Parturition and subsequent uterine health and fertility in sows

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ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Veterinary Medicine of the University of Helsinki, for public examination in the Auditorium XII, University main building, on 15 September 2017, at 12 noon.
To Venla, Valtteri and Frida
ACKNOWLEDGEMENTS

I would first like to thank my thesis supervisors Olli Peltoniemi and Claudio Oliviero of the Department of Production Animal Medicine at the University of Helsinki as well as Nicoline Soede of the Department of Animal Sciences at the Wageningen University and Research for their unfailing support and assistance.

Prof. Peltoniemi was an excellent supervisor and consistently allowed this dissertation to be my own work, approved my own research ideas and steered me in the right direction whenever he thought I needed it. Furthermore, he also was an excellent life-coach and friend during the last years. His door was always open and he was always available whenever I ran into troubles or had questions.

Prof. Soede was also more than just an excellent supervisor. She welcomed me with great hospitality every time I visited her institution. Although going through a difficult time herself, she always found time and energy to enormously increase the scientific quality of my research through interesting and productive discussions. She has impressed me the most and I wish to be one day as outstanding in research as she is.

I am also very grateful to Dr. Oliviero for his supervision who also always greatly supported me with advice and solutions when facing practical issues during my trials.

I would also like to thank my two pre-examiners, Prof. Geert Opsomer of the Department of Obstetrics, Reproduction and Herd Health at the Ghent University, and Dr. Ylva Sjunnesson of the Department of Clinical Sciences, Division of Reproduction at the Swedish University of Agricultural Sciences for taking the time to read through this thesis.

A very special gratitude goes out to all my workmates of the pig research group at the University of Helsinki for help and discussion whenever it was needed as well as to all my co-authors of my scientific papers, Jinhyeon Yun, Mikael Niku, Mari Heinonen, Anna Valtros, Päivi Rajala-Schultz and Johannes Kauffold, for their valuable input and comments. It was fantastic to have the opportunity to work with them!

I also would like acknowledge my deepest gratitude to the farmers, funding agencies and sponsors. Without them, none of the studies would have been possible. The farmers Jari and Veera Ollikkala and Elina and Mikko Yrjövuori were always welcoming me on their pig farms for conducting my research. The Finnish Ministry of Agriculture (2017/312/2011), Vetcare Oy, Atria Oy, Figen Oy and Hankkija Oy provided me with the major funding for the studies. The Finish Veterinary Foundation, The Finnish Foundation of Veterinary Research and the Doctoral Programme in Clinical Veterinary Medicine of the University of Helsinki provided me with additional research and travel grants. Also I would like to thank Agfa HealthCare Finland Oy for providing me with a PACS system.

Finally, and most importantly, I must express my very profound gratitude to my parents, parents-in-low and the rest of my family for their support throughout my years of study and through the process of researching and writing this thesis. Especially, I would like to deeply thank Jenni, my beloved wife, and Venla, Valtteri and Frida, my beloved kids. They have been my eternal cheerleaders and their support, encouragement, quiet patience, smiles and unwavering love were undeniably the bedrock upon which this thesis has been built.
This thesis is based on the following publications:


The publications are referred to in the text by their roman numerals.
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Abstract
There has been a steady increase in litter size, and consequently farrowing duration, in modern pig production during recent decades. At the same time, annual removal rates due to reproductive failure have increased and an association with litter size demonstrated. Underlying physiological mechanisms for the associations between these phenomena are not well established. It is plausible that the increase in farrowing duration is causally linked with the increase in litter size because it takes longer to farrow a larger litter. Therefore, it is possible that prolonged farrowing negatively affects subsequent reproductive performance of sows.

Postpartum dysgalactia syndrome (PDS), the major puerperal disease complex of sows, can cause subfertility, but it is not clear that this is linked with prolonged farrowing. However, PDS and prolonged farrowing represent similar risk factors regarding increased backfat, constipation, low dietary fiber, and restricted movement in farrowing crates. In some studies there was evidence of a connection between prolonged farrowing and increased incidence of general clinical symptoms such as fever and anorexia. Therefore, it is feasible that prolonged farrowing increases the risk for PDS, which in turn increases the risk of subsequent subfertility.

In order to understand better these tentative relationships it makes sense to look at the different symptoms individually, it being most likely that prolonged parturition in particular affects the incidence of metritis. Puerperal metritis is an acute inflammation and infection of the uterus that occurs during the first week after parturition and is characterized by an enlarged uterus that results from fluid accumulation. In other species, including the cow, a long and difficult parturition increases the risk of puerperal metritis, which in turn delays uterine involution and subsequently reduces fertility by disturbing postpartum ovarian follicular growth and ovulation. Furthermore, long and difficult parturitions can cause retained placenta, which is of major consequence in the etiology of postpartum metritis.

We hypothesized that prolonged farrowing decreases subsequent fertility, that is, pregnancy rate. Furthermore, we hypothesized that prolonged farrowing causes retention of placentae and metritis, delays uterine involution and perturbs follicular growth after weaning. We also hypothesized that sows that undergo prolonged farrowing release less oxytocin at subsequent estrus in response to boar stimulation. It is also known that prolonged parturition is linked with reduced oxytocin concentrations. We therefore speculated that this is the case also at subsequent breeding. At that time, oxytocin is needed for sperm transport to the site of fertilization and formation of the sperm reservoirs and thus, impairment of transport may have a negative effect on the establishment of pregnancy.

Furthermore, corpus luteum function is important for establishment and maintenance of pregnancy. The primary function of the corpus luteum is secretion of progesterone, the pregnancy hormone, and this function depends on other hormones active before embryonic attachment and maternal recognition. Whether corpus luteum function is compromised in sows with prolonged farrowing was not investigated in this study, but validation and testing of a transvaginal ultrasound-guided biopsy method for luteal tissue was done. This represents an easy and reliable tool for future investigations of corpus luteum function.

In the first part of the study we determined the farrowing duration (time between expulsion of first and last piglet) of sows and explored whether there is a negative effect on subsequent post-weaning pregnancy rate (n = 148). In the following part, we explored the relationship between farrowing duration and placenta expulsion (n = 142), and postpartum uterine size and intrauterine fluid (n = 99). For that, placenta expulsion was
observed until 24 h after birth of the last piglet and ultrasonography was used during the first week postpartum to examine the uteri of the sows. Uterine size and intrauterine fluid were used as indicators for initial uterine involution and puerperal metritis. Furthermore, we determined the farrowing duration of sows (n = 30) and monitored the subsequent follicular development using transrectal ultrasound twice a day between the third day after weaning and ovulation. At estrus, blood samples were collected in the presence of a boar in order to determine the endogenous oxytocin concentrations and release patterns.

