a prolonged uterotonic effect could not be demonstrated through a single administration of any of the two drugs, more frequent administrations of OT or CB could be considered to achieve this. However, the determination of an optimal period, over which the uterus could work at an elevated level without exhausting the smooth muscle cellular functions, the effectiveness of a single or repeated use of these drugs in different dosages, or the use of possible other routes of administration (e.g. intrauterine, [11]), all remain to be studied, before any recommendation can be given for their application in the field. On the basis of our documented rapid decrease in uterine contractility during the first two days after normal calving (control group of this study; [7]), it appears that the time window for such treatments is relatively short, i.e. it should take place within 48 hours after calving. More importantly, further investigations should be carried out to clarify the necessity and possible clinical benefits of uterotonic treatments after uncomplicated calvings, where such treatments are targeted towards an improved uterine involution and shortening of the service period. In this respect, it would be interesting to investigate, whether blocking of uterine contractility by relaxants, such as clenbuterol [45,46], would, on the other hand, slow down the process of uterine involution.

The data of the present study indicates, that using the described dosage and method of administering of the drugs, their effectiveness in enhancing uterine contractility was almost identical, if treatments occurred on the first day postpartum. Furthermore, because by the fourth hour after treatment, the levels of contractility in the two treated groups have effectively dropped to that of the control group, no further effect on the contractility of the uterus should be expected from the drugs beyond this time.

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5. References

1. Gier HT, Marion GB. Uterus of the cow after parturition: involutional changes. *Am J Vet Res* 1968;29:83-96.
2. Morrow DA, Roberts SJ, McEntee K, Gray HC. Postpartum ovarian activity and uterine involution in dairy cattle. *J Am Vet Med Assoc* 1966;149:1596-1609.
3. Rasbech NO. Den normale involutio uteri hos koen (The normal involutio uteri in the cow). *Nord Vet Med* 1950;2:655-687.
4. Buch NC, Tyler WJ, Casida LE. Postpartum estrus and involution of the uterus in an experimental herd of Holstein-Friesian cows. *J Dairy Sci* 1955;38:73-79.
5. Wagner WC, Hansel W. Reproductive physiology of the post partum cow. I. Clinical and histological findings. *J Reprod Fertil* 1969;18:493-500.
6. Morrow DA, Roberts SJ, McEntee K. A review of postpartum ovarian activity and involution of the uterus and cervix in cattle. *Cornell Vet* 1969;59:134-154.
7. Bajcsy ÁC, Szenci O, Doornenbal A, van der Weijden GC, Csorba C, Kocsis L, Szűcs I, Ostgard S, Taverne MAM. Characteristics of bovine early puerperal uterine contractility recorded under farm conditions. *Theriogenology* 2004. in press.
8. Eiler H, Hopkins FM, Armstrong-Backus CS, Lyke WA. Uterotonic effect of prostaglandin $F_{2\alpha}$ and oxytocin on the postpartum cow. *Am J Vet Res* 1984;45:1011-1014.
9. Sobiraj A, Hermülheim A, Herfen K, Schulz S. Einfluss verschiedener Uterotonika auf den Nachgeburtsabgang bei Rindern nach konservativen und operativen geburtshilflichen Eingriffen. *Tierärztl Umsch* 1998;53:392-399.
10. Kündig H, Thun R, Zerobin K. Die Uterusmotorik des Rindes während Spätgravidität, Geburt und Puerperium. II. Medikamentelle Beeinflussung. *Schweiz Arch Tierheilkd* 1990;132:515-524.
11. Eulenberger K, Wilhelm J, Schulz J, Gutjahr S, Wohanka K, Däberitz H. Uterotonika im Puerperium des Rindes. *Monatsh Veterinärmed* 1986;41:371-377.
12. Ko JCH, McKenna DJ, Whitmore HL, Chen CY, Gustafsson BK, Smith RP. Effects of estradiol cypionate and natural and synthetic prostaglandins on myometrial activity in early postpartum cows. *Theriogenology* 1989;32:537-543.
13. Lindell J-O, Kindahl H, Jansson L, Edqvist L-E. Post-partum release of prostaglandin $F_{2\alpha}$ and uterine involution in the cow. *Theriogenology* 1982;17:237-245.
14. Lindell J-O, Kindahl H. Exogenous prostaglandin $F_{2\alpha}$ promotes uterine involution in the cow. *Acta Vet Scand* 1983;24:269-274.
15. Thompson FN, Page RD, Cook CB, Caudle AB. Prostaglandin $F_{2\alpha}$ metabolite levels in normal and uterine-infected postpartum cows. *Vet Res Commun* 1987;11:503-507.
16. Bosu WTK, Liptrap RM, Leslie KE. Peripartal changes in plasma progesterone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ concentrations in Holstein cows with or without retained foetal membranes. *Anim Reprod Sci* 1984;7:497-510.
17. Giama I, Elze K, Eulenberger K. Untersuchungen zur postpartalen Uterusmotilität des Rindes 2. Mitt.: Uterusmotilität im Frühpuerperium des Rindes nach Oxytozinapplikation. *Monatsh Veterinärmed* 1976;31:940-942.
18. Burton MJ, Dziuk HE, Fahning ML, Zemjanis R. Effects of oestradiol cypionate on spontaneous and oxytocin-stimulated postpartum myometrial activity in the cow. *Br Vet J* 1990;146:309-315.
19. Starke A, Fricke H-P, Elze K. Ein Behandlungsverfahren zur Stimulation der Uterusinvolution im Frühpuerperium des Rindes mittels Cloprostenol und Carbetocin. *Tierärztl Umsch* 1998;53:730-739.
20. Bernhard A, Schulz J, Gutjahr S, Eulenberger K. Indikationen für die Anwendung eines Depotoxytozin-Präparates in der tierärztlichen Praxis. *Tierärztl Umsch* 1993;48:446-453.
21. Eulenberger K, Stubbe J, Böhme W, Liebaug E. Zur metaphylaktischen und therapeutischen Anwendung eines Depotoxytocin-Präparates im Puerperium des Rindes. *Monatsh Veterinärmed* 1987;42:738-742.

22. Armstrong-Backus CS, Hopkins FM, Eiler H. The uterotonic effect of prostaglandin $F_{2\alpha}$ and oxytocin on the postpartum cow. In: *Abstracts* 64th Ann Meet Conf Res Workers Anim Dis 1983. Chicago. (93.abstract) 17.
23. Zerobin K. Die Uterusbewegungen bei Kühen während der Geburt und der Nachgeburtphase. *Schweiz Arch Tierheilkd* 1970;112:544-560.
24. Wachs EA, Gorewit RC, Currie WB. Half-life, clearance and production rate for oxytocin in cattle during lactation and mammary involution. *Domest Anim Endocrinol* 1984;1:121-140.
25. Fuchs A-R, Behrens O, Helmer H, Liu C-H, Barros C-M, Fields MJ. Oxytocin and vasopressin receptors in bovine endometrium and myometrium during the estrous cycle and early pregnancy. *Endocrinology* 1990;127:629-636.
26. Fuchs A-R, Helmer H, Behrens O, Liu H-C, Antonian L, Chang SM, Fields MJ. Oxytocin and bovine parturition: a steep rise in endometrial oxytocin receptors precedes onset of labor. *Biol Reprod* 1992;47:937-944.
27. Cort N, Einarsson S, Viring S. Actions of oxytocin and a long-acting carba oxytocin analog on the porcine myometrium in vitro and in vivo. *Am J Vet Res* 1979;40:430-432.
28. Cort JH, Cash JD, Jost K, Schwartz IL, Mulder JL. The cyclic neurohypophysial peptides: From structure to clinical use. In: MacIntyre I, Szelke M (eds): *Molecular Endocrinology*. Amsterdam: Elsevier/North-Holland Biomedical Press; 1977.337-349.
29. Barth T, Krejcí I, Vanécková J, Jošt K, Rychlík I. Prolonged action of deamino-carba analogues of oxytocin on the rat uterus in vivo. *Eur J Pharmacol* 1974;25:67-70.
30. Barth T, Krejcí I, Kupková B, Jošt K. Pharmacology of cyclic analogues of deamino-oxytocin not containing a disulphide bond (carba analogues). *Eur J Pharmacol* 1973;24:183-188.
31. Jošt K, Šorm F. The effect of the presence of sulphur atoms on the biological activity of oxytocin; Synthesis of deamino-carba[6]-oxytocin and deamino-dicarba-oxytocin. *Collect Czech Chem Commun* 1971;36:234-245.
32. Barth T, Jošt K, Rychlík I. Milk-ejecting and uterotonic activities of oxytocin analogues in rats. *Endocrinol Exp* 1975;9:35-42.
33. Cort N, Einarsson S, Schams D, Vilhardt H. Blood concentrations of oxytocin equivalents after single injections of deamino-1-monocarba-[2-O-methyltyrosine]-oxytocin in lactating sows. *Am J Vet Res* 1981;42:1804-1806.
34. Vě ník Z, Holub A, Zralý Z, Kummer V, Holčák V, Jošt K, Cort JH. Regulation of bovine labor with a long-acting carba-analog of oxytocin: A preliminary report. *Am J Vet Res* 1979;40:425-429.
35. Atke A, Vilhardt H. Uterotonic activity and myometrial receptor affinity of 1-deamino-1-carba-2-tyrosine(O-methyl)-oxytocin. *Acta Endocrinol (Copenh)* 1987;115:155-160.
36. Bajcsy ÁC, van der Weijden GC, Doornenbal A, Breeveld-Dwarkasing VNA, de Jong RC, Szenci O, Taverne MAM. Validation of pressure measurements and electromyography of the bovine uterus during the early postpartum period. *Am J Vet Res* 2004; accepted for publication.
37. STATISTICA (data analysis software system), Version 6.1. StatSoft Inc. Tulsa, OK, USA, 1984-2004; www.statsoft.com
38. Fuchs A-R, Helmer H, Chang SM, Fields MJ. Concentration of oxytocin receptors in the placenta and fetal membranes of cows during pregnancy and labour. *J Reprod Fertil* 1992;96:775-783.
39. Garfield RE, Rabideau S, Challis JRG, Daniell EE. Ultrastructural basis for maintenance and termination of pregnancy. *Am J Obstet Gynecol* 1979;133:308-315.
40. Guay P, Lamothe P. Metritis following parturition. Serum progesterone and 17β-oestradiol levels. The significance of the corpus luteum and the advisability of using a luteolytic agent as a treatment. *Can Vet J* 1980;31:18-20.
41. Roberts JS, McCracken JA, Gavagan JE, Soloff MS. Oxytocin-stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometrium in vitro: correlation with estrous cycle and oxytocin-receptor binding. *Endocrinology* 1976;99:1107-1114.
42. Schams D. Luteal peptides and intercellular communication. *J Reprod Fertil Suppl* 1987;34:87-99.

43. Fuchs A-R, Rollyson MK, Meyer M, Fields MJ, Minix JM, Randel RD. Oxytocin induces prostaglandin $F_{2\alpha}$ release in pregnant cows: influence of gestational age and oxytocin receptor concentrations. *Biol Reprod* 1996;54:647-653.
44. Taverne MAM, de Schwartz NCM, Kankofer M, Bevers MM, van Oord HA, Schams D, Gutjahr S, van der Weijden GC. Uterine responses to exogenous oxytocin before and after pre-partum luteolysis in the cow. *Reprod Domest Anim* 2001;36:267-272.
45. Zerobin K, Kündig H. The control of myometrial functions during parturition with a β_2-mimetic compound, Planipart®. *Theriogenology* 1980;1:21-35.
46. Arbeiter K, Thurnher M. Über die Wirkung des Sympathikomimetikums Planipart® (NAB 365) auf den Geburtsablauf beim Rind. *Tierärztl Umsch* 1977;8:423-427.

CHAPTER 6

The effect of oxytocin on the peripheral plasma prostaglandin $F_{2\alpha}$-metabolite levels in early postpartum dairy cows

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To be submitted

Abstract

A study was performed in postpartum cows to demonstrate the oxytocin-induced changes in peripheral plasma 15-ketodihydroprostaglandin $F_{2\alpha}$ (prostaglandin $F_{2\alpha}$–metabolite) concentrations after a single oxytocin treatment with a therapeutical dose. Cows without placental retention were treated between 13 and 15 h after normal parturition with 50 IU oxytocin intramuscularly, (OT-IM group; n = 11), or intravenously (OT-IV group; n = 2), or intramuscular saline solution was applied as control (CON group; n = 13). Based on the plasma prostaglandin $F_{2\alpha}$–metabolite results, obtained through frequent blood samplings (in 10-min intervals starting 1 h before and ending 2 h after treatment), no significant differences could be observed between cows, treated intramuscularly either with oxytocin or saline. Although the positive effect of oxytocin on prostaglandin (PG) release in the two cows after an intravascular treatment was slightly different, they provided evidence for such a stimulation. These findings also suggest that more factors could be responsible for an effective induction of the PG release during this period. Therefore, an indirect effect through a local stimulation of the PG release after an OT treatment might additionally contribute to its direct uterotonic effect in early postpartum cows.