The results show that sows with a prolonged parturition (> 300 min) were 3.4 (odds; \( P = 0.027 \)) more likely not to be pregnant. Farrowing duration was highly associated with retained placenta (369 ± 202 min; \( P = 0.021 \) and \( P = 0.004 \)). Otherwise, farrowing duration conformed to a quadratic relationship with the number of expelled placental parts (\( P = 0.001 \)), placental expulsion duration (time between expulsion of first and last placental part; \( P = 0.002 \)) and time between expulsion of last piglet and last placental part (\( P = 0.024 \)). On the other hand, use of oxytocin increased number of expelled placental parts (LSmeans ± SD, 3.8 ± 0.2 vs. 2.9 ± 0.3, \( P = 0.035 \)), decreased the placental expulsion duration (172 ± 44 vs. 328 ± 26 min, \( P = 0.011 \)) and time between expulsion of last piglet and last placental part (148 ± 48 vs. 300 ± 24 min, \( P = 0.025 \)). Furthermore, prolonged farrowing (\( \beta \pm SE. \ Wald \chi^2 \), Odds: 2.0 ± 0.5, 13.1, 7.6; \( P = 0.001 \)), obstetrical intervention (1.5 ± 0.7, 4.4, 4.3; \( P = 0.036 \)) and two or more stillborn pigs (1.4 ± 0.7, 3.8, 3.9; \( P = 0.052 \)) increased the risk of having enlarged uterine size whereas oxytocin administration (- 1.5 ± 0.7, 4.7, 0.2, \( P = 0.040 \)) decreased the risk. Two or more stillborn piglets (2.6 ± 0.9, 8.7, 13.7; \( P = 0.003 \)), obstetrical intervention (1.8 ± 0.8, 5.0, 6.0; \( P = 0.025 \)), prolonged farrowing (1.7 ± 0.8, 4.3, 5.7; \( P = 0.039 \)) and impaired placenta expulsion (3.3 ± 1.7, 4.0, 26.9; \( P = 0.044 \)) were associated with intrauterine fluid. After weaning, OT concentrations were higher in sows with prolonged farrowing than in sows with shortened farrowing (LSmeans ± SD, 28.0 ± 7.7 vs. 20.6 ± 7.7, \( P < 0.01 \)). OT concentrations correlated with diameters of the follicles measured 5 d after weaning and when the follicles reached their maximum size after weaning (\( r = 0.61 \), \( P < 0.01 \) and \( r = 0.57 \), \( P < 0.01 \), respectively).

The results show that sows with prolonged farrowing can show subsequent subfertility, which may be linked with compromised uterine health. Sows with prolonged farrowing are at higher risk of impaired placental expulsion, puerperal metritis and impaired uterine involution. Using exogenous oxytocin after parturition may be useful in sows with prolonged farrowing in order to lower the risk for retained placenta and subsequent metritis. Obstetrical intervention should be careful and done under antiseptic conditions. Proper parturition management should be used in order to prevent piglets being stillborn. Against expectations, sows with prolonged farrowing duration had higher oxytocin concentrations and larger follicles at subsequent estrus. This merits further investigation.

In addition, we adopted and tested a transvaginal ultrasound-guided biopsy method for luteal tissue, which can be used to study corpus luteum function. No effects were observed on the cyclicity and morphology of the reproductive organs of the sows: not on corpus luteum function, pregnancy rate, gestation length or subsequent litter size. A biopsy was obtained for 50% of all attempts. Sows from which at least one biopsy was obtained were older (parity 5.0 ± 2.8 vs. 2.2 ± 0.4; mean ± SD), heavier (290 ± 26 vs. 244 ± 27 kg) and had more backfat (11.4 ± 2.7 vs. 6.4 ± 2.5 mm; \( P < 0.05 \) for all) compared with sows from which no samples could be obtained.
Introduction and literature review
1. Introduction

There has been a steady increase in litter size and consequently farrowing duration in modern pig production over recent decades (Rutherford et al., 2013; Edwards and Baxter, 2015; Tab 1). Both trends are probably causally linked, many authors having reported that it takes a longer time to farrow larger litters than it does smaller litters (Fahmy and Friend, 1981; Van Dijk et al., 2005; van Rens and van der Lende, 2004). Concurrently, the annual removal rate of gilts and sows has increased and an association with litter size has been established (Andersson et al., 2016; Fig. 1); with the most common reason for removal being conception failure (Lucia et al., 2000). Nevertheless, it has not been determined whether farrowing duration is a causal factor in this association and would have a negative impact on subsequent reproductive health and fertility.

Table 1. Data from Danish Pig Research Center (PRC) annual reports (Rutherford et al., 2013) presenting the increase in litter size between 1996 and 2011.
In other species, e.g. cow (Parkinson, 2009), mare (LeBlanc, 2008b; Paccamonti and Pycock, 2009) and bitch (Fellows et al., 2012), prolonged and difficult parturition is known to increase the risk of subsequent subfertility due to compromised uterine function postpartum. Uterine function can be compromised because of inflammation (Gilbert et al., 1998), infection (Savc et al., 2016b) or incomplete involution (Savc et al., 2016a; Mee et al., 2009). The most severe impairment of uterine function is when postpartum metritis occurs. Postpartum metritis is an acute infection of the uterus occurring during the first week after parturition. Many predisposing factors have been identified and summarized as factors at parturition which contribute to 1) prolonged dilatation of the cervix – prolonged duration of parturition and delayed uterine involution; 2) bacterial invasion of the uterus – dystocia and obstetrical intervention; 3) intra-uterine bacterial growth – retention of fetuses or placentae; and 4) delayed uterine clearance – decreased uterine contractions (Sheldon et al., 2006; Troedsson & Liu, 1990; Linde-Fosberg & Eneroth, 2005; LeBland, 2008).

The majority of postpartum uterine infections begin with bacterial contamination of the uterine lumen. In the case of a difficult parturition, trauma to the uterus and bacterial contamination are increased and a uterine contamination can become a uterine infection. The presence of pathogenic bacteria in the uterus causes further inflammation and lesions of the endometrium and delays uterine involution. Subsequently, as in cattle, uterine bacterial infection, bacterial products and/or the associated inflammation suppress postpartum pituitary LH secretion and therefore perturb postpartum ovarian follicular growth and ovulation (Peter et al., 1988, 1989; Sheldon et al., 2002; Opsomer et al., 2000).
Savc (2016b) used vaginal mucus scoring and ultrasonographic assessment of uterine content at first pre-breeding examination after parturition as predictors of future reproductive performance in dairy cattle. Conception rate to first service, breeding window, and positive pregnancy status were significantly worse for cows with a uterine score of 3 to 4 (≥ 0.2 cm of mixed echogenicity fluid with hyperechoic particles in the lumen without infolding of the endometrium) and a positive vaginal mucus score (pus) compared with cows with a uterine score of 1 to 2 (small volume ≤ 0.2 cm or moderate volume ≥ 0.2 cm ≤ 0.5 cm of anechoic fluid in the lumen with infolding endometrium) and a negative vaginal mucus score (no or clear mucus, or mucus with specks of purulent material) (Fig. 2).

![Figure 2. Kaplan–Meier survival curve for all 493 cows which shows days after earliest service date (ESD) until the cow got pregnant of all animals with the different uterine scores (UTSs). UTS scores of two and higher were defined as uteri with abnormal content and these animals had a decreased likelihood of getting in calf. Pregnancy rate hazard for cows with UTS0, 1, 2, 3, and 4 were 1, 0.84, 0.53, 0.78, 0.28, respectively (P < 0.001; Šavc et al., 2016b).](image)

Thus, the general aim of this thesis was to explore whether prolonged parturition decreases subsequent pregnancy rates and whether similar associations between parturition and puerperal disorders such as placental retention, metritis, and delayed uterine involution, as describes above for the cow and other species, exist also in sows. We speculated that modern hyperprolific sows with increased farrowing duration have higher risks of experiencing puerperal disorders as well as impaired resumption of ovarian cyclical activity and fertility after subsequent weaning.
In the first parts of this literature review, normal parturition, normal puerperium and normal return of ovarian cyclical activity after weaning will be described. An outline is presented in Figure 3. In the last part of this literature review, the focus will be about diseases of the puerperium and possible associations with previous parturition and subsequent ovarian cyclical activity.

<table>
<thead>
<tr>
<th>Event</th>
<th>Duration</th>
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<tbody>
<tr>
<td>Previous pregnancy</td>
<td>~ 115 days</td>
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<tr>
<td>Parturition</td>
<td></td>
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<tr>
<td>1. Stage: Dilatation of the cervix</td>
<td>&lt; 12 hours</td>
</tr>
<tr>
<td>2. Stage: Expulsion of the piglets</td>
<td>&lt; 5 hours</td>
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<tr>
<td>3. Stage: Expulsion of the placenta</td>
<td>&lt; 4 hours</td>
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<tr>
<td>Puerperium</td>
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<tr>
<td>Puerperal vulvar discharge</td>
<td>&lt; 2 days</td>
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<tr>
<td>Elimination of bacterial contamination</td>
<td>&gt; 28 days</td>
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<td>Uterine involution</td>
<td>&lt; 21 days</td>
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<tr>
<td>Restoration of the endometrium</td>
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<td>Resumption of ovarian activity</td>
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<td>Lactation</td>
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<td>Follicular recruitment and growth</td>
<td>&lt; 7 days</td>
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<td>Ovulation and Breeding</td>
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<td>Return of cyclical activity</td>
<td></td>
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<tr>
<td>Establishment of subsequent pregnancy</td>
<td>~ 115 days</td>
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Figure 3. Overview of reproductive events between two subsequent pregnancies and their normal durations.
2. Parturition

Sufficient corpus luteum function is important for maintenance of pregnancy. The primary function of the corpus luteum is to release at sufficient concentrations progesterone. Progesterone is released in pulses which is triggered by pulsatile LH release from the pituitary gland (Ellendorf et al., 1979). Progesterone concentrations become independent of LH and decrease about one to two days before parturition, following activation of the pituitary adrenal axis of the fetuses and subsequent increase in cortisol levels in the fetal plasma (Randall, 1983). These events are mainly mediated by intrauterine production of prostaglandin F2α (Whittle et al., 2000). The increased cortisol levels in the fetal plasma enhance the expression of prostaglandin synthetase within the fetal trophoblast cells of the placenta. As a result, the concentration of prostaglandin E2 increases, which stimulates the placential conversion of pregnenolone to estrogen in the placenta. The change in the steroid balance (decrease of progesterone and increase in estrogen) enhances the expression of prostaglandin synthetase within the endometrium, leading to increased prostaglandin F2α within the uterine wall (Fig. 4). An overview of the hormonal changes around parturition is presented in Figure 5.

Figure 4. Endocrine events associated with onset of parturition and increased uterine contractility (Taverne and Noakes, 2009).
Another result of the change in steroid balance is production of oxytocin and oxytocin receptors. Oxytocin, prostaglandins and estrogens have local positive effects on myometrial contractility and dilatation of the cervix, and general effects on nest-building behavior. Within 24 h before the birth of the first piglet, sows isolate themselves from the rest of the group and have an innate need to build a nest where the litter is to be born (Jensen, 1986; Jensen & Redbo, 1987). This nest-building behavior is characterized by collecting branches, leaves or grass and by rooting and pawing activity (Jensen & Redbo, 1987). The behavior is expressed at highest activity 3–8 h before the birth of the first piglet and gradually ceases prior to the birth of the first piglet (Hartsock & Barczewski, 1997; Westin, 2014).

The first phase of farrowing starts about 12 h before expulsion of the first piglet and is marked by cervical dilatation and increased myometrical activity. The second phase of farrowing involves abdominal straining of the sow in lateral recumbency and expulsion of the piglets. This stage can last for 4 to 6 h, with piglets born at 20 min intervals on average. During the third stage of farrowing the placentae are expelled. This expulsion may already start before the end of the second stage and takes from 20 min up to 12 h in sows, but is usually completed within 4 h (Jones, 1966). A study in gilts reported that placenta expulsion may take from 15 min up to 8 h, but is, on average, completed within 2.5 h (van Rens and van der Lende, 2004).
Studies on factors affecting placental expulsion are scarce. To date, only the study in gilts investigated factors affecting onset, defined as time of expulsion of the first placental part relative to the last piglet, and duration of placenta expulsion duration, defined as time duration between expulsion of first and last placental part (van Rens and van der Lende, 2004). A placental part was defined as an individual placenta or two or more intergrown placentae. The number of expelled placental parts was smaller than the number of piglets, with a curvilinear relationship between relative placental parts and the number of piglets (Fig. 6). Farrowing duration was the only factor affecting the onset and duration of placenta expulsion. An increase in farrowing duration was significantly related to an increase in duration of placental expulsion (Fig. 7a) and to a decrease in the onset of placenta expulsion (Fig. 7b). On average, the onset of placenta expulsion occurred simultaneously with the expulsion of the last piglet, but could occur as early as 3 h before and as late as 2 h after the last piglet. In 40% of the gilts placenta expulsion started before, in 11% of the gilts simultaneously and in 49% after the birth of the last piglet. Litter size, gestation length, percentage of males in the litter, average and total litter weight, bodyweight and genotype of the sow had no effect on placental expulsion. Moreover, reports on incidence rates and causes of retained placentas or remnants of the placentas are also scarce. It is considered rare and a retained fetus as the only cause (Jones, 1966).

One possible explanation for the association between farrowing and placental expulsion duration is oxytocin. Oxytocin is one of the most important modulators for uterine contractions. Therefore, sufficient concentrations during parturition are needed for rapid and successful expulsion of piglets and placentae. The baseline concentration of oxytocin and the frequency of its pulsatile release start to increase about 6 h before parturition, peak 2-4 h after start of parturition and decrease thereafter (Castrén et al., 1993; Oliviero et al., 2008). Furthermore, there are pulsatile releases between births of the piglets (Oliviero et al., 2008). The farrowing duration was shown to correlate negatively with peripheral oxytocin levels. Sows with long farrowings had lower average basal and peak levels as well as post-expulsion pulse concentration of oxytocin during farrowing compared with sows with short farrowings (Castrén et al., 1993; Oliviero et al., 2008).

In domesticated species other than the pig, dystocia and subsequent reduction in uterine contractions are known to cause retained placentae. Retained placentae is of major importance in the etiology of postpartum metritis, which is considered to be one of the most significant causes of subsequent infertility (LeBlanc, 2008b; Parksinson, 2009; Fellows et al., 2012). In the pig, studies on consequences of retained placentae are lacking.

Therefore, the second aim of the study was to investigate whether an increase in farrowing duration is related to an increase in duration of placental expulsion also in sows as observed in gilts (van Rens and van der Lende, 2004). Furthermore, we speculated that in sows with prolonged farrowing placental expulsion can be impaired and therefore retained placentae occurs.
Figure 6. Relation of number of placental parts found per gilt (expressed as percentage of total number of piglets born (TNB) in relation to TNB; van Rens and van der Lende, 2004).

Figure 7. Relation of placenta expulsion duration (a) and time interval between expulsion of last piglet and first placental part (b) to farrowing duration (X-axes log scale; Y-axis in (a) log scale; van Rens and van der Lende, 2004).
3. Puerperium

The puerperium is that period after the completion of parturition, often including the third stage of parturition, when the genital tract is returning to its normal non-pregnant state (Noakes, 2009). It is important that the puerperium proceeds normally, since it is practice under most conventional production systems to wean piglets and breed sows fairly soon after puerperium is completed (Fig. 4). Therefore, any extension of the puerperium may have negative effects on subsequent fertility as it has, e.g., in cattle (Šavc et al., 2016b).

The most important events of the puerperium are elimination of bacterial contamination, uterine involution and restoration of the endometrium, and resumption of ovarian function (Noakes, 2009; Fig. 4). Initial uterine involution is rapid during the first week postpartum when the uterine weight is reduced by 65% (Palmer et al., 1965). After that, uterine involution is continuous and progresses uniformly, and is completed during the third week postpartum (Palmer et al., 1965; Kudlác and Groch, 1979; Busch, 2007; Fig. 8 and 9). Compared with the uterus, involution of the cervix is very rapid and completed within one week (Busch, 2007; Kudlác and Groch, 1979). As a result, resorption of uterine content needs to occur. During the initial period, uterine weight loss is mainly due to atrophy and resorption of intraluminal fluid, whereas after the initial period, uterine weight loss is mainly due to changes in the myometrium, notably a reduction in cell numbers, cell size and amounts of connective tissue (reorganization) (Busch, 2007).