Keywords: oxytocin; plasma prostaglandin $F_{2\alpha}$–metabolite; puerperal cow

1. Introduction

Oxytocin is one of the drugs, most frequently used to stimulate uterine contractility in early postpartum cows. We recently demonstrated, that intramuscular injection with either oxytocin or carbetocin (a long-acting synthetic oxytocin analogue), if administered between 14 and 16 hours after uncomplicated calvings, only temporarily stimulated myometrial contractions [1]. This raises the question whether oxytocin only acts directly through stimulation of the myometrial oxytocin receptors (OTR), or does it also function via an indirect pathway, in which OTRs in the endometrium respond indirectly to OT with a prostaglandin (PG) release, which then additionally could induce a contractile response in the myometrium through a paracrine route [2].

There have been few in vitro and in vivo studies reporting on the uterine response in cows in terms of PG release, to treatments with various doses of OT. Oxytocin (in doses of 10, 100 or 1000 μU) stimulated the $PGF_{2\alpha}$ secretion in a dose-dependent manner in an in vitro incubated endometrial tissue taken from heifers in estrous, however a similar effect could not be demonstrated in tissue from Days 19-20 of the oestrous cycle [3]. Low, intravascularly applied physiological doses of OT (0.8, 1.6 or 3.2 IU) were found not to result in an increased $PGF_{2\alpha}$ release in uterine venous blood in late pregnant cows [4]. Others reported a dose- and stage-dependent release of $PGF_{2\alpha}$ during gestation, induced by higher, pharmacological intra-vascular doses of OT (10 or 100 IU) [5]. Also cows, at later stages of the postpartum period (Days 10, 20 or 30), were found to respond with markedly elevated plasma prostaglandin $F_{2\alpha}$-metabolite (15-ketodihydroprostaglandin $F_{2\alpha}$) concentrations to the intravascular treatment with 30, 150 and 300 IU OT, but the magnitudes of the PG releases were found to decrease with the advancing postpartum period [6]. However, so far, the PG response to OT treatment on the first day after normal calving has not been tested.

In order to accurately characterize uterine $PGF_{2\alpha}$ synthesis and release, the changes in plasma $PGF_{2\alpha}$ concentration can be directly measured in utero-ovarian venous blood [7]. Because in cattle, prostaglandin $F_{2\alpha}$-metabolite is a reliable estimator of the changes in central $PGF_{2\alpha}$ concentration [8-11], this invasive type of blood collection can be substituted by the determination of the peripheral plasma concentration of 15-ketodihydroprostaglandin $F_{2\alpha}$ (prostaglandin $F_{2\alpha}$-metabolite). However, due to the relatively short half-life of prostaglandin $F_{2\alpha}$-metabolite – the mean value varying between 7 and 18 min in cattle [9,12,13] –, frequent sampling is required to correctly determine its concentrations in the peripheral plasma.

The normally observed postpartum increase in peripheral plasma prostaglandin $F_{2\alpha}$-metabolite concentrations did not occur in hysterectomized cows where the operation was performed on the day of parturition. The low prostaglandin $F_{2\alpha}$-metabolite levels during the postoperative days in such cows clearly demonstrated, that the uterus is the main source of F series prostaglandins ($PGF_{2\alpha}$ and/or prostaglandin $F_{2\alpha}$-metabolite) during the postpartum period [12,14], with the most active $PGF_{2\alpha}$ synthesis and metabolism taking place in the caruncular tissue [12]. Fetal cotyledons were shown to contain a potent inhibitor of prostaglandin synthesis during bovine pregnancy [15]. Therefore, it can be supposed that by the birth of the calf and

shedding of the fetal placenta after parturition, this inhibitory effect ceases, which then contributes to the increase in prostaglandin $F_{2\alpha}$ synthesis in the maternal caruncular tissue during the immediate postpartum phase [8].

The higher initial prostaglandin $F_{2\alpha}$-metabolite concentrations postpartum [16] point to a more intensive prostaglandin synthesis and metabolism in cows with normal involution, than in cows with uterine infections [17], or retained fetal membranes [18-20] during the early stage of the puerperium. Consequently, a faster completion of the involutional processes takes place, leading to a more rapid decline in uterine prostaglandin synthesis, assuming the uterotonic effect of the prostaglandins during this period [21].

Early reports from the seventies, on isolated pregnant rat uteri, showed a close association between uterine PG release and uterine muscle activity [22,23]. Since then, the stimulating effect of PGs on smooth muscle cells is well accepted, although it depends on the applied dose, the route of application or the stage of the reproductive cycle. Because the uterotonic effect of PGs is very similar to that induced by OT, it is often called an oxytocic effect. However, with cows, the data about the effects of PGs on myometrial activity are rather conflicting. While a low dose of $PGF_{2\alpha}$ (5 ng/ml of bath fluid), applied in vitro, did not result in a linear correlation with uterine activity at different stages of the oestrous cycle or ovarian dysfunctions, such as cystic ovaries [24], the in vivo results with therapeutical doses (25 mg $PGF_{2\alpha}$, im.) showed a certain inconsistency. Although PG treatment did not increase intrauterine pressure changes at oestrus, it did result in a sustained contracture of 6-8 min duration after treatment on Day 7 [25]. It has also been observed that differences exist, in terms of the elicited myometrial effects, depending on the manner of the application routes of $PGF_{2\alpha}$. Prostaglandin $F_{2\alpha}$ was reported to increase uterine motility at all stages of the oestrous cycle [26], and also during dioestrus if applied intravenously [27] or intramuscularly [28], but it had no effect on uterine activity in early postpartum cows [29]. The lack of further stimulation of uterine activity in cows during oestrus after the application of $PGF_{2\alpha}$ [25], might be due to the high endogenous prostaglandin production during oestrus [30], which possibly already maintains maximal uterine activity during this stage. This relative insensitivity of the myometrium to external prostaglandins could be due to the full occupancy of prostaglandin receptors in the myometrium.

The aim of the present study was to examine the oxytocin-induced changes in peripheral plasma prostaglandin $F_{2\alpha}$-metabolite concentrations in postpartum cows, after a single oxytocin treatment (50 IU), administered intramuscularly or intravenously between 13 and 15 h after calving.

2. Materials and methods

2.1. Cows and treatment

Twenty six postpartum cows with 0, 1 or 2 previous lactations were randomly assigned into three groups according to their treatment between 13 and 15 h after parturition – after they had shed the fetal membranes. Cows in the first group were intramuscularly treated with 50 IU oxytocin (OT-IM group; n = 11; 5 ml Oxytocine® NCP injectable solution [Newco Pharma Inc, Clifton, NJ, /10 IU oxytocin synth./ml/; Exp.: 03-2006, Lot: 4566]); those in the second group, intravenously with 50 IU oxytocin (OT-IV group; n = 2; from the same drug as was used for the OT-IM group); while those in the third group, intramuscularly with a saline solution (CON group; n = 13; 5 ml 0.9% NaCl solution). Blood samples were taken at 10-min intervals from the jugular vein, starting 60 min before and ending 120 min after the administration of the treatment. Blood was collected through a previously inserted plastic venous catheter (Vygonüle S type iv. cannula with PTFE Catheter; 75 mm long, size G12, internal diameter 2.6 mm [VYGON GmbH & Co KG, Aachen, Germany]) into sodium heparinated tubes (7 ml Vacutainer tubes; 119 IU Na-heparin/tube [Belliver Industrial Estate, Plymouth. PL6 7BP, UK]). The catheter was flushed after each use with heparin containing saline solution, to prevent obstruction. The first 3-5 ml of blood from each sample were discarded. Immediately after withdrawal, the blood samples were placed on crushed ice. Centrifugation took place within 1 hour of collection, at +4°C for 10 min at 4000 rpm. The supernatant was harvested and stored below –20°C until the analyses could be performed.

The use of cows in this experiment was approved by the Committee for Animal Experiments of the Pest County Veterinary and Food Control Station (Nr. 99/003/03).

2.2. Hormone analysis

15-Ketodihydroprostaglandin $F_{2\alpha}$ concentrations were measured by a direct homologous, double antibody radioimmunoassay, according to Granström and Kindahl [31]. The sensitivity of the method was 60 pmol/l, the inter-assay coefficient of variation was 14%, and the intra-assay coefficient of variation ranged between 6.6% and 11.7% for the different ranges of the standard curve.

2.3. Statistical analysis

The treatment and time dependent changes in the prostaglandin $F_{2\alpha}$-metabolite data were analyzed by using a two-way repeated measures analysis of variance procedure, where group was taken as an independent variable and time as a repeated measures factor (19 levels). The level of significance was set to $P<0.05$, and 95% confidence intervals (CI) were calculated [32]. Data of the two cows of the OT-IV group (Cow A and B) are presented individually.

3. Results

Figure 1 presents the mean plasma prostaglandin $F_{2\alpha}$-metabolite levels and 95% confidence intervals for the samples taken before and after treatment in the OT-IM group and CON group. Neither time nor treatment appeared to have a significant effect on prostaglandin $F_{2\alpha}$-metabolite levels. Therefore, the data for all the animals from these two groups were pooled, and the mean and SEM values for each sampling time were calculated and plotted in Fig. 2, together with the individual plasma prostaglandin $F_{2\alpha}$-metabolite levels of the two cows of the OT-IV group. It appeared that after an intravenous bolus injection of 50 IU oxytocin, one of the cows of this group (Cow A) responded with higher absolute plasma prostaglandin $F_{2\alpha}$-metabolite levels, but also the other cow (Cow B) showed elevated levels at +20, +30 and +60 min after treatment.

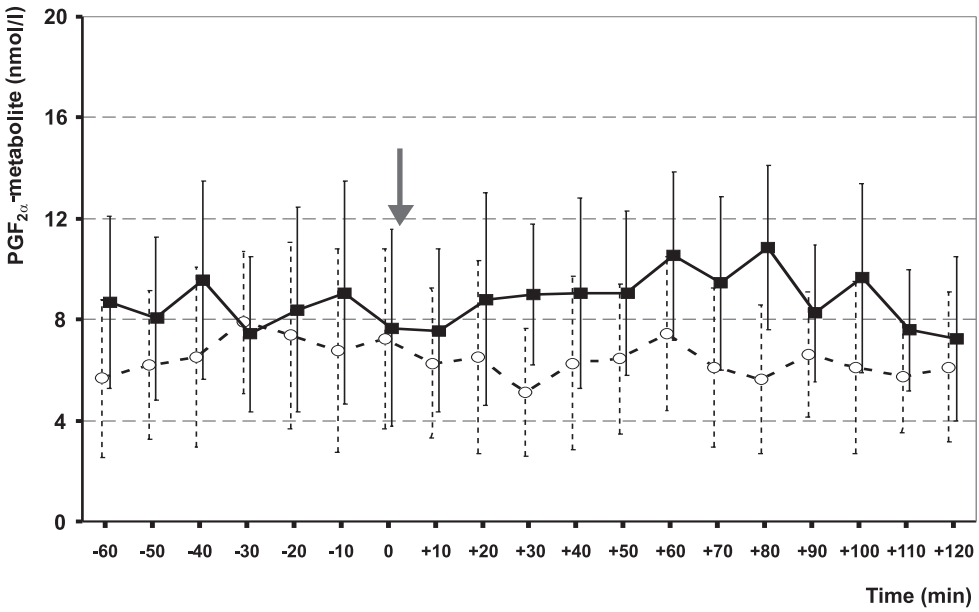

Fig. 1. Mean peripheral plasma levels of 15-ketodihydroprostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$-metabolite) in cows without retained fetal membranes, before and after treatment (arrow) with 50 IU oxytocin im. (n = 11, ■, solid line), or 5 ml saline im. (n = 13, ○, dotted line), between 13 and 15 h after normal calving.

Vertical bars denote 95% CI.