Figure 8. Uterine weight loss after parturition (Kudlác and Groch, 1979)
At the end of uterine involution, during the third week postpartum, many authors reported an increase in the uterine weight of about 5% (Palmer et al., 1965; Kudlac and Groch, 1979; Busch, 2007). The same observation was made regarding the length of the uterine horns (Busch, 2007). Busch (2007) interpreted the increase in length and weight of the uterus during week three postpartum as proliferation due to increased estrogen production by growing follicles and as a sign of completed uterine involution. Follicular development during early lactation is suppressed and characterized by a large population of small follicles (< 3 mm) and a small population of medium-sized follicles (3-4 mm) (Britt, 1985). During the second week postpartum the number of small follicles increases and during the third week the overall number of follicles and the number of medium-sized follicles increases (Busch, 2007; Fig. 10). During this time also the weight of the ovaries increases concomitantly with the increase in uterine weight and length (Busch, 2007), which is due to an increase in the number of follicles and an increase of the diameter of the follicular pool (Lucy et al., 2001; Busch et al., 2007).
This shift in the follicular pool occurs not only alongside completion of uterine involution but also alongside restoration of LH-pulsatility. After parturition, the ovarian axis is inhibited. Weaning sows immediately after parturition, or at less than 3 weeks postpartum, would result in a higher incidence of anestrus and ovulation failure (Soede et al., 2011). Sows that fail to ovulate either show follicular regression with a short/intermittent estrus or develop ovarian cysts. If sows are weaned at more than 3 weeks, WEI seems to be normal and about 98% of sows show estrus, and 98% of which ovulate (Knox and Rodriguez Zas, 2001).
Anestrus and cystic ovaries in sows weaned at less than 3 weeks can be due to the lack of a preovulatory LH-surge. In cyclic gilts, the occurrence of cystic follicles has been related to a lack of the preovulatory LH-surge (Reviewed by Ryan and Reaside, 1991). The lack of the LH-surge is believed to be due to high levels of corticosteroids (Ryan and Raeside, 1991) or an impaired responsiveness to a positive feedback of estradiol-17β produced by the follicles at the hypothalamic-pituitary level (Soede et al., 2011). The impaired responsiveness may be related to depleted LH stores after farrowing that are progressively restored during lactation (Bevers et al., 1981). On the other hand, mean concentrations of circulating LH are high during the first two to three days after parturition and only decrease thereafter (Tokach et al., 1992; De Rensis et al., 1993; Fig. 11). These concentrations and the number of LH pulses remain low during day four to fourteen and gradually increase subsequently (Shaw and Foxcroft, 1985; De Rensis et al., 1993; Fig 11). In addition to this restoration of LH-pulsatility, a pre-ovulatory LH surge will not occur before weaning in most of the sows because of suckling and piglet proximity (De Rensis et al., 1993).

If and how uterine involution affects resumption of ovarian function and restoration of LH concentration and pulsatility is not known. The restoration of LH-pulsatility and shift in the follicular pool occur around the same time as when uterine involution ends; during the third week postpartum. Therefore, uterine involution could also play a role. On the other hand, no data are available on the interaction between uterine involution and ovarian activity in pigs (Quesnel & Prunier, 1995).

Furthermore, studies on factors affecting uterine involution are lacking. We speculated that uterine clearance after prolonged parturition may be impaired; it was shown that prolonged parturition is associated with reduced oxytocin concentration (Castren et al., 1993; Oliviero et al., 2008), a hormone considered to be important in uterine contractions and therefore clearance. Thus, it is plausible that sows with prolonged parturition may have more uterine content to absorb because of reduced uterine clearance postpartum and, therefore, this may have a negative effect on the duration of uterine involution.
4. Return of cyclical activity

After weaning, pulsatile LH release shifts from a lesser frequency of a greater amplitude pattern to a greater frequency of a lesser amplitude pattern (Fig. 12). This shift is important for selection of antral follicles and subsequent follicular development and ovulation (Kemp et al., 1998; Knox et al., 2005; Soede et al., 2011). Once LH has stimulated follicular development, these follicles start to produce 17β-oestradiol, which triggers the LH surge (Fig. 13), causing ovulation of the follicles. Ovulation takes place at 30 ± 3h (mean ± SD) after the peak of the LH surge (Soede et al., 1994).

Few factors affecting the LH surge have been described. For instance, Turner and Tilbrook (2005) concluded that prolonged increase in cortisol levels (sustained stress) can result in a delayed LH surge or even prohibit it through reduction in GnRH output. Furthermore, lactation weight loss and feed intake during lactation have a major effect on follicular growth and LH-release after weaning as reviewed by Soede and Kemp (2015). Sows in bad metabolic state due to high milk production or low feed intake have a high degree of inhibition of LH secretion before weaning and therefore decreased LH concentration and pulsatility after weaning (reviewed by Quesnel and Prunier, 1995).

Also lactation length is affecting the return of cyclical activity after weaning (Soede and Kemp, 2015). A short lactation length of less than 21 days can cause anestrus, ovulation failure or follicular cysts, as discussed in the last chapter. On the other hand, Kirkwood et al. (1984) showed that sows weaned at day 35 of lactation had a higher pre-ovulatory LH surge than sows weaned at 10 d of lactation.

Figure 12. Pulsatile LH-patterns on the day before weaning (A) and the day of weaning (B) in a primiparous sow. Weaning took place at day 24 of lactation (Soede et al., 2011).
At estrus, increased myometrial activity is needed for sperm transport from the site of deposition, the cervix and caudal part of the uterine body, towards the sperm reservoir, located at the utero-tubal junction and the caudal part of the oviduct. Myometrial contractions start to increase in frequency and amplitude from 15 to 22/h and from 20 to 40 mmHg at the end of the proestrus, reach their maximum during estrus, and decrease thereafter (Langendijk et al., 2002). Myometrial activity is, for example, in landrace sows, constant throughout the estrus (Hoang-Vu, 1987), but sow-dependent variation has been demonstrated and is positively correlated with tissue and plasma concentrations of certain hormones, such as estradiol and oxytocin, and sensory-related stimulus, including presence of the boar (Langendijk et al., 2005). The presence of a boar increases oxytocin release as well as uterine activity, with a causal link between both (Langendijk et al., 2002 and 2003, Madej et al., 2005). Langendijk et al. (2003) assessed the effects of exogenous oxytocin on uterine activity and compared three different sexual stimuli in their effects on endogenous oxytocin release and uterine activity. Exogenous oxytocin increased frequency, amplitude or both of uterine contractions. Overall, frequency of contractions increased by 5/h and amplitude of contractions by 32 ± 5.8 mmHg after administration of exogenous oxytocin. Furthermore, boar presence increased the frequency of contractions by 3/h and the amplitude of contractions by 10 mmHg, especially when the frequency of spontaneous contractions before boar introduction was below 26/h (Fig. 14).
This increase in uterine contractility was related to the release of endogenous oxytocin. Boar introduction in combination with application of manual pressure on the back of the sow caused a clear, immediate, surge-like release of oxytocin, with mean peak concentrations of 80 pg/ml (Fig. 15). Oxytocin concentrations rose above basal level within 1 min and reached maximum concentrations between 1 and 3 min after introduction of the boar. Oxytocin levels returned to basal levels 20-30 min later. This observation was confirmed by the study of Madej et al. (2005), who showed that boar presence and high human stimulation (reverse riding test and manipulation of costae-, scapulae- and inguinal area) increased oxytocin within 10 min after initiation of stimulation compared with only application of manual pressure on the back of the sow. Furthermore, Langendijk et al. (2002) showed that also the intrauterine infusion of estrogens increased frequency of uterine contractions.
Therefore, speculated that sows with prolonged farrowing have perturbed follicular growth after subsequent weaning and oxytocin release during boar stimulation at subsequent estrus. As discussed, oxytocin release and subsequent uterine contractions are not only needed at parturition but also during breeding at estrus when they are important for ovulation and sperm transportation towards the sperm reservoirs. Furthermore, oxytocin release during the weaning-to-estrus interval is important for follicular growth. We speculated that if prolonged farrowing can be associated with reduced oxytocin concentrations then also with impaired follicular growth after subsequent weaning and reduced oxytocin release during boar stimulation at subsequent estrus.
5. Puerperal diseases