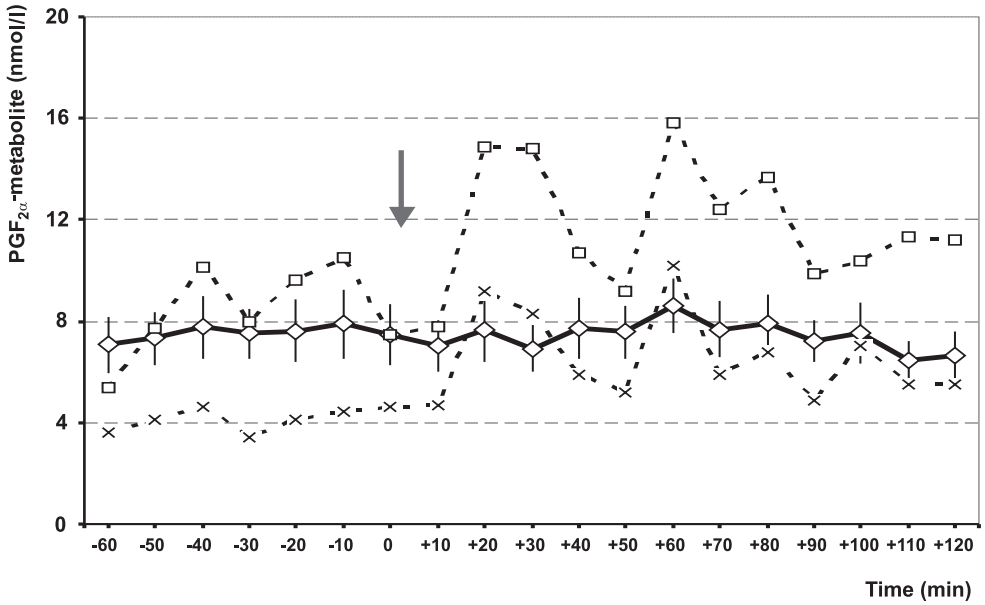

Fig. 2. Mean (±SEM) values of 15-ketodihydroprostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$-metabolite) concentrations in peripheral plasma relative to the time of administering the treatment between 13 and 15 h after normal calving.

Treatment (arrow) immediately followed the blood sampling at time zero. Values were calculated from the pooled data (solid line: mean, vertical bars: ±SEM) for cows treated either with 50 IU oxytocin, or 5 ml saline solution im. The dotted lines represent the individual values from the two cows (□: Cow A; x: Cow B), which had been treated intravenously with a bolus injection of 50 IU oxytocin.

4. Discussion

We found in this study that a single intramuscular injection of 50 IU oxytocin, administered between 13 and 15 h after parturition, did not significantly alter prostaglandin $F_{2\alpha}$-metabolite concentrations in the peripheral plasma in early postpartum cows without fetal membrane retention. However, in Cows A and B, which had the OT administered intravenously, plasma prostaglandin $F_{2\alpha}$-metabolite levels nearly doubled their pretreatment values at 20 min after the intravascular treatment.

The explanation as to why a challenge with a frequently used dose of OT (50 IU, im.) on the first postpartum day did not increase prostaglandin $F_{2\alpha}$-metabolite concentrations of the peripheral plasma, could be a rather complex one. Both the applied dose, the method of

administering it, the high basal level of PGs during this period, the site of blood sampling and/or the availability of free OT receptors, are all factors, which might have contributed to the failure of OT to affect on uterine prostaglandin release.

Although the intramuscularly applied dose of OT in this study did not seem to elevate the prostaglandin $F_{2\alpha}$-metabolite concentrations in peripheral plasma significantly, the mean values did rise slightly in the OT-IM group and continued to fluctuate about the pretreatment mean values of the CON group between 20 and 80 min after treatment (Fig. 1). The absence of a significant difference could partly be explained by the high variability of the prostaglandin $F_{2\alpha}$-metabolite concentrations among the individual cows, as shown by the large 95% CI for both groups. This is in agreement with a previous report [33]. A further explanation as to why an intramuscular treatment with OT did not result in significant changes in the prostaglandin release might be due to the already high level of PG synthesis during the early postpartum period. High plasma prostaglandin levels during this stage in cattle have been reported in earlier studies [16,21,34]. The additional stimulation by a therapeutical OT dose on PG release is most probably negligible when compared to the high basal (physiological) PG concentrations. However, the use of intravascular OT treatment did produce a discernable effect on PG release in Cow A, which was also present, although less explicitly, in Cow B. While the increase in the absolute values of plasma prostaglandin $F_{2\alpha}$-metabolite for Cow A, 20 min after intravenous treatment, was much greater than that for Cow B, both showed a nearly 100% relative increase over their pretreatment values (Fig. 2). This suggests that intravenously administered OT can stimulate the prostaglandin release, probably because it reaches its receptors in a higher concentration, while the intramuscularly applied OT enters from the injection site into the blood stream in a prolonged way, according to the actual absorption speed. It is also possible that a proportion of the intramuscular oxytocin degrades at the injection site. Because of the prolonged (and the probably decreased) absorption of the intramuscular injection, only a smaller proportion of the applied dose will therefore reach the target OT receptors. Additionally, the intramuscularly administered OT undergoes a major dilution in the vascular system after absorption, which contributes to a major reduction of OT molecules reaching the free OT receptors.

As already indicated above, both pharmacological intravascular doses of OT (10 or 100 IU) during gestation [5] or similar subcutaneous doses of OT (0.33 IU/kg BW) during the various stages of the oestrous cycle [35], resulted in a dose- and stage-dependent prostaglandin release in cows. The stimulating results on PG release in these cases can be due to a lower basal prostaglandin level, which can also lead to easier measurements of lower elevations in the peripheral prostaglandin $F_{2\alpha}$-metabolite concentrations as compared to the early postpartum phase.

The purpose of this study was to find an additional explanation for the stimulatory (uterotonic) effect of intramuscularly applied OT on the early postpartum uterus, as we recently reported from a field experiment where cows were intramuscularly treated with 50 IU oxytocin [1]. Our present findings provide some evidence for the existence of an indirect pathway through stimulation of PG release that might support the direct uterotonic effect of OT. However, before we can further substantiate this interpretation, we still need additional information on the possible changes in the number of OT and PG receptors in bovine uterine tissues during the early postpartum stage.

Prostaglandins that are produced locally within the uterine wall, could diffuse to the myometrium by a paracrine route [2], which does not necessarily result in the appearance of PGs in peripheral blood. To demonstrate a more pronounced prostaglandin response in peripheral blood, an even higher intravenous OT dose, such as used by others during bovine pregnancy [5], might also be required to be used during the early postpartum stage.

In conclusion, although an intramuscularly applied dose of 50 IU oxytocin, by locally stimulating PG release in the uterine wall, might contribute indirectly to the uterotonic effect in cows on their first day postpartum, the amount of the released PGs appearing in the blood does not seem to be sufficient for its accurate determination in peripheral plasma.

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5. References

1. Bajcsy ÁC, Szenci O, van der Weijden GC, Doornenbal A, Maassen F, Bartyik J, Taverne MAM. The effect of a single oxytocin or carbetocin treatment on uterine contractility in early postpartum dairy cows. *Theriogenology* 2004; accepted for publication with a need for minor modifications.
2. Lye SJ, Challis JRG. Paracrine and endocrine control of myometrial activity. In: Gluckman PD, Johnston BM, Nathanielsz PW (eds): *Research in Perinatal Medicine (VIII). Advances in fetal physiology: Reviews in Honor of G.C. Liggins.* Ithaca, New York: Perinatology Press, 1989;361-375.
3. Lafrance M, Goff AK. Control of bovine uterine prostaglandin $F_{2\alpha}$ release in vitro. *Biol Reprod* 1990;42:288-293.
4. Taverne MAM, de Schwartz NCM, Kankofer M, Bevers MM, van Oord HA, Schams D, Gutjahr S, van der Weijden GC. Uterine responses to exogenous oxytocin before and after pre-partum luteolysis in the cow. *Reprod Domest Anim* 2001;36:267-272.
5. Fuchs A-R, Rollyson MK, Meyer M, Fields MJ, Minix JM, Randel RD. Oxytocin induces prostaglandin $F_{2\alpha}$ release in pregnant cows: influence of gestational age and oxytocin receptor concentrations. *Biol Reprod* 1996;54:647-653.
6. Del Vecchio RP, Chase Jr CC, Bastidas P, Randel RD. Oxytocin-induced changes in plasma 13,14 dihydro-15-keto prostaglandin $F_{2\alpha}$ concentrations on days 10, 20 and 30 postpartum in the bovine. *J Anim Sci* 1990;68:4261-4266.
7. Fairclough RJ, Hunter JT, Welch RAS. Peripheral plasma progesterone and utero-ovarian prostaglandin F concentrations in the cow around parturition. *Prostaglandins* 1975;9:901-914.
8. Guilbault LA, Thatcher WW, Foster DB, Caton D. Relationship of 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ concentrations in peripheral plasma with local uterine production of F series prostaglandins and changes in uterine blood flow during the early postpartum period of cattle. *Biol Reprod* 1984;31:870-878.
9. Kindahl H, Edqvist L-E, Bane A, Granström E. Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ during the normal oestrous cycle and early pregnancy in heifers. *Acta Endocrinol (Copenh)* 1976;82:134-149.
10. Kindahl H, Granström E, Edqvist LE, Neely D, Hughes J, Stabenfeldt G. The advantages of measuring a prostaglandin $F_{2\alpha}$ metabolite in peripheral blood in studies of the physiological role of prostaglandin release during luteolysis in domestic animals. In: *Proceedings* VIIIth Int Congr Anim Reprod AI 1976. Krakow. Vol. III:145-148.
11. Kindahl H, Edqvist L-E, Granström E, Bane A. The release of prostaglandin $F_{2\alpha}$ as reflected by 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ in the peripheral circulation during normal luteolysis in heifers. *Prostaglandins* 1976;11:871-878.
12. Guilbault LA, Thatcher WW, Drost M, Hopkins SM. Source of F series prostaglandins during the early postpartum period in cattle. *Biol Reprod* 1984;31:879-887.
13. Williams WF, Lewis GS, Thatcher WW, Underwood CS. Plasma 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) in pregnant and nonpregnant heifers prior to and during surgery and following intrauterine injection of $PGF_{2\alpha}$. *Prostaglandins* 1983;25:891-899.
14. Lindell J-O, Kindahl H, Edqvist L-E, Tufvesson G. Effect of hysterectomy on the postpartum prostaglandin levels in the cow. *Acta Vet Scand* 1982;23:144-146.
15. Shemesh M, Ailenberg M, Lavi S, Mileguir F. Regulation of prostaglandin biosynthesis by an endogenous inhibitor from bovine placenta. In: Schwartz NB, Hunzicker-Dunn M (eds): *Dynamics of Ovarian Function.* New York: Raven Press, 1981;161-166.
16. Edqvist L-E, Kindahl H, Stabenfeldt G. Release of prostaglandin $F_{2\alpha}$ during the bovine peripartal period. *Prostaglandins* 1978;16:111-119.
17. Thompson FN, Page RD, Cook CB, Caudle AB. Prostaglandin $F_{2\alpha}$ metabolite levels in normal and uterine-infected postpartum cows. *Vet Res Commun* 1987;11:503-507.

18. Madej A, Kindahl H, Larsson L, Edqvist L-E. Sequential hormonal changes in the postpartum dairy cow. *Acta Vet Scand* 1986;27:280-295.
19. Bosu WTK, Liptrap RM, Leslie KE. Peripartal changes in plasma progesterone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ concentrations in Holstein cows with or without retained foetal membranes. *Anim Reprod Sci* 1984;7:497-510.
20. Nakao T, Gamal A, Osawa T, Nakada K, Moriyoshi M, Kawata K. Postpartum plasma PGF metabolite profile in cows with dystocia and/or retained placenta, and effect of fenprostalene on uterine involution and reproductive performance. *J Vet Med Sci* 1997;59:791-794.
21. Lindell J-O, Kindahl H, Jansson L, Edqvist L-E. Post-partum release of prostaglandin $F_{2\alpha}$ and uterine involution in the cow. *Theriogenology* 1982;17:237-245.
22. Vane JR, Williams KI. The contribution of prostaglandin production to contractions of the isolated uterus of the rat. *Br J Pharmacol* 1973;48:629-639.
23. Chan WY. Relationship between the uterotonic action of oxytocin and prostaglandins: oxytocin action and release of PG-activity in isolated nonpregnant and pregnant rat uteri. *Biol Reprod* 1977;17:541-548.
24. Patil RK, Sinha SN, Einarsson S, Settergren I. The effect of prostaglandin $F_{2\alpha}$ and oxytocin on bovine myometrium in vitro. *Nord Vet Med* 1980;32:474-479.
25. Cooper MD, Foote RH. Effect of oxytocin, prostaglandin $F_{2\alpha}$ and reproductive tract manipulations on uterine contractility in Holstein cows on days 0 and 7 of the estrous cycle. *J Anim Sci* 1986;63:151-161.
26. Rodriguez-Martinez H, Ko J, McKenna D, Weston PG, Whitmore HL, Gustafsson BK, Wagner WC. Uterine motility in the cow during the estrous cycle. II. Comparative effects of prostaglandins $F_{2\alpha}$, E_2, and cloprostenol. *Theriogenology* 1987;27:349-358.
27. Stolla R, Schmid G. Auswirkungen natürlicher und synthetischer $PGF_{2\alpha}$-Präparate auf die Uteruskontraktilität des Rindes. *Berl Münch Tierärztl Wochenschr* 1990;103:198-202.
28. Hirsbrunner G, Küpfer U, Burkhardt H, Steiner A. Effect of different prostaglandins on intrauterine pressure and uterine motility during diestrus in experimental cows. *Theriogenology* 1998;50:445-455.
29. Ko JCH, McKenna DJ, Whitmore HL, Chen CY, Gustafsson BK, Smith RP. Effects of estradiol cypionate and natural and synthetic prostaglandins on myometrial activity in early postpartum cows. *Theriogenology* 1989;32:537-543.
30. Shemesh M, Hansel W. Levels of prostaglandin F (PGF) in bovine endometrium, uterine venous, ovarian arterial and jugular plasma during the estrous cycle. *Proc Soc Exp Biol Med* 1975;148:123-126.
31. Granström E, Kindahl H. Radioimmunoassay of the major plasma metabolite of $PGF_{2\alpha}$, 15-keto-13,-14-dihydro-$PGF_{2\alpha}$. *Methods Enzymol* 1982;86:320-339.
32. STATISTICA (data analysis software system), Version 6.1. StatSoft Inc. Tulsa, OK, USA, 1984-2004; www.statsoft.com
33. Madej A, Kindahl H, Woyno W, Edqvist L-E, Stupnicki R. Blood levels of 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ during the postpartum period in primiparous cows. *Theriogenology* 1984;21:279-287.
34. Eley DS, Thatcher WW, Head HH, Collier RJ, Wilcox CJ. Periparturient endocrine changes of conceptus and maternal units in jersey cows bred for milk yield. *J Dairy Sci* 1981;64:312-320.
35. Silvia WJ, Taylor ML. Relationship between uterine secretion of prostaglandin $F_{2\alpha}$ induced by oxytocin and endogenous concentrations of estradiol and progesterone at three stages of the bovine estrous cycle. *J Anim Sci* 1989;67:2347-2353.

CHAPTER 7

General discussion

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1. Introduction

The main purpose of the studies described in this thesis was to develop an accurate, non-invasive measurement and analyzing technique, for quantifying uterine contractility in cows during the early postpartum period. By being able to apply it even under on-farm conditions, the activity of the puerperal uterus can be monitored under physiological circumstances and the effects of drugs, administered to alter myometrial contractility, can be more accurately evaluated.

2. Discussion of the results

2.1. Establishment of a non-invasive fixation method

One of our first and rather basic results is described in **Chapter 3**, in which a non-invasive fixation method of the intrauterine pressure (IUP) recording devices within the uterine cavity is introduced. As mentioned earlier, the use of non-fixed devices could result in either their displacement within the uterine cavity or even their complete shedding from the uterus, making the IUP recordings unreliable. As reported for the bovine postpartum uterus, it is also difficult to make proper comparisons between subsequent recordings of IUP signals if they are recorded from different locations in the uterus, because these might be quite different in terms of their contractility patterns [1,2]. Alternatively, surgical intervention would be needed to properly fix the IUP recording unit to the uterine wall [1,3]. To overcome this problem, we developed a simple fixation method, the so-called "*virtual knot*" system. After a transcervical insertion, the measuring device is fixed to a caruncle of the previously gravid uterine horn in such a way that, at the end of the experiment, no intravaginal or intrauterine manipulation is needed for its removal. Thus, this system ensures that repeated measurements can be made and the data compared without the need for a surgical intervention.

2.2. The development of digital data acquisition and analyzing methods, with a post-measurement filtering capability

Initially, uterine activity was only recorded and evaluated using analogue methods, which have a number of drawbacks. As thoroughly discussed in **Chapters 2 and 3**, the advancement of computer technique enabled the development of specific digital data acquisition and analyzing softwares, based on a general purpose graphical program package LabVIEW™ 5.0 (National Instruments Corp, Austin, TX). To validate the digital system, using a 40 Hz sampling frequency, simultaneous data collection from two electromyographic (EMG) electrodes and two different IUP measurement systems were made that had been installed on the postpartum bovine

uterus. Signals, originating either from the EMG electrodes, or the IUP transducers, were first preamplified, and after an analogue-digital (A-D) conversion, stored in the computer for later analysis. The fast processors, and the large random access memories and hard disk drives available in current computers, enable long, uninterrupted recordings to be made. Two-hour **(Chapter 3)**, and even twelve-hour **(Chapter 5)** recordings (recorded at 6-hour intervals) were made without resulting in any difficulties with the handling of the data during the later analysis.

Despite attempts to automatically correct the drift in the basal tone [4], digital evaluation techniques still don't always allow a distinction to be drawn between signals of uterine mechanical activity and random noise [5]. Such noise can be generated quite often, when animals are coughing, bellowing, straining, eructating, randomly moving, urinating or defecating during recording sessions, and these actions may cause artefacts. Such events should therefore be noted during the recording phase, as by using these remarks during the evaluation phase, obvious artefacts can be recognized and excluded.

Digital data allows the pressure curves to be smoothed by filtering. Low-pass filtering was used to eliminate artefacts, caused by circuit frequency fluctuations, or small pressure changes caused by the animal [4]. Filtering the data recorded by the open tip catheter system, yielded very similar results to those recorded with the more expensive, Konigsberg type microtransducer, as was described in detail in **Chapter 3**. This made the analysis of the curves easier.

2.3. A disposable catheter for digital IUP recordings proved to be applicable even under on-farm conditions

Once the disposable open tip catheter, connected with a digital data acquisition and analyzing system, had been tested and approved, a 4 Hz sampling frequency was subsequently used during IUP data collection from animals kept on large-scale cattle farms **(Chapters 4 and 5)**. Regular flushing with saline solution prevented blockage of the tips of the catheters. The differentiation between uterine contractions and artefacts, such as caused by abdominal straining, can be facilitated by a continuous observation of the animal, or by using differential pressure measurements on the basis of a second catheter, introduced into the anterior vagina. Such an approach has recently been used in non-pregnant mares [6-8].

The technical requirements for conducting experiments with IUP measurements on a cattle farm are relatively simple: an adequate portable computer, provided with special programs for data acquisition and analysis, and a disposable pressure transducer. The quantitative IUP data, obtained from the uterus of postpartum cows with this system, were found to reach an accuracy, equal to that of the much more expensive microtransducer system. Because our experimental approach is non-invasive, there is no need to take the animals permanently out of the production. It is in fact only necessary to restrain them for the time, during which the recordings are made **(Chapters 4 and 5)**. This on-farm method, therefore, allows the studying of physiological, pathophysiological and pharmacological aspects of uterine contractility in animals kept under practical, production circumstances.

2.4. The level of myometrial contractility in untreated cows without fetal membrane retention decreased rapidly, during the first 48 hours after calving

A rapid decrease occurred in uterine contractility during the first 48 h after normal calving in cows without fetal membrane retention (NRFM), in particular between 12 and 24 h (**Chapters 4 and 5**). This decline might have several causes. Plasma oxytocin levels rapidly decline after expulsion of the calf and a reduced secretion of oxytocin, as mirrored by the very low plasma oxytocin levels during the first 4 days [9], could offer one possible explanation. Moreover, the extensive decline in uterine oxytocin receptors (OTR) from parturition until the first day postpartum, measured in cows [10], suggests a further decline in their concentration, although, at present, no additional data are available on changes in OTR concentration during the following days of the puerperium. Although not measured, it seems very unlikely that the rapid disappearance of oxytocin from the circulation after calving would explain the observed decline of uterine contractility between 12 and 24 h and of the subsequently low level of uterine activity during the period up to 48 h postpartum.

Another possible explanation for the decline in uterine activity, observed during the early postpartum period, could be associated with a decreasing effect of prostaglandins (PGs) on the myometrium. It is known from early in vitro studies with pregnant rat uteri that a close association exists between uterine PG release and uterine muscle activity [11,12]. Based on the levels of the major $PGF_{2\alpha}$ metabolite (15-ketodihydroprostaglandin $F_{2\alpha}$) in peripheral blood, which is considered to be a reliable estimator of (uterine) $PGF_{2\alpha}$ synthesis and release [13-16], the postpartum uterus of cows is exposed to very high levels of PG's during the early postpartum days after normal calving [17]. This raises the question as to whether the number of prostaglandin receptors in the myometrium decline rapidly after expulsion of the calf, thus explaining the observed reduction of uterine motility during this period. However, as far as we know, at present, there aren't any published data reporting about changes in prostaglandin receptors in the postpartum uterus of cows.

Although progesterone inhibits myometrial activity, luteolysis occurs already before parturition, resulting in progesterone levels to drop before and during calving and to remain low during the early postpartum days. Therefore, it is very unlikely that progesterone plays any role in decreasing contractility during these first days.

Another explanation as to why uterine contractility declines so rapidly after normal calving, is based on the hypothesis that expression of iNOS (the inducible isoform of nitric oxide synthase) in the myometrium increases after calving [18]. iNOS promotes the production of nitric oxide (NO), an active inhibitor of the myometrium [19]. However, there is preliminary evidence indicating that iNOS expression in the myometrium is elevated on Day 6, but not on Day 3, after calving [18].

An additional explanation for the decline in uterine activity could be related to the number of functioning gap junctions between myometrial cells [20]. If the number of these low-resistance locations between neighbouring myometrial cells decreases, the ion transport is hindered and muscle cells are not excited to contract [21]. Such a decline in postpartum uterine

contractility was found in rats, when gap junctions were almost completely lost by 24 h postpartum [22]. It remains to be studied whether such a situation is also valid for early postpartum cows.

Finally, since milking was always completed at least one hour before starting the IUP recordings, any possible effect due to milking, on uterine contractility, could not be observed in any of our experiments performed with early postpartum NRFM cows **(Chapters 4 and 5)**.

2.5. The early postpartum IUP values varied considerably between individual cows

We found considerable variation in the levels of postpartum uterine contractility between individual cows used in the various studies of this thesis. Although the studies described in **Chapters 4 and 5** were performed in large-scale dairy cattle farms, enabling us to establish quite homogeneous groups, a high level of biological diversity appears to characterize the contractility of the postpartum uterus of the cow. Despite our efforts to standardize conditions as far as possible during the selection of the animals and with the experimental protocols, there were cows, from which the recorded data deviated from the overall mean values of the group. Therefore, in some cases, the results for these animals had to be evaluated individually, as described for example in **Chapter 3**. This natural variability in spontaneous postpartum myometrial contractility makes it rather questionable if standard treatment protocols to influence postpartum uterine involution make sense, when they are thought to operate by enhancing the level of contractility of the uterus. In fact, personal judgement about individual animals before and after treatment, still remains a determining factor.

2.6. Relationship between blood Ca^{2+}-concentrations and uterine contractility in early postpartum cows

Calcium exists in three major forms in the extracellular compartment: as protein-bound (45%), complex-bond (5%) and as free ionized calcium (50%) [23]. It became clear in 1934 that it is the ionized form, which is the physiologically active form of the extracellular calcium [24], most accurately reflecting the amount available for its uptake and utilization by the tissues, and thus more rapidly indicating the disturbances in calcium metabolism. Kvart et al. (1982) classified the different degrees of hypo- and hypercalcemia in cows, on the basis of their serum ionized calcium (Ca^{2+})-concentration. He considered the normocalcemic range to lie between 1.06 and 1.26 mmol/l [25]. It was also found that minor differences exist between bovine serum and plasma Ca^{2+}-contents, with 0.05 mmol/l higher values being measured in the serum originating from the same blood sample [26].

A direct association between hypocalcemia, and decreased uterine activity was clearly demonstrated by experimentally inducing hypocalcemia in various species, including humans, during their parturition and immediate postpartum phases [27-31]. After experimentally

inducing a severe hypocalcemia (to plasma Ca^{2+}-levels as low as 0.45 mmol/l) in a pregnant cow, both FREQ and AMP of the contractions from the gravid uterine horn decreased quickly, and simultaneously, and the clinical signs of milk fever appeared [29]. A marked negative effect of milk fever on uterine activity has also been reported in spontaneously diseased, NRFM cows [32,33]. However, if severe hypocalcemic cows were intravenously treated with calcium borogluconate, after their gradual return to consciousness, a slow return of the uterine contractions could be measured, both in cows without [33], and with fetal membrane retention [34]. These results demonstrate the essential role of normocalcemic conditions in the maintenance of a proper level of uterine contractility.