One of the most important puerperal diseases in sows is postpartum dysgalactia syndrome (PDS), recently reviewed by Maes et al. (2010). PDS is a multifactorial disease and characterized by insufficient colostrum and milk production during the first days after farrowing. Martineau et al. (1992) summarized a list of symptoms for the herd, piglets and sow. Local symptoms for the sow can include mastitis and/or vaginal discharge due to metritis and general symptoms can include fever and/or anorexia.

PDS is mediated by different pathways, including endotoxin production by bacteria in the gut, mammary gland and/or uterus (Martineau et al., 1992). In a study by Bostedt (1998), 24.4% of gilts with feverish puerperal illness exclusively had signs of mastitis, in 29.5% there was a combination of mastitis and inflammatory affection of the genital tract and in 46.1% a solitary infection of the reproductive tract. E. coli, Staphylococcus spp. and Streptococcus spp. were the most predominant bacteria isolated from the genital tract. The current hypothesis is that interactions between endotoxins produced by Gram-negative bacteria in the gut and alterations of the immune and endocrine functions play a central role in the development of PDS (Maes et al., 2010). In dairy cows, it was shown that those cows suffering significant immunosuppression around parturition were both predisposed to uterine disease and less able to clear the infections on their own (LeBlanc, 2008a). The impaired immune system also puts the animal at increased risk of developing retained fetal membranes and suppression of appetite, all with repercussions on subsequent health and reproductive performance (Dobson et al., 2008).

There are studies on sows showing that prolonged parturition can be linked with a delayed decline in progesterone (Oliviero et al., 2008). Progesterone is known to impair not only uterine contractility but also the immune response. Furthermore, a study by Tummaruk et al. (2013) showed that farrowing duration can affect the incidence of postparturient fever and reduced appetite. The percentage of sows with fever during the first 24 h postpartum increased from 40 to 100% when the farrowing duration increased from <2 to >4 h (Fig. 16). On day 3 postpartum, the percentage of sows with reduced appetite and a farrowing duration of 4-8 h was higher than for those with a farrowing duration of 2-3 h (Fig. 17). A study by Bostedt et al. (1998) examined gilts suffering from feverish puerperal illness. Also in this study, the farrowing duration had a significant effect. 85.9% of gilts with consequent puerperal illness had farrowing durations of longer than 6 h, whereas 78.8% of control gilts of the same age finished parturition in <3 h. Other significant factors affecting the incidence of feverish puerperal illness were frequency of obstetrical intervention and stillbirth rate. The frequency of obstetrical intervention measured in the group of patients was 27% and only 9.5% in the group of control sows. In addition to puerperal fever, 75% of the gilts were anorexic and 66% had abnormal fecal consistency as general symptoms.
Figure 16. Percentage of sows with fever (rectal temperature ≥39.0 °C) on the day of parturition in relation to farrowing duration. Different letters (a-c) differ significantly (Tummaruk et al., 2013).

Figure 17 4. Percentage of reduced appetite (daily food intake relative to provided food) sows at Days 1, 2, and 3 postpartum by farrowing duration (Tummaruk et al., 2013).
Furthermore, there is evidence that prolonged parturition and PDS are associated with similar risk factors. Risk factors for PDS were reviewed by Maes et al. (2010), and are constipation, ad libitum feeding, high body condition, restricted movement, high ambient temperature and heat stress, rapid changes in the housing only a few days before parturition, farrowing induction and no farrowing supervision. Risk factors for prolonged farrowing were reviewed by Peltoniemi and Oliviero (2015) and include high body condition, restricted movement, and constipation.

Subsequently, PDS has been associated with reduced subsequent fertility (Hoy, 2006). Sows with PDS had a higher rate of anestrus and late onset of estrus, a higher return and mortality rate, more abortions and smaller litters at subsequent farrowing (Hoy, 2006). An overview of the current knowledge about associations between prolonged farrowing, puerperal disease and subsequent fertility is presented in Figure 18.
Figure 18. Associations between prolonged farrowing, puerperal disease and subsequent fertility
6. Corpus luteum function and biopsy of luteal tissue

The primary function of the corpus luteum is the production and secretion of progesterone. Therefore, corpus luteum function is important for establishment and maintenance of the pregnancy. The amount of progesterone is related to total luteal mass ($r = 0.26-0.45$; Athorn et al., 2012) and further depends on the blood flow and the capacity of the luteal tissue to synthesize progesterone (Niswender et al., 2000). This capacity depends on several hormones, such as LH (Peltoniemi et al., 1995; Tast et al., 2000; Virolainen et al., 2003), growth factors (Miller et al., 2003), enzymes (Bao and Garverick, 1998), and pathways (Niswender et al., 2000), as well as management and environmental factors, such as pre-mating nutrition (Wientjes et al., 2011), post-mating nutrition (Athorn et al., 2012), season (Langendijk and Peltoniemi et al., 2013), and lactation weight loss (Hoving et al., 2012).

The maximum size of the corpora lutea are established at day 8-9 of the pregnancy and maintained autonomous until day 10-12 (Fig. 19). Meduri et al. (1996) showed a decrease in density of LH receptors in luteal cells, which increased again 6 d after ovulation. This finding is indicative of an LH-independent autonomous time period during early corpus luteum development. After that, maintenance of corpus luteum function seems to be dependent on LH (Fig. 19; Peltoniemi et al., 1995; Tast et al., 2000; Virolainen et al., 2004). Chronic treatment with a GnRH agonist from days 14 to 21 of pregnancy eliminated LH secretion and resulted in a decrease in progesterone secretion and loss of pregnancy (Peltoniemi et al., 1995).

![Figure 19. Conceptual diagram illustrating the dynamics in luteal mass (cross sectional area of corpora lutea, closed symbols) and systemic progesterone (open symbols) during LH independent and LH dependent stage of the luteal phase in pregnant sows (Langendijk, 2015)]
The LH release pattern during the luteal phase is similar to the release pattern of a lactating sow, thus characterized by a lesser frequency of greater amplitude LH pulses. Furthermore, it seems that also progesterone release by corpora lutea occurs in a pulsatile pattern related to preceding LH pulses (Fig. 20).

As discussed earlier, in other species like the cow, difficult calving can subsequently interrupt LH release and therefore negatively interfere with ovulation and establishment of pregnancy. Considering that LH seems to play an important role in maintaining luteal function in sows, it should be possible to study corpus luteum function in sows with prolonged parturition. Besides blood sampling for LH, luteal tissue collection for gene expression analysis of LH receptors, for example, may be an interesting approach to study corpus luteum function. In order to do so, it would be useful to have a practical and minimally invasive method available that allows repeated sampling of luteal tissue over a period of time. Until now, luteal tissue could be sampled only once, for example after euthanasia (Conley and Ford, 1989), or with labor-intensive and invasive methods, such as ovariectomy under general anesthesia (Forni et al., 2003; Ribeiro et al., 2007). In other species, for instance cows (Kot et al., 1999) and horses (Beg et al., 2005), an alternative method, termed transvaginal ultrasound-guided biopsy, is used. This method requires only local anesthesia (Tsai et al., 2001), if any, and can be performed by one to two persons within a few minutes (Beg et al., 2005).

Therefore, a secondary aim of this study was to develop and test a similar ultrasound-guided biopsy method as used in other species such as cows and horses. Furthermore, we aimed to investigate whether there are adverse effects of this method on subsequent ovarian function and reproductive performance. We also aimed to investigate if sufficient amounts and quality of luteal tissue can be obtained for subsequent genetic studies.
Aims and hypotheses of the study
I

The first aim of the study was to investigate the correlation between farrowing duration and subsequent pregnancy rate in sows breed after a lactation of about four weeks and a weaning-to-estrus interval of less than seven days (TRIAL-I).

We hypothesized that sow with a prolonged parturition have a lower pregnancy rate compared with sows with a normal parturition.

II

The second aim of the study was to investigate the correlation between farrowing duration and placental expulsion duration as well as retention of placentae (TRIAL-II).

We hypothesized that prolonged parturition prolongs placenta expulsion duration compared with normal parturition. Furthermore, we hypothesized that sows with severely prolonged parturition experience retained placentae.

III

The third aim of the study was to investigate the correlation between farrowing duration and the uterine size (indicator for uterine involution) and presence of intrauterine fluid (indicator for metritis) during the first week postpartum (TRIAL-III).

We hypothesized that prolonged parturition causes an increase in intrauterine fluid and uterine size as signs of an increased risk for metritis and delayed uterine involution compared with normal parturition.

IV

The fourth aim of the study was to investigate whether sows with prolonged farrowing have perturbed follicular growth after subsequent weaning and oxytocin release during boar stimulation at subsequent estrus (TRIAL-IV).

We hypothesized that sows with prolonged parturition have smaller follicles at weaning and ovulation compared with sows with normal parturition. Furthermore, we hypothesized that these sows release less oxytocin at estrus during boar stimulation compared with sows with normal parturition.
The fifth aim of the study was to adopt and validate a similar ultrasound-guided biopsy method for pigs as used for other species, such as cows and horses. Furthermore, the aim was to investigate whether adequate amounts of luteal tissue for genetic studies can be obtained without adverse effects on ovarian function and reproductive performance of the sows.

Figure 21. Overview of the aims, hypothesis, and the outline of the studies
Materials and methods
1. Animals, housing, and management

The trials were conducted on two different pig herds (HERD-I and HERD-II) in southern Finland. HERD-I included a sow pool farm with 280 Yorkshire x Large White crossbred sows and four satellite farms (Peltoniemi et al., 2009). HERD-II included a fully integrated farm with about 400 Yorkshire x Large White crossbred sows. TRIAL-I took place in the sow pool farm and two of the satellite farms (SATELLITE-I and SATELLITE-II) in HERD-I. TRIAL-II and TRIAL-III took place in SATELLITE-I of HERD-I and TRIAL-IV in the sow pool farm and SATELLITE-I of HERD-I and HERD-II. TRIAL-V was conducted in the sow pool farm of HERD-I.

In the sow pool farm of HERD-I, sows were housed during gestation in groups of twenty in pens (20 x 5m) with straw bedding. Sows had ad libitum access to water from a nipple drinker and were fed a standard pregnancy diet (Emakko Pekoni, Suomen Rehu, Finland) two times per day via an automatic liquid feeding system. Sows were transported from the sow pool farm to SATELLITE-I or SATELLITE-II about three weeks before the expected date of farrowing where they were group-housed in the waiting unit for about two weeks. About one week before the expected date of farrowing, sows were moved from the waiting unit to the farrowing and lactation unit. This unit was cleaned and disinfected before the sows were moved in and consisted of farrowing pens with adjustable farrowing crates on semi-slatted floors. Sows in SATELLITE-I had no bedding material whereas sows in SATELLITE-II had sawdust bedding. Feed consisted of a commercial diet for lactation (Emakkotäysrehu, RehuxOy, Finland) and was served three times a day via an automatic dry feeding system. After about four weeks of lactation, the sows were returned to the sow pool farm.

In HERD-II, approximately 400 crossbred Yorkshire x Large White crossbred sows were housed during gestation in groups of 8 to 10 (8 x 5 m) with peat bedding where they had ad libitum access to water from a nipple drinker and were fed a standard pregnancy diet (Emakko Täysreu, Rehux Oy, Finland) two times a day via an automatic liquid feeding system. Sows were moved from the gestation unit into the farrowing and lactation unit about one week before expected date of farrowing where they received a commercial lactation diet (Emakko Pekoni Plus, Suomen Rehu, Finland) as dry feed three times a day. This unit was cleaned and disinfected before the sows were moved in and consisted of farrowing pens with adjustable farrowing crates on fully-slatted plastic floors. After about four weeks of lactation, the sows were moved to the breeding unit.

Farrowings were not induced in any of the sows included in the study. In TRIAL-II and TRIAL-III, farm workers supervised the farrowings and injected 0.5 – 1.0 ml oxytocin (Vetox Vet 10 IU/ml, Vetcare Oy, Finland or Partoxin Vet 10 IU/ml, Pharmaxim, Finland) during the parturition if indicated. An indication was given if: 1) the birth canal was open and unobstructed; 2) a fetus was not present or present and well positioned cranially in the uterine cervix; 3) the sow gave an expulsion effort (contractions of abdominal musculature) but no piglet was delivered over a period of at least 30 min. These criteria were based on the recommendation of Gilbert (1999).
2. Sample size and study design

In TRIAL-I, parturitions of 148 randomly assigned multiparous sows were recorded in SATELLITE-I (n = 65) and SATELLITE-II (n = 83) of HERD-I during eighteen consecutive months in eleven batches. Sows were excluded from TRIAL-I if they experienced obstetrical intervention or administration of exogenous oxytocin during parturition or did not come to heat within 7 d after weaning. After weaning, sows were brought in the sow pool farm to a corridor in groups of five for heat detection twice a day, starting from second day after weaning until standing heat. In the corridor, sows had nose-to-nose contact with boars. After standing heat was detected, the sows were separated from the others by a fence, further stimulated (five-point stimulation) and artificially inseminated with semen of 10 Hampshire x Duroc crossbred boars (3 x 10⁹ spermatozoa in each extended AI dose). Artificial insemination was repeated about 16-24 h later. At four and seven weeks after insemination, a pregnancy examination was performed (transabdominal ultrasound; Tringa 505; Esaote).

In TRIAL-II, parturitions of 142 randomly assigned multiparous sows were recorded in SATELLITE-I of HERD-I during twelve consecutive months in ten batches. Sows were excluded from TRIAL-II if they experienced obstetrical intervention. On the other hand, administration of endogenous oxytocin was allowed and recorded. Two to three days after parturition, an ultrasound examination of the uterus was performed once per sow and the uterus was inspected for signs of retained piglets and placentae.

In TRIAL-III, parturitions of 99 randomly assigned multiparous sows were recorded in SATELLITE-I of HERD-I during twelve consecutive months in eight batches. Obstetrical intervention and oxytocin administration were allowed and therefore not considered to be exclusion criteria. During the first week after parturition, an ultrasound examination of the uterus was performed once per sow and uterine size and fluid accumulation was determined.