Despite the clear association between the clinical form of severe hypocalcemia and impaired uterine activity in early postpartum cows, the magnitude of the decline in the extracellular calcium concentration that would result in establishing a general set point value, below which a decline in uterine activity will be induced, has not been established so far. As mentioned in **Chapter 4**, about 50% of the cows involved in our study, experienced a slight form of hypocalcemia throughout the entire course of the study. Such a decline in blood Ca^{2+}-concentration has been known to occur immediately after calving in healthy cows [35], and was also noted in our own study in Hungary [36]. The lack of significant correlations between blood Ca^{2+}-concentration and the IUP parameters as described in **Chapter 4**, was most likely due to the level of hypocalcemia not being severe enough, during the first 48 h after calving, to reduce or even block uterine activity in NRFM cows.

The determination of a general set point, based on a blood Ca^{2+}-concentration, below which the uterine motility is abolished, therefore, does not seem to be a realistic approach. Instead, the determination of a hypocalcemic range, based on the measured blood Ca^{2+}-concentrations, seems to be more practical, but even that needs to be judged carefully. This is not only because of the individual variability of the IUP parameters measured in the normally calved, healthy cows **(Chapter 4)**, but also because of many other factors that influence the physiology of periparturient cows, among which various metabolic disturbances might play a determinant role [37-39].

Severe hypocalcemia leads to reduction of uterine activity and because this will delay uterine involution, it also causes economic losses, even when the animals have been cured. Such delay can also occur with milder cases of hypocalcemia if uterine motility is affected [40].

Although hypocalcemia, by itself, is a very important causative factor causing decreased uterine activity during the early postpartum period, there are many other factors, which can also contribute to this. A strong relationship between the simultaneous occurrence of milk fever and RFM [41] and a significantly prolonged interval to the first postpartum ovulation [42], have been reported. But also several other risk factors, such as a high milk production, a negative energy balance [43], dystocia [44,45], different forms of uterine infections [46-48] have frequently been reported to be responsible for, or occurring simultaneously with, a delayed uterine involution.

2.7. The similar uterotonic effects of oxytocin and carbetocin

Uterotonic drugs are often used in early postpartum cows to facilitate involution, by stimulating myometrial activity in order to remove excessive fluid and debris from the uterine lumen. From observations on NRFM cows, uterine contractions were found to decline rapidly, with the most expressed reduction taking place between 12 and 24 h after calving, and a very low level of spontaneous contractility remaining at 48 h **(Chapter 4;** [49]**)**. Oxytocin (OT) is one of the most frequently applied drugs during this early puerperal period [50-55].

Basically, two factors determine the biological effect of OT and its analogues – the time needed for excretion or metabolism, and the number of OT specific receptors. Oxytocin-analogues, like carbetocin (CB), appeared to have a prolonged biological activity in cows [51,56,57]. From our observation with NRFM cows after normal parturition **(Chapter 5)**, a single treatment with either OT (50 IU) or CB (0.35 mg), administered intramuscularly between 14 and 16 h postpartum, significantly increased the FREQ and TAUC values of the pressure cycles, mainly during the first post-treatment hour. The oxytocin-like effect of both drugs resulted in an increase in the frequency of myometrial contractions, without significantly altering the characteristics of the pressure cycles. This suggests that, in early postpartum cows, apart from the spontaneous uterine contractility and the individual variability, activity of the myometrium can still be enhanced. This is best mirrored by the TAUC values, which represent the total amount of work done by the uterus within a certain time interval.

That the immediate effects of OT and CB are similar, as described in **Chapter 5**, can be due to their similar affinity to the membrane receptors in the myometrial cells. Although data to support this assumption has not been found for cows, CB has been shown to bind to the uterine receptors in isolated rat myometrial strips [58].

Carbetocin, in the dose recommended by the manufacturer, did not induce a more prolonged uterotonic effect than OT **(Chapter 5)**. Since the duration of the effects from a drug also depends on its molecular structure and this determines its biological half-life, these findings were quite unexpected. Our findings, therefore, imply that, despite a reported prolonged half-life of carbetocin [59], other factors such as the applied dose or route of administration, still need further investigation. Yet our findings are in agreement with those of Eulenberger et al. [60], who suggested that like with OT, no further beneficial effect of CB can be expected from the use of either drugs in terms of ensuring an undisturbed puerperium or improving fertility in dairy cows after normal parturition.

2.8. Oxytocin and the endometrial prostaglandin release

The results from **Chapter 6** suggest that, additionally to a direct myometrial effect of oxytocin as demonstrated in **Chapter 5,** no significant contribution to myometrial stimulation can be expected from an oxytocin-stimulated prostaglandin release, when oxytocin is injected intramuscularly. Prostaglandin $F_{2\alpha}$-metabolite determinations in samples from the venous outflow of the uterus have to be performed, before this conclusion can be substantiated. However, intravascular treatment with OT resulted in increased plasma $PGF_{2\alpha}$-metabolite levels

in two cows, which suggests that a high bolus of OT stimulated the PG synthesis and release in the uterus. Mutual interactions between stimulation of endometrial PG release and myometrial activity by a paracrine route need further investigations with bovine myometrial tissues in vitro. In addition, there is a need to identify and quantify expression of oxytocin and prostaglandin receptors in the postpartum uterus of the cow.

3. Main outcomes and conclusions of this thesis

1. The most frequently used methods for measuring intrauterine pressure (IUP) changes in the uterus of the cow during the postpartum period, and the available reports on spontaneous and drug-induced uterine activity, have been overviewed.

2. A non-invasive fixation method (the "*virtual knot*") for transcervically inserted IUP measuring devices, which only requires intrauterine manipulation at the start of the observation period, has been described.

3. Digital data acquisition and analyzing methods with post-measurement filtering capability have been developed for IUP and EMG (electromyographic) measurements.

4. A disposable catheter and pressure transducer system, connected with computerized recording and analyzing equipment, using a sampling frequency of 4 Hz, appeared applicable under farm conditions. It enabled the characterization of the mechanical activity of the uterus during the early postpartum period in cows. It appeared to be an accurate tool for measuring temporal changes in uterine contractility during the first two days after uncomplicated calving in cows, without retained fetal membranes (NRFM).

5. The mean contraction frequency (FREQ), amplitude (AMP) and area under the curve (AUC) of the myometrial contractions, measured in untreated, NRFM cows on a large-scale dairy farm, decreased continuously and significantly during the first 48 h, with the most marked decline occurring between 12 and 24 h.

6. The changes in IUP values varied considerably among individual untreated cows.

7. Significant correlations were not found between the characteristics of uterine contractility and blood Ca^{2+}-concentrations, in healthy puerperal cows.

8. A single treatment with either oxytoxin (50 IU, im.) or carbetocin (0.35 mg, im.) only induced very similar short-term uterotonic effects, as characterized by the temporarily increased values of contraction frequency (FREQ) and of the total area under the curve (TAUC).

9. No further effects of these two drugs on the contractility of the uterus could be observed beyond the fourth hour after treatment.

10. The effectiveness of OT or CB, in enhancing uterine contractility, was almost identical. A more beneficial clinical effect on uterine involution after using carbetocin is, therefore, unlikely.

11. A single intramuscular oxytocin treatment (50 IU), applied between 13 and 15 h after normal calving, did not induce significant changes in 15-ketodihydroprostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$-metabolite) concentrations in peripheral plasma.

12. Intravascular treatment with oxytocin provided evidence for a stimulation of uterine prostaglandin (PG) release. An indirect uterotonic effect of oxytocin might additionally contribute to its direct effect on the myometrium.

4. References

1. Zerobin K. Die Uterusbewegungen bei Kühen während der Geburt und der Nachgeburtsphase. *Schweiz Arch Tierheilkd* 1970;112:544-560.
2. Taverne MAM. Uterine motility in the post partum female. In: *Proceedings* Xth Int Congr Anim Reprod AI 1984. Illinois.Vol.IV:XI-1-8.
3. Kündig H, Thun R, Zerobin K, Bachmann B. Die Uterusmotorik des Rindes während Spätgravidität, Geburt und Puerperium. I. Die Spontanmotorik. *Schweiz Arch Tierheilkd* 1990;132:77-84.
4. Beck NFG, Carter MC, Jansen CAM, Joyce PL, Krane EJ, Nathanielsz PW, Steer P, Thomas AL. A method for the quantitative assessment of uterine activity in the pregnant sheep. *Proc Physiol Soc* 1977;9P-10P.
5. Braaksma JT, Veth AFL, Eskes TKAB, Stolte LAM: Digital evaluation of uterine contraction records. In: Josimovich JB (ed): *Uterine Contraction-Side Effects of Steroidal Contraceptives. Volume 1. Problems of human reproduction: A Wiley-Interscience series.* New York: John Wiley & Sons, 1973;9-18.
6. Goddard PJ, Allen WE, Gerring EL. Genital tract pressures in mares. I. Normal pressures and the effect of physiological events. *Theriogenology* 1985;23:815-827.
7. Goddard PJ, Allen WE. Anterior vaginal and intrauterine pressures in mares. *Theriogenology* 1988;30:83-89.
8. Gutjahr S, Paccamonti DL, Pycock JF, Taverne MAM, Dieleman SJ, van der Weijden GC. Effect of dose and day of treatment on uterine response to oxytocin in mares. *Theriogenology* 2000;54:447-456.
9. Schams D, Schmidt-Polex B, Kruse V. Oxytocin determination by radioimmunoassay in cattle. I. Method and preliminary physiological data. *Acta Endocrinol (Copenh)* 1979;92:258-270.
10. Fuchs A-R, Helmer H, Behrens O, Liu H-C, Antonian L, Chang SM, Fields MJ. Oxytocin and bovine parturition: a steep rise in endometrial oxytocin receptors precedes onset of labor. *Biol Reprod* 1992;47:937-944.
11. Vane JR, Williams KI. The contribution of prostaglandin production to contractions of the isolated uterus of the rat. *Br J Pharmacol* 1973;48:629-639.
12. Chan WY. Relationship between the uterotonic action of oxytocin and prostaglandins: oxytocin action and release of PG-activity in isolated nonpregnant and pregnant rat uteri. *Biol Reprod* 1977;17:541-548.
13. Guilbault LA, Thatcher WW, Foster DB, Caton D. Relationship of 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ concentrations in peripheral plasma with local uterine production of F series prostaglandins and changes in uterine blood flow during the early postpartum period of cattle. *Biol Reprod* 1984;31:870-878.
14. Kindahl H, Edqvist L-E, Bane A, Granström E. Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ during the normal oestrous cycle and early pregnancy in heifers. *Acta Endocrinol (Copenh)* 1976;82:134-149.
15. Kindahl H, Granström E, Edqvist LE, Neely D, Hughes J, Stabenfeldt G. The advantages of measuring a prostaglandin $F_{2\alpha}$ metabolite in peripheral blood in studies of the physiological role of prostaglandin release during luteolysis in domestic animals. In: *Proceedings* VIIIth Int Congr Anim Reprod AI 1976. Krakow. Vol. III:145-148.
16. Kindahl H, Edqvist L-E, Granström E, Bane A. The release of prostaglandin $F_{2\alpha}$ as reflected by 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ in the peripheral circulation during normal luteolysis in heifers. *Prostaglandins* 1976;11:871-878.
17. Lindell J-O, Kindahl H, Jansson L, Edqvist L-E. Post-partum release of prostaglandin $F_{2\alpha}$ and uterine involution in the cow. *Theriogenology* 1982;17:237-245.