In TRIAL-IV, parturitions of 19 randomly assigned multiparous sows were recorded in HERD-II during three consecutive months in four batches. Furthermore, parturitions of another 11 randomly assigned multiparous sows were recorded in SATELLITE-I of HERD-I during three consecutive months in three batches. Sows were excluded from TRIAL-IV if they experienced obstetrical intervention or administration of exogenous oxytocin during parturition. After weaning, the subsequent follicular development was monitored twice a day between the third day after weaning and ovulation for all sows in either the sow pool farm of HERD-I or in HERD-II. Furthermore, sows were brought to a corridor in groups of five for heat detection twice a day starting from third day after weaning until standing heat was established. The day after that, blood samples were collected in the presence of a boar in order to determine the endogenous oxytocin concentrations and release patterns (Method described in detail in 4.7).
TRIAL-V was conducted in the sow pool farm of HERD-1 and consisted of two sub-trials (TRIAL-Va and TRIAL-Vb). In TRIAL-Va, four randomly assigned cycling multiparous sows were chosen and their day of ovulation was determined with the aid of transrectal ultrasonography. After that, a transvaginal ultrasound-guided biopsy was performed nine and fifteen days after ovulation for three consecutive estrous cycles. The obtained samples were submitted to the laboratory for histological staining and evaluation. Before each biopsy and shortly before each ovulation, a transrectal ultrasound examination of both ovaries was performed to determine the average size of the corpora lutea and follicles. In TRIAL-Vb, two groups of randomly assigned multiparous sows were chosen, their day of ovulation determined, and artificially inseminated twice with the semen of the same boar. One group served as control group and the other group was subjected to two TUBs. The first was conducted 10 d and the second 13 d after ovulation. The obtained samples were submitted to the laboratory for RNA extraction and determination of the quality and quantity of the extracted RNA. Before each second TUB, a transrectal ultrasound examination of both ovaries was performed to determine the average size of the corpora lutea. At day 28 after ovulation, a pregnancy examination was performed with the aid of transabdominal ultrasonography (MyLab OneVet, Esaote). At farrowing, gestation length and litter size were determined.

An overview of the animals used in the trials is presented in Table 2.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Batch</th>
<th>Herd</th>
<th>Sample size</th>
<th>Parity</th>
<th>Breed</th>
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<td>2 – 8</td>
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<tr>
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<td>Herd I: sow pool + SATELLITE II</td>
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<td>2 – 8</td>
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<tr>
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<td>2 – 5</td>
<td></td>
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<tr>
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<td>99</td>
<td>2 – 5</td>
<td></td>
</tr>
<tr>
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<td>2 – 5</td>
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<tr>
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<td>1 – 11</td>
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<tr>
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<td>Herd I: sow pool</td>
<td>52</td>
<td>2 – 5</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Overview of the animals used in the trials
3. Farrowing observations

In TRIAL-I, sows were monitored with a digital video recorder (AVC 787; Avtech) connected to infrared cameras (TS-6030PSC, Actech). The cameras were placed on the ceiling above each pen about 2 d before expected farrowing. The recordings were viewed afterwards and the farrowing duration (defined as the time interval between the expulsion of the first and the last piglet) was determined.

In TRIAL-II, TRIAL-III, and TRIAL-IV, sows were monitored with WLAN IP cameras (NICECAM420WL, Niceview Corp.) and IP-camera software (Blue Iris v.2.64, Perspective Software Corp.). The cameras were placed behind and 1.5 m above the sow about 2 d before expected farrowing (Fig. 22). The videos were viewed afterwards on a stationary computer with a media player (Windows media player 12.0, Microsoft Corp.), starting from the expulsion of the first piglet until 24 h after expulsion of the last piglet. In TRIAL-IV, the farrowing duration was determined. In TRIAL-II and TRIAL-III, the farrowing duration and the following placenta expulsion traits were determined: number of expelled placental parts (number of expelled placental parts from first to last expulsion), placenta expulsion duration (defined as the time interval between the expulsions of the first and the last placental parts), first placental part expulsion (time of expulsion of the first placental part relative to the last piglet) and last placental part expulsion (time of expulsion of the last placental part relative to the last piglet). Placental part was defined as ‘placental mass’ that was observed to be expelled at once. A ‘placental mass’ consists of an individual placenta or two or more placentae. Furthermore, it was recorded whether the sow had total retained placentae (no expelled placental parts during the observation period) or partially retained placentae (expulsion of last placental part observed before the expulsion of the last piglet) and whether oxytocin was administered during parturition.

Figure 22. Position of the cameras during parturition.
4. Sonographic evaluation of the uterus

The uterus was examined in TRIAL-II and TRIAL-III with a 4.3 MHz convex array ultrasound probe (MyLab OneVet, SC3421 Vet, Esaote) through the right flank of the standing sow.

In TRIAL-II the examination was performed 2-3 d after parturition once for each sow. The uterus was inspected for signs of retained piglets and placentae. Retained placentae were defined as hyperechoic structures within the lumen of the uterus and/or the endometrium.

In TRIAL-III, the examination was performed between the second and seventh day after farrowing once in each sow. The uterus was imaged cranially of the bladder and the clips were saved, and later exported in DICOM format and analyzed on a stationary computer using IMPAX 6.5.5 picture archiving and communication system (Agfa Healthcare, Belgium). The uterine size (cross-sectional area in cm²) at five different locations were measured (Fig. 23) and averaged. Furthermore, it was recorded if fluid accumulation was present at any location of the uterine horns. For that, as much as possible of the uterus was pictured with the ultrasound. When uterine fluid accumulation was detected then the uterine size was determined at cross-sections without fluid accumulation. Postpartum uterine size was used as an indicator of initial uterine involution and postpartum uterine fluid accumulation was used as an indicator of puerperal metritis.

Figure 23. Determination of uterine size (cross-sectional area in cm²) at one location of one uterine horn. The uterus was imaged cranially of the bladder und dorsally of the intestine. A – sectional area of the uterine horn; B – urinary bladder; C – intestine.
5. Sonographic evaluation of the ovaries

The ovaries were examined in TRIAL-IV and TRIAL-V with a 10.0 MHz linear array ultrasound probe (MyLab OneVet, SV3513, Esaote) through the rectum of the standing sow. The ultrasound examinations were saved, exported in DICOM format and analyzed on a stationary computer using IMPAX 6.5.5 picture archiving and communication system (Agfa Healthcare, Belgium).

In TRIAL-IV, both ovaries of each sow were scanned twice a day, once in the morning and once in the afternoon, starting from 3 d after weaning until ovulation. The sizes (diameter in mm) of the five biggest follicles of each ovary were measured and averaged (Fig. 24A).

In TRIAL-V, both ovaries of each sow were scanned once a day starting from the third day after weaning until ovulation. The day of ovulation was defined as Day 0. Furthermore, in TRIAL-Va, ovaries were scanned at day 9, 15 and 21 of three consecutive estrus cycles and the sizes (area in cm²) of the five biggest corpora lutea (Fig. 24B) of each ovary at day 9 and 15 and the sizes (diameter in mm) of the five biggest follicles at day 21 were measured and averaged. In TRIAL-Vb, ovaries were scanned at day 10 and 13 and the size (area in cm²) of the five biggest corpora lutea of each ovary were measured and averaged.

Figure 24. Determination of the size of one follicle (diameter in mm; A) and one corpus luteum (area in cm²; B).
6. Sonographic evaluation of backfat

Sonographic evaluation of backfat was used in TRIAL-V. Backfat was determined from all sows at weaning with a 10.0 MHz linear array ultrasound probe (MyLab OneVet, SV3513, Esaote). The probe was placed above the last rib on the back of the sow, 5-6 cm to the right and left of the backbone (P2 position). An image of each position was saved, exported in DICOM format and analyzed on a stationary computer using IMPAX 6.5.5 picture archiving and communication system (Agfa Healthcare, Belgium). The thickness of the fat layer (mm) was measured in each image at three different places and averaged (Fig. 25).

Figure 25. Determination of backfat (thickness in mm). The ultrasound probe was placed above the last rib on the back of the sow, 5-6 cm to the right and left of the backbone. A – fat layer; B – Backfat layer, C – muscle layer.
7. Blood collection and oxytocin assay

In TRIAL-IV, blood samples were collected at estrus before, during, and after boar presence to determine plasma oxytocin concentrations.

For that, the sows were catheterized non-surgically through the auricular vein, approximately 2 d after weaning. Each sow was confined in a stall and restrained with a rope snare around the upper jaw. The rope snare was tied to the stall and an i.v. catheter (14 G, 57 mm) was inserted into the auricular vein. Through the catheter, 0.5 m of a 1.5 m long PVC tube (o.d. 1.5 mm × i.d. 1.0 mm; SteriHealth) was passed and the outer end was sealed with a cap, attached to the ear with adhesive tape, and the remaining tube placed into a pouch that was attached to the neck with adhesive tape.

After catheterization, the vinyl tubing was flushed daily with a saline (0.9% NaCl) solution containing heparin (5 IU/ml) until collection of the blood samples. Blood sample collection occurred the day after detection of standing estrus. On the day of collection, three blood samples were obtained before boar presence (minutes -15, -10, and -5), eight samples during boar presence (minutes 0, 1, 2, 3, 4, 6, 8, and 10) and seven samples after boar presence (minutes 15, 20, 25, 30, 40, 50, and 60). Thus the boar was present for 10 min. Minute 0 was the moment of boar introduction.

Before collection of each blood sample, the first 2 ml of blood drawn up was discarded. The following blood was collected with single-use vacuum needles into ice-chilled EDTA tubes (HERD-II) containing 500k IU/ml of aprotinin. All blood samples were immediately centrifuged for 10 min at 2000 rpm. The plasma was separated into 1.5 ml microtubes and stored at -80 °C until analysis of oxytocin concentrations in the lab.

In the lab, blood samples were purified using two different methods. For HERD-I: 500 μl of plasma was precipitated with 1 ml of acetone (Chromasolve Plus, for HPLC ≥ 99.9%, 650501/Sigma), and extracted in 2 ml of petroleum ether (Purist. p.a., ACS reagent, boiling point range 40-60 °C, 77399/SIGMA) twice. Extracted plasma samples were evaporated in a vacuum evaporator (RVC 2-25 CD, Martin Christ) at 40 °C, and stored at -80 °C for later analysis of oxytocin. For HERD-II: Oasis HLB columns (Oasis HLB 1 cc Vac Cartridge, 30 mg, Waters Corporation) columns were conditioned with 1 ml of methanol and 1 ml of water, then 1 ml of pig plasma with 20 μl of phosphoric acid was added to the columns. Columns were washed with 1 ml of 10% acetonitrile containing 0.1% trifluoroacetic acid (TFA) in water and eluted with 1 ml of acetonitrile:water (80:20). Eluted samples were evaporated in a nitrogen stream at 37 °C and stored at -80 °C for later analysis of oxytocin.

After purification, oxytocin concentrations were measured using an enzyme-linked immunosorbent assay kit (Oxytocin ELISA kit, Enzo Life Sciences). Purified plasma samples were reconstituted with 250 μl of assay buffer supplied in the ELISA kit, and analyzed in duplicates according to the assay kit instruction. Sensitivities of the plasma oxytocin assay were 7.8 pg/ml and 3.9 pg/ml, average mass recovery were 89.6% and 90.1%, intra-assay CVs were 14.5% and 9.6%, and inter-assay CVs were 15.1% and 7.4% for HERD-I and HERD-II, respectively.
8. Transvaginal ultrasound-guided biopsy of the corpus luteum

A novel method for collection biopsies of the porcine corpus luteum was tested and validated in TRIAL-V. During each biopsy, a maximum of two attempts was made. If the first attempt was successful, the biopsy was over. If the first attempt was not successful, a second attempt was made.

A semiautomatic Tru-Cut biopsy needle (25 cm long, 16 gauge; Ultimate PMO 16-25; Zamar, Croatia) was used. The biopsy needle consisted of an inner needle with a 1 cm specimen notch and an echogenic trocar tip, and an outer cutting cannula. Before the procedure, animals were confined in a crate, feces were removed from the rectum and the vulva was washed three times with a povidone–iodine solution (7.5% Betadine; Leiras, Helsinki). The biopsy needle was then inserted into a needle guide (DBSE12X Biopsy kit; Esaote) that was 18 cm in length and placed on to a 6.8-MHz microconvex array probe (Endocavity probe, SE3123; Esaote) that was 30 cm in length. The probe was connected to an ultrasound device (MyLab One VET; Esaote), placed in the vagina and held in place with one hand. The other hand was placed in the rectum to localize the ovary. In the early pregnant sow, or during the luteal phase in cycling sows, the ovary is located laterally and ventrally of the uterine body, approximately 5–6 cm cranially of the pelvic inlet (Constantinescu, 2007). To facilitate localization of the ovary, the suspensory ligament of the ovary, which suspends the ovary and extends from the abdominal roof as the cranial boundary of the broad ligament (König and Liebich 2004), was palpated and followed ventrally until the ovary was reached. Furthermore, to alleviate grabbing of the ovary, the ligament was pulled on to bring the ovary more caudally and dorsally of the uterine body. Thereafter, the ovary was held between the index and middle fingers by the site of attachment of the proper ligament, a short and strong ligament between the ovary hilus and the cranial end of the uterine horn (Constantinescu, 2007), with the ovary on the palm side of the hand. Subsequently, the ovary was relocated into the pelvic cavity towards the caudal part of the cervix (Fig. 26). At the same time, the probe was inserted deeper into the vagina towards the ovary, until the ovary became visible on the ultrasound screen. When visible, the ovary was pushed against the face of the probe and the biopsy needle was introduced approximately 5 mm through the vaginal wall into the ovary. The echogenic tip of the needle allowed visual confirmation on the ultrasound screen of the correct position of the biopsy needle in the ovary. After the biopsy needle was in place, the trigger was pushed and the inner needle advanced 1 cm into the ovarian tissue, exposing the specimen notch, followed by the outer cannula, which cut and trapped ovarian tissue inside the notch (Fig. 27). After the sample had been obtained, the biopsy needle and ultrasound probe were withdrawn from the vagina.
In TRIAL-Va, biopsies were stained for histological evaluation of their composition. After biopsy collection, they were drop-fixed in 10% formalin solution. After 24 h, the biopsies were embedded in paraffin and cut with a microtome into 4 mm sections before being mounted on glass slides. The slides were deparaffinized with xylene and ethanol, and stained with hematoxylin–eosin.

In TRIAL-Vb, RNA was extracted from the obtained biopsies and their quality and quantity were evaluated. After biopsy collection, they were stored in RINalater (Sigma-Aldrich Life Sciences) until RNA extraction (GenElute; Sigma Aldrich Life Sciences) according to manufacturer’s instructions. For assessment of RNA quality, the RNA integrity number (RIN) was determined (Agilent 2100 Bioanalyzer, RNA Nano LabChip; Agilent Technologies,) and for determination of RNA concentration, a Qubit fluorometer (Qubit RNA HS assay kit; ThermoFisher Scientific) was used. Furthermore, the concentrations were multiplied by the volume of the RNA sample to calculate the total amount of RNA extracted per sample.
Figure 27. The transvaginal ultrasound-guided biopsy. (a) The ovary is pushed against the vaginal wall and the face of the probe. The biopsy needle is pushed approximately 5 mm through the vaginal wall into one corpus luteum (CL). (b) The echogenic tip (black arrow) of the needle allows visual confirmation on the ultrasound screen of the correct positioning of the biopsy needle in the ovary. (c) After the biopsy needle is in place, the trigger is pushed and the inner needle advances 1 cm into the ovarian