18. De Jong R. Characterisation of uterine contractility of the postpartum cow and the role of nitric oxide in its regulation. *MSc Thesis*, Faculty of Veterinary Medicine, Utrecht University: Utrecht, The Netherlands, 2002.
19. Yallampalli C, Garfield RE, Byam-Smith M. Nitrit oxide inhibits contractility during pregnancy but not during delivery. *Endocrinology* 1993;133:1899-1902.
20. Garfield RE, Sims S, Daniel EE. Gap junctions: Their presence and necessity in myometrium during parturition. *Science* 1977;198:958-960.
21. Garfield RE, Rabideau S, Challis JRG, Daniel EE. Ultrastructural basis for maintenance and termination of pregnancy. *Am J Obstet Gynecol* 1979;133:308-315.
22. Wathes DC, Porter DG. Effect of uterine distension and oestrogen treatment on gap junction formation in the myometrium of the rat. *J Reprod Fertil* 1982;65:497-505.
23. Watson F, Anbar M. Determination of ionized calcium and total calcium in human serum with the Nova 7 Calcium Analyzer. *Health Care Instrum* 1985;1:74.
24. McLean FC, Hastings AB. A biological method for the estimation of calcium ion concentration. *J Biol Chem* 1934;107:337-343.
25. Kvart C, Björsell KA, Larsson L. Parturient paresis in the cow. Serum ionized calcium concentrations before and after treatment with different calcium solutions – classification of different degrees of hypo- and hypercalcemia. *Acta Vet Scand* 1982;23:184-196.
26. Kvart C, Larsson L. Studies on ionized calcium in serum and plasma from normal cows. Its relation to total serum calcium and the effects of sample storing. *Acta Vet Scand* 1978;19:487-496.
27. Robalo Silva J, Noakes DE. The effect of experimentally induced hypocalcaemia on uterine activity at parturition in the ewe. *Theriogenology* 1984;21:607-623.
28. Ayliffe TR, Noakes DE, Robalo Silva J. The effect of experimental induced hypocalcaemia on uterine activity in the sow during parturition and post-partum. *Theriogenology* 1984;21:803-822.
29. Al-Eknah MM, Noakes DE. A preliminary study on the effect of induced hypocalcaemia and nifedipine on uterine activity in the parturient cow. *J Vet Pharmacol Ther* 1989;12:237-239.
30. Wurth Y, van der Weyden GC, Taverne MAM, van Oord R. The effect of the calcium-antagonist nifedipine on uterine contractility of the dog before, during and after parturition. *Tijdschr Diergeneesk Suppl I* 1986;111:6-7.
31. Forman A, Gandrup P, Andersson KE, Ulmsten U. Effects of nifedipine on spontaneous and methylergometrine-induced activity post partum. *Am J Obstet Gynecol* 1982;144:442-448.
32. Martin LR, Williams WF, Russek E, Gross TS. Postpartum uterine motility measurements in dairy cows retaining their fetal membranes. *Theriogenology* 1981;15:513-524.
33. Jordan WJ. The puerperium of the cow: a study of uterine motility. *J Comp Pathol Ther* 1952;62:54-68.
34. Bajcsy ÁC. Uterine contractility in a cow suffering from milk fever. Unpublished data. 2001.
35. Blum JW, Ramberg CFJ, Johnson KG, Kronfeld DS. Calcium (ionized and total), magnesium, phosphorus, and glucose in plasma from parturient cows. *Am J Vet Res* 1972;33:51-56.
36. Szenci O, Chew BP, Bajcsy ÁC, Szabó P, Brydl E. Total and ionized calcium in parturient dairy cows and their calves. *J Dairy Sci* 1994;77:1100-1105.
37. Reid IM, Roberts CJ, Manston R. Reduced fertility associated with fatty liver in high yielding dairy cows. *Vet Sci Commun* 1979;3:231-236.
38. Goff JP, Horst RL. Physiological changes at parturition and their relationship to metabolic disorders. *J Dairy Sci* 1997;80:1260-1268.
39. Zurek E, Foxcroft GR, Kennelly JJ. Metabolic status and interval to first ovulation in postpartum dairy cows. *J Dairy Sci* 1995;78:1909-1920.
40. Kamgarpour R, Daniel RCW, Fenwick DC, McGuigan K, Murphy G. Post partum subclinical hypocalcaemia and effects on ovarian function and uterine involution in a dairy herd. *Vet J* 1999;158:59-67.
41. Pelissier CL. Herd breeding problems and their consequences. *J Dairy Sci* 1972;55:385-391.

42. Risco CA, Drost M, Thatcher WW, Savio J, Thatcher MJ. Effects of calving-related disorders on prostaglandin, calcium, ovarian activity and uterine involution in postpartum dairy cows. *Theriogenology* 1994;42:183-203.
43. Butler WR. Symposium: Optimizing protein nutrition for reproduction and lactation. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *J Dairy Sci* 1997;81:2533-2539.
44. Nakao T, Gamal A, Osawa T, Nakada K, Moriyoshi M, Kawata K. Postpartum plasma PGF metabolite profile in cows with dystocia and/or retained placenta, and effect of fenprostalene on uterine involution and reproductive performance. *J Vet Med Sci* 1997;59:791-794.
45. Oltenacu PA, Britt JH, Braun RK, Mellenberger RW. Relationships among type of parturition, type of discharge from genital tract, involution of cervix, and subsequent reproductive performance in Holstein cows. *J Dairy Sci* 1983;66:612-619.
46. Mateus L, da Costa LL, Bernardo F, Silva JR. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. *Reprod Domest Anim* 2002;37:31-35.
47. Tennant B, Peddicord RG. The influence of delayed uterine involution and endometritis on bovine fertility. *Cornell Vet* 1968;58:185-192.
48. Lewis GS. Symposium: Health problems of the postpartum cow. Uterine health and disorders. *J Dairy Sci* 1997;80:984-994.
49. Bajcsy ÁC, Szenci O, van der Weijden GC, Doornenbal A, Maassen F, Bartyik J, Taverne MAM. The effect of a single oxytocin or carbetocin treatment on uterine contractility in early postpartum dairy cows. *Theriogenology* 2005; accepted with minor modifications.
50. Zerobin K. Die uterusmotorischen Ablaeufe waehrend Geburt und Puerperium beim Rind und deren Beeinflussbarkeit. In: *Proceedings* XI. Int Congr Dis Cattle 1980. Tel-Aviv.Vol.II:1157-1164.
51. Eulenberger K, Wilhelm J, Schulz J, Gutjahr S, Wohanka K, Däberitz H. Uterotonika im Puerperium des Rindes. *Monatsh Veterinärmed* 1986;41:371-377.
52. Armstrong-Backus CS, Hopkins FM, Eiler H. The uterotonic effect of prostaglandin $F_{2\alpha}$ and oxytocin on the postpartum cow. In: *Abstracts* 64th Ann Meet Conf Res Workers Anim Dis 1983. Chicago. (93.abstract) 17.
53. Giama I, Elze K, Eulenberger K. Untersuchungen zur postpartalen Uterusmotilität des Rindes. 2. Mitt.: Uterusmotilität im Frühpuerperium des Rindes nach Oxytozinapplikation. *Monatsh Veterinärmed* 1976;31:940-942.
54. Burton MJ, Dziuk HE, Fahning ML, Zemjanis R. Effects of oestradiol cypionate on spontaneous and oxytocin-stimulated postpartum myometrial activity in the cow. *Br Vet J* 1990;146:309-315.
55. Eiler H, Hopkins FM, Armstrong-Backus CS, Lyke WA. Uterotonic effect of prostaglandin $F_{2\alpha}$ and oxytocin on the postpartum cow. *Am J Vet Res* 1984;45:1011-1014.
56. Bernhard A, Schulz J, Gutjahr S, Eulenberger K. Indikationen für die Anwendung eines Depotoxytozin-Präparates in der tierärztlichen Praxis. *Tierärztl Umsch* 1993;48:446-453.
57. Eulenberger K, Schulz J, Gutjahr S, Strohbach U, Strohbach C, Randt A. Beeinflussung der Geburt bei Schwein und Rind mit Oxytocin, Carbetocin und Carazolol. *Wien Tierärztl Mschr* 1993;80:276-279.
58. Atke A, Vilhardt H. Uterotonic activity and myometrial receptor affinity of 1-deamino-1-carba-2-tyrosine(O-methyl)-oxytocin. *Acta Endocrinol (Copenh)* 1987;115:155-160.
59. Jošt K, Šorm F. The effect of the presence of sulphur atoms on the biological activity of oxytocin; Synthesis of deamino-carba^{6}-oxytocin and deamino-dicarba-oxytocin. *Collect Czech Chem Commun* 1971;36:234-245.
60. Eulenberger K, Stubbe J, Böhme W, Liebaug E. Zur metaphylaktischen und therapeutischen Anwendung eines Depotoxytocin-Präparates im Puerperium des Rindes. *Monatsh Veterinärmed* 1987;42:738-742.

CHAPTER 8

Summary - Samenvatting - Összefoglaló

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This thesis deals with uterine contractility in dairy cows during the first few days after calving. The individual studies, presented in the separate chapters, were designed to get answers to certain methodological problems and biological questions associated with the myometrial function of early postpartum dairy cows.

Chapter 1 gives a general introduction to the topic uterine contractility during the bovine early postpartum period. After introducing the scope of the thesis, the events in the uterus, following the birth of the calf, are briefly summarized. This chapter then outlines some introductory ideas on the role myometrial contractions play in evacuation of the uterus after calving. The available possibilities for recording uterine contractility are introduced, also mentioning some of the characteristic features associated with the different recording circumstances. From these it seems, that subject to several limitations, intrauterine pressure (IUP) measurements can serve as a proper tool for characterizing uterine contractility. However, a non-invasive, accurate intrauterine pressure recording and analysis system for use in postpartum cows that enables recordings even on the farm, was not found in the literature. Not only had these methodological questions not been solved so far, but also, very little data had been published on postpartum uterine contractility in cows. Therefore, accurate quantitative information about the physiological changes in uterine function after normal calvings in cows on large-scale farms, was still missing. The following part of Chapter 1 briefly deals with these aspects. This part is followed by an introduction of possibilities of medically stimulating uterine contractility, describing the mechanisms, through which they act in postpartum cows. Finally, the aims of the individual studies of the thesis are outlined, introducing them by chapters.

Chapter 2 reviews the development of techniques for measuring uterine activity and summarizes the most frequently used data recording, analyzing and evaluation methods. Uterine contractility measurements, based on recordings of intrauterine pressure during the normal and abnormal bovine postpartum period, are detailed in a chronological order. At the end of this chapter there is a review of those studies, in which either uterine relaxants were used during parturition but not in the postpartum period, or uterine stimulants during the postpartum period, focusing on the use of oxytocin, prostaglandin $F_{2\alpha}$, and their analogues.

This chapter shows that various techniques are used to characterize uterine contractility. Not only the IUP data of the postpartum cows report a certain variability, similar, partly inconsistent results were obtained from those studies, in which uterine contractility was medically influenced.

Chapter 3 outlines how electromyographic (EMG) measurements, using two previously surgically imbedded bipolar electrodes on the surface of the pregnant uterus, were compared with the measurements from two different IUP recording methods (open tip and microtransducer systems), in cows after parturition. Simultaneous recordings were made in these cows during the immediate postpartum period, after parturition had been induced with prostaglandin $F_{2\alpha}$ at day 274 of gestation. The development of a transcervically introduced,

reliable, non-invasive method for the fixation of the IUP measuring units (the *„virtual knot"*), which restricts intrauterine manipulation to the start of the observation period, is described. Appropriate acquisition and analyzing softwares, including a post-measurement filtering capability based on a 40 Hz sampling frequency have also been developed. This study gave highly comparable, but not identical results, obtained from the comparison of the two EMG signals and it also showed good correlations between the different pressure measuring systems, and between the IUP and EMG recordings.

Thus an appropriate digital technique, for characterizing the mechanical activity of the early postpartum uterus, in untreated or treated cows, based on low-cost, disposable IUP recording units, has become available for use, even under on-farm conditions.

Chapter 4 details a study, performed on a large-scale dairy farm in which the digital (4 Hz sampling frequency), non-invasive, open tip catheter system was successfully applied to quantifying intrauterine pressure changes in untreated, healthy, early postpartum cows, which did not retain their fetal membranes. Mean frequency (FREQ), amplitude (AMP) and area under the curve (AUC) of the myometrial contractions, decreased continuously and significantly during the first 48 hours postpartum, with the largest decline occurring between 12 and 24 hours. However, these changes in the IUP values varied considerably between individual cows. The possible relationships between the characteristics of uterine contractility and blood Ca^{2+}-concentrations were also investigated, but no significant correlations were found.

It was concluded that the IUP recording technique, applied under on-farm conditions, appears to be an accurate tool for measuring temporal changes in uterine contractility during the first two days after uncomplicated calvings.

Chapter 5 describes a study aimed at evaluating to what extent a single treatment with either oxytocin (50 IU im.) or carbetocin (0.35 mg im.), 14 to 16 h after normal parturition, influences uterine contractility during the first and second days postpartum, in dairy cows. Uterine contractility was characterized by measurements derived from the digital recording of IUP, using a transcervically introduced open tip catheter system.

Mean contraction frequency (FREQ) and total area under the curve (TAUC) showed a significant short-term treatment effect (until 4 h after treatment) in both treated groups. After peaking significantly during the 1st post-treatment hour, the values of these parameters for these two groups remained higher during the 2nd h, returning to the initial levels again during the 3rd h, and reaching the level of the control group by the 12th h. Other parameters of the pressure cycles (AMP, DUR /duration/ and AUC) were not significantly affected by the treatments. The long-term analysis (12 to 36 h post-treatment) revealed no significant differences in any of the IUP parameters, due to the treatments. The effectiveness of oxytocin and carbetocin in enhancing uterine contractility, using the described dosage, method and time of administration, was almost identical. No further effects on the contractility of the uterus were expected from the drugs beyond the 4th hour after treatment, because by that time, the levels of contractility in the two treated groups had effectively dropped to that of the saline-treated control group.

Because the use of both drugs resulted in similar uterotonic effects in healthy, early postpartum cows, it cannot be expected, that the use of carbetocin in preference to oxytocin will result in a more beneficial clinical effect on uterine involution during this period.

Chapter 6 details an investigation of the oxytocin-induced changes in peripheral plasma 15-ketodihydroprostaglandin-$F_{2\alpha}$ (prostaglandin $F_{2\alpha}$-metabolite) concentrations, in postpartum cows given a single oxytocin treatment (50 IU), administered intramuscularly or intravenously between 13 and 15 h after calving. No significant differences could be observed in the results of those cows treated intramuscularly with oxytocin, from those treated with a saline solution. However, the fact that in the two cows given intravascular oxytocin treatment, a positive effect on prostaglandin (PG) release could be measured, despite their slight differences, provides evidence that such a stimulation did occur.

Therefore, it can be hypothesized that an indirect effect of intramuscular oxytocin treatment, through the local stimulation of a PG release, might make an additional contribution to its direct uterotonic effect in early postpartum cows. However, when treatment occurred on the first day postpartum, the quantity of the released prostaglandins appearing in the circulation after such an intramuscular treatment did not seem to be large enough for its detection in peripheral plasma.

Chapter 7 contains a general discussion, in which the results of the individual studies from this thesis are discussed in the context of the most current knowledge available in the literature. A list containing the main outcomes and conclusions of the thesis closes this chapter.

Dit proefschrift heeft betrekking op de contractiliteit van de baarmoeder van melkkoeien gedurende de eerste dagen na het afkalven. De afzonderlijke experimenten, zoals die in de opeenvolgende hoofdstukken worden gepresenteerd, werden ontworpen om oplossingen te vinden voor enkele methodologische problemen en antwoorden te geven op biologische vraagstellingen die zijn verbonden met de functie van het myometrium bij koeien gedurende de vroege periode postpartum.

Hoofdstuk 1 geeft een algemene inleiding op het onderwerp „contractiliteit van de baarmoeder" in de periode kort na het afkalven. Nadat de kaderstelling van het proefschrift is uiteengezet worden de belangrijkste processen die zich in de baarmoeder na de geboorte van het kalf afspelen kort samengevat. Dit hoofdstuk schetst vervolgens enkele inleidende gedachten met betrekking tot de rol die samentrekkingen van de baarmoeder zouden kunnen spelen bij het ledigen van de uterus na de partus. De mogelijkheden die beschikbaar zijn om contracties van de baarmoeder te registeren worden geïntroduceerd, waarbij tevens ook enkele specifieke kenmerken worden vermeld die zijn verbonden met de verschillende omstandigheden waaronder wordt geregistreerd. Hieruit blijkt dat intra-uteriene drukmeting, ondanks een aantal beperkingen, een geschikte methode is om de contractiliteit van de baarmoeder te karakteriseren. In de literatuur werden echter geen methoden aangetroffen waarmee op een niet invasieve en nauwkeurige wijze de intra-uteriene drukveranderingen ook onder stalomstandigheden bij postpartum koeien kunnen worden geregistreerd en geanalyseerd. Naast het feit dat er voor deze methodologische problemen nog geen oplossing voor handen was, werd tevens vastgesteld dat er zeer weinig gepubliceerde gegevens beschikbaar zijn met betrekking tot contracties van de baarmoeder na het afkalven. Dat betekent dat er geen nauwkeurige en kwantitatieve informatie is aangaande fysiologische veranderingen in de functie van het myometrium na het normaal afkalven bij koeien die op grote bedrijven worden gehouden. Hoofdstuk 1 gaat vervolgens kort op deze kwantificering in en introduceert vervolgens enkele mogelijkheden waarmee uteruscontracties medicamenteus kunnen worden gestimuleerd en de wijze waarop deze beïnvloeding bij koeien tijdens de postpartum periode tot stand komt. Tenslotte worden de doelstellingen van de afzonderlijke hoofdstukken van dit proefschrift uiteengezet.

Hoofdstuk 2 geeft een samenvatting van de ontwikkeling van technieken waarmee uterusactiviteit kan worden gemeten en van de meest frequent gebruikte methoden waarmee registratie, analyse en evaluatie van gegevens worden uitgevoerd. Metingen van baarmoeder contracties, die zijn gebaseerd op de registratie van intra-uteriene drukveranderingen gedurende een normaal en abnormaal verlopende postpartum fase bij koeien, worden in chronologische volgorde besproken. Aan het einde van dit hoofdstuk wordt een overzicht gegeven van studies waarin ofwel de effecten van uterusverslappende, dan wel die van uterusstimulerende middelen tijdens of na het afkalven werden onderzocht. Daarbij wordt met name aandacht geschonken aan het gebruik van oxytocine en prostaglandine $F_{2\alpha}$, of synthetische analogen van deze middelen. Dit hoofdstuk toont aan dat er verschillende technieken worden toegepast om de contractiliteit van de baarmoeder te karakteriseren. Gegevens over intra-uteriene

drukveranderingen bij koeien tijdens de postpartum fase laten een zekere mate van variabiliteit zien. Ook is er sprake van een (gedeeltelijke) inconsistentie met betrekking tot de effecten die werden gemeten na toediening van genoemde medicamenten.

In *Hoofdstuk 3* wordt uiteengezet hoe bij koeien na de partus electromyografische (EMG) metingen, waarbij gebruik wordt gemaakt van twee tevoren op het oppervlak van de drachtige uterus aangebrachte bipolaire elektroden, worden vergeleken met twee verschillende typen intra-uteriene drukmetingen (IUP): een zogenaamde (disposable) „open-tip catheter" systeem en een „micro-transducer" systeem. Nadat op dag 274 van de dracht de partus met prostaglandine $F_{2\alpha}$ was geïnduceerd, worden gelijktijdig EMG en IUP registraties gemaakt tijdens de eerste dagen die volgen op de partus. Er wordt een methode beschreven waarmee op een betrouwbare, niet-invasieve (trans-cervicale) wijze een fixatie kan plaats vinden van de intra-uterien aangebrachte IUP sensoren. Hiermee is een intra-uteriene manipulatie alleen nog maar noodzakelijk aan het begin van de observatieperiode. Er worden software programma's ontwikkeld voor de acquisitie en analyse van data, inclusief een digitale filtering op de met 40 Hz ingelezen, A-D geconverteerde, data. Deze studie laat zien dat er sprake is van in sterke mate vergelijkbare, maar niet volledig identieke resultaten wanneer de gekwantificeerde signalen van de twee EMG elektroden onderling worden vergeleken. Ook worden goede correlaties aangetoond tussen de twee IUP systemen en tussen de gekwantificeerde IUP en EMG signalen. Door gebruik te maken van een goedkope disposable IUP sensor hebben we dus nu een geschikte, digitale methode waarmee de mechanische activiteit van de baarmoeder tijdens de vroege periode postpartum kan worden gekarakteriseerd, zowel bij onbehandelde als behandelde dieren, zelfs bij koeien die onder boerderij omstandigheden worden gehouden.

In *Hoofdstuk 4* wordt een studie beschreven die is uitgevoerd op een grootschalig melkvee bedrijf en waarbij de IUP veranderingen tijdens de vroege periode postpartum worden gekwantificeerd bij gezonde, onbehandelde koeien die niet aan de nageboorte stonden. Hierbij wordt met succes gebruik gemaakt van het niet-invasieve, open-tip IUP systeem, met een digitale sampling frequentie van 4 Hz. De gemiddelde frequentie (FREQ), amplitudo (AMP) en oppervlak onder de curve (AUC) van de contracties dalen geleidelijk en significant gedurende de eerste 48 uur postpartum, waarbij de grootste afname plaats vindt tussen 12 en 24 uur. Echter, er is sprake van een grote mate van variabiliteit in deze veranderingen tussen individuele koeien. Bij het zoeken naar een mogelijke relatie tussen de karakteristieken van de uteruscontracties en Ca^{2+} concentraties in het bloed worden echter geen significante correlaties vastgesteld. Er wordt geconcludeerd dat de IUP registratie techniek zoals die onder bedrijfsomstandigheden werd toegepast, een nauwkeurig instrument is voor het meten van chronologische veranderingen in contractiliteit van de baarmoeder tijdens de eerste twee dagen na een normaal verlopen partus.

Hoofdstuk 5 doet verslag van een experiment dat beoogt na te gaan in welke mate een eenmalige behandeling met ofwel oxytocine (50 I.U. i.m.) dan wel carbetocine (0.35 mg i.m.), gegeven tussen 14 en 16 uur na het normaal afkalven, van invloed is op de contracties van de

baarmoeder tijdens de eerste en tweede dag postpartum. Hierbij wordt opnieuw gebruik gemaakt van de digitale IUP registratie methode waarbij een open-tip sensor transcervicaal wordt aangebracht. Beide behandelingen beïnvloeden op de korte termijn (tot 4 uur na behandeling) zowel de gemiddelde FREQ als het gemiddelde oppervlak onder de curven van alle drukverhogingen (TAUC). Na een significante piekwaarde gedurende het eerste uur na behandeling, blijven de waarden voor deze twee parameters hoger (dan die in de controle groep) tijdens het 2e uur, keren terug naar de uitgangswaarden tijdens het derde uur en bereiken het niveau van de dieren in de controlegroep tijdens het 4e uur na behandeling. Andere karakteristieken van de drukcurven (AMP, duur en AUC) worden door de behandelingen niet beïnvloed. Analyse van de effecten op langere termijn (tussen 12 en 36 uur na behandeling) laat zien dat geen van de IUP parameters door de twee preparaten significant wordt beïnvloed. Het blijkt dus dat met de gebruikte doseringen, wijze en tijdstip van toediening, de effectiviteit van oxytocine en carbetocine voor de stimulatie van de baarmoedercontractiliteit nagenoeg gelijk is. Omdat vanaf vier uur na behandeling de contractiliteit bij beide behandelingen het niveau had bereikt van dat bij met fysiologische zoutoplossing behandelde controledieren, kon ook geen verder effect worden verwacht voorbij het vierde uur na toediening. Aangezien beide middelen resulteerden in identieke effecten op de baarmoeder bij gezonde koeien tijdens de vroege periode postpartum, kan niet worden verwacht dat het gebruik van carbetocine voorkeur geniet boven dat van oxytocine wanneer een gunstiger klinisch effect op de involutie van de baarmoeder wordt beoogd.

Hoofdstuk 6 rapporteert over veranderingen in de perifere plasma concentraties van 15-ketodihydroprostaglandin-$F_{2\alpha}$ (prostaglandine $F_{2\alpha}$-metaboliet) bij koeien die tussen 13 en 15 uur na het afkalven met een enkele intramusculaire of intraveneuze dosis (50 I.U.) van oxytocine worden behandeld. Er werden geen significante verschillen in plasma niveaus gevonden tussen dieren die intramusculair met oxytocine of fysiologisch zoutoplossing werden behandeld. Echter de waarneming dat bij twee koeien die het oxytocine intraveneus kregen toegediend wél een stijging van de prostaglandine (PG) release werd gevonden, wijst erop dat oxytocine een stimulerende werking heeft. Daarom wordt verondersteld dat een indirect effect van een intramusculaire oxytocine behandeling, middels een locale stimulatie van de PG release, een additionele bijdrage levert aan het uterusstimulerende effect tijdens de vroege postpartum periode. Wanneer echter de behandeling op de eerste dag postpartum intramusculair plaats vindt zal de hoeveelheid PG die in de perifere circulatie terechtkomt te klein zijn om te kunnen worden gedetecteerd.

Hoofdstuk 7 geeft een algemene beschouwing waarin de resultaten van de afzonderlijke studies van dit proefschrift worden bediscussieerd in het licht van de beschikbare informatie uit de literatuur. Dit hoofdstuk eindigt met de opsomming van de belangrijkste bevindingen en conclusies van het proefschrift.

A disszertáció a tejelő tehenek méhkontrakció-vizsgálatának témakörével foglalkozik az ellést követő néhány napban. A külön fejezetekben tárgyalt egyes vizsgálatokat úgy terveztük, hogy általuk választ kaphassunk az ellést közvetlenül követő időszakban a tejelő tehenek méhizomzatának működésével kapcsolatos bizonyos módszertani és biológiai kérdésekre.

Az *1. fejezet* általános bevezetést nyújt az ellést közvetlenül követő időszakban a méhkontrakciókkal kapcsolatos ismeretekbe szarvasmarhában. A disszertáció témakörének ismertetése után a méhben a borjú megszületését követően végbemenő változások rövid összefoglalása kerül sorra. Ezt követően, néhány bevezető gondolat megemlíti a méhösszehúzódásoknak a méhtartalom ellést követő eltávolításában játszott szerepét. A méhkontrakció-mérések lehetséges változatainak vázlatos összefoglalását az eltérő felvételi körülményekkel kapcsolatos jellemzők ismertetése követi. Az irodalmi adatok vizsgálati eredményeiből azt a következtetést lehet levonni, hogy bár a méh belső nyomásának mérése során számos tényező hatását figyelembe kell venni, az eljárás alkalmasnak tűnik a méhösszehúzódások mérésére. Ugyanakkor fontos megemlíteni, hogy olyan, a tehenek belső méhnyomásának mérésén és elemzésén alapuló, nem invazív eljárás, amely az ellést követő időszakban akár szarvasmarhatelepen is alkalmazható, a szakirodalomban nem ismeretes. Ám nemcsak megoldatlan módszertani kérdések jellemezik e tárgykört, hanem a szarvasmarhában az ellést követő időszak méhműködését jellemző szűkös irodalmi háttér is. Így hiányoznak azok a pontos, számszerű adatok is, amelyek nagyüzemekben tartott tehenek méhműködésének élettani változásait írják le, szabályos ellést követően. Az 1. fejezet ezt követő részében röviden ezek ismertetése történik. Ezután a méhkontrakciók gyógyszeres fokozásának rövid ismertetése következik, utalva a szerek hatásmechanizmusaira is az ellést követő időszakban, tehenekben. A bevezető fejezetet végül a disszertáció egyes vizsgálati céljainak fejezetenkénti áttekintése zárja.

A *2. fejezet* a méhaktivitás mérési technikájának fejlődését tárgyalja, és összefoglalja a felvételek, elemzések és értékelések során leggyakrabban alkalmazott módszereket. Ezt követi a belső méhnyomás mérésén alapuló méhkontrakció-változások időrendi ismertetése. E fejezet végén azon szakirodalmi adatok kerülnek áttekintésre, amelyekben vagy méh-elernyesztő hatású készítmények hatását tesztelték az ellés során, vagy olyan, a méhtevékenységet fokozó készítményekét az ellést követő időszakban, mint például az oxytocin, a prosztaglandin $F_{2\alpha}$ és származékaik.

Ez a fejezet ismerteti a méhkontraktilitás jellemzésére használatos különféle módszereket. Megállapítható, hogy nemcsak a tehenek ellést követő időszakában jellemzi a belső méhnyomást egy bizonyos variabilitás, hanem hasonló, részben egymásnak ellentmondó eredményekről számolnak be azok a vizsgálatok is, amelyekben a méhösszehúzódásokat gyógyszeresen befolyásolták.

A *3. fejezet* azt mutatja be, miként sikerült a vemhes méh felszínére sebészi úton előzőleg beültetett bipoláris elektródákkal végzett elektromiográfiás (EMG) mérések eredményeit két különböző, a méh belső nyomásának felvételén alapuló módszer (ún. nyitott végű katéter- és mikrotranszducer-rendszerek) eredményeivel összehasonlítani a tehenek ellést követő

időszakában. A vizsgált állatokban a vemhesség 274. napján az ellés megindítása prosztaglandin $F_{2\alpha}$-val történt, majd az ellést közvetlenül követő időszakban a fenti mérések egyidejűleg kerültek elvégzésre. Egy olyan, a belső méhnyomás mérésére szolgáló, a nyakcsatornán át bevezetett katéter rögzítésére kifejlesztett, megbízható, nem invazív eljárás (a *„virtuális csomó”*) kerül bemutatásra, amely az eszköz kézzel végzett, méhen belüli rögzítését a vizsgálati időpont kezdetére korlátozza, egyúttal eltávolítása sem igényel további, méhen belüli kézi segítséget. Ugyancsak kifejlesztésre kerültek olyan, 40 Hz-es mintavételi frekvencián alapuló adatfelvevő és –elemző szoftverek, amelyek képesek a felvett nyomásgörbék utólagos értékelésére és szűrésére is. A két EMG-jel összehasonlító elemzése ugyan egymáshoz nagy mértékben hasonló, de nem teljesen egyező eredményeket adott. Egymással szorosan összefüggő értékek adódtak mind a két különböző nyomásmérő rendszer, mind a belső méhnyomás és az elektromiográfia felvételeinek összehasonlítása kapcsán.

A vizsgálat eredményeként tehát egy, a gyógyszeres kezelésben nem részesült és kezelt tehenek ellést követő korai időszakában egyaránt alkalmazható, a belső méhnyomás mérésére akár telepi körülmények között is jól használható, viszonylag olcsó, egyszer használatos mérőegységgel működő, digitális eljárás került kifejlesztésre.

A *4. fejezet* egy olyan, nagyüzemben végzett vizsgálatról számol be, amelyben a digitális (4 Hz-es mintavételi frekvencia), nem invazív, nyitott végű katéter-rendszer sikeresen került alkalmazásra a méh belső nyomásának mérésére szabályosan ellett, egészséges, magzatburkukat elvetett, nem kezelt tehenekben. A méhösszehúzódások átlagos gyakorisága (FREQ), amplitúdója (AMP) és a görbe alatti területek átlagos nagysága (AUC) folyamatosan és szignifikánsan csökkent az ellést követő első 48 órában oly módon, hogy a csökkenés 12 és 24 óra között volt a legnagyobb mértékű, ugyanakkor ezek a paraméterek lényegesen különböztek egymástól az egyes tehenek esetében. A méh méhösszehúzódások jellege, és a vér Ca^{2+}-koncentrációja közötti kapcsolat nem jelzett szignifikáns összefüggést.

Az eredmények alapján az a következtetés vonható le, hogy a méh belső nyomásának mérésére telepi körülmények között használt módszer alkalmas szabályosan ellett tehenek ellést követő első két napjában a méhkontrakciók pontos vizsgálatára.

Az *5. fejezet* egy olyan vizsgálatot ismertet, amelynek célja annak megfigyelése és értékelése volt, milyen mértékben befolyásolja a méh kontraktilitását az 1. és 2. napon a szabályosan ellett tejhasznú tehenekben 14-16 órával az ellést követően végzett egyszeri oxytocin- (50 IU im.), vagy carbetocin- (0,35 mg im.) kezelés. A méh kontraktilitásának jellemzése a méh belső nyomásának digitális mérése alapján történt egy, a nyakcsatornán át behelyezett, nyitott végű katéter-rendszer segítségével.

A méhkontrakciók átlagos gyakorisága (FREQ) és az összesített görbe alatti területek nagysága (TAUC) rövid hatás tekintetében (a kezelést követő első 4 óra vizsgálata) mindkét kezelt csoportban szignifikáns hatást jelzett. Ezen paraméterek értékei a két kezelt csoportban a kezelést követő első órában szignifikánsan emelkedtek, a kiindulási értékek fölött maradtak a második, és azok szintjére csökkentek a harmadik órában, majd a kezelést követő 12. órára

a kontroll csoport értékére süllyedtek. A nyomásgörbék egyéb jellemzőit (AMP, DUR /görbék átlagos időtartama/ és AUC) a kezelések nem befolyásolták. Elhúzódó hatás tekintetében (a kezelést követő 12-36 óra közötti időszak vizsgálata) a kezelések egyetlen vizsgált paraméter esetében sem okoztak szignifikáns különbségeket. Az oxytocin és a carbetocin méh-kontraktilitást fokozó hatása, ha azokat az említett adagban, módon és időben alkalmaztuk, közel azonos volt. A kezelést követő 4. óra után további, a kezelésekkel összefüggő, a méh-kontrakciókra gyakorolt hatás nem volt várható, mert addigra a két kezelt csoportban a kontraktilitás mértéke visszaesett a fiziológiás sóoldattal kezelt kontroll csoport szintjére.

Mivel a két készítmény hasonló hatást váltott ki egészséges tehenek méhére az ellést követő korai időszakban, nem várható, hogy a carbetocin az oxytocinhoz képest kedvezőbb klinikai hatást gyakorolna a méh involúciójára ebben az időszakban.

A *6. fejezet* egy olyan vizsgálat eredményeit közli, amelyben az oxytocin hatását vizsgálatuk a perifériás vérplazma 15-ketodihydroprosztaglandin-$F_{2\alpha}$ (prosztaglandin $F_{2\alpha}$-metabolit) koncentrációjának alakulására az ellést követő időszakban. Ehhez 13-15 órával az ellést követően egyszeri intramuszkuláris, vagy intravénás oxytocin-kezelést (50 NE) alkalmaztunk. Az intramuszkuláris kezelés hatása nem tért el lényegesen a fiziológiás sóoldattal kezelt kontroll csoport értékeitől. Ugyanakkor az a tény, hogy az érpályába fecskendezett oxytocin mindkét kezelt tehénben a prosztaglandin (PG)-elválasztás mérhető emelkedését eredményezte, a köztük mért kis különbségek ellenére is azt bizonyítja, hogy az oxytocin PG-elválasztást serkentő hatása létezik.

Ezért feltételezhető, hogy az intramuszkuláris oxytocin-kezelés közvetett úton, a PG-elválasztás helyi fokozásával hozzájárulhat az oxytocin közvetlen méhtevékenységet fokozó hatásához az ellést követő korai időszakban, tehenekben. Egyúttal megemlítendő, hogy amennyiben az adott intramuszkuláris kezelés az ellést követő első napon történt, az elválasztott prosztaglandinok vérkeringésben is mérhető mennyisége látszólag nem volt elég nagy ahhoz, hogy az a perifériás vérplazmában is kimutatható legyen.

A *7. fejezet* az eredmények általános megvitatását tartalmazza, melyben a disszertáció egyes, különálló vizsgálatainak eredményei a jelen szakirodalmi ismeretek tükrében kerülnek megbeszélésre. Ezt a fejezetet a disszertáció főbb eredményeinek és következtetéseinek felsorolása zárja.

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Bedankt – Thank you – Köszönöm:

Csaba

Curriculum vitae

Árpád Csaba Bajcsy was born in Körmend, Hungary on June 25, 1963. After finishing secondary school in 1981 (Nagy Lajos Gimnázium, Szombathely), he started studying veterinary medicine the following year, at the University of Veterinary Science in Budapest. He received his diploma in 1987. From then until 1992, he worked at the University of Veterinary Science, as a research scientist at the Central Laboratory, where his main activity focused on the etiology, diagnosis and prevention of metabolic disorders in dairy cows kept on large-scale farms. Until 1993, he continued this work at the Department of Animal Hygiene. From there he transferred to the Department of Obstetrics and Reproduction and became more involved in teaching and clinical work. In 1994 he became a PhD-student of the University of Veterinary Science, Budapest obtaining a PhD-degree from his Alma Mater in 1999. The title of his PhD was: *Changes in ionized calcium concentrations with special regard to the peripartal period in cattle.* In the same year he started his second PhD-project under the Utrecht International PhD Programme at Utrecht University, the Netherlands. His topic focused on the physiology and clinical aspects relating to uterine contractility in postpartum cows. On January 1, 2000, his parent institution became the Faculty of Veterinary Science, Szent István University. With opening the new Large Animal Clinic at Üllő in 2001, he became a staff member of that unit, while maintaining his part time position at the Department of Obstetrics and Reproduction. In 2002, he was appointed to a senior scientist position. In 2003, he was awarded a 3-year Bolyai János Research Scholarship by the Hungarian Academy of Science. In 2004, he became a Diplomate of the European College of Bovine Health and Management. His collaborations with other veterinary institutions have produced several scientifically rewarding outcomes. At his home institution he is involved in giving lectures and practical demonstrations in Hungarian, English and German.

Instead of an

Epilogue

„Mi dolgunk a világon? küzdeni
Erőnk szerint a legnemesbekért.

…

Ez jó mulatság, férfi munka volt!”

Vörösmarty Mihály: Gondolatok a könyvtárban
(1844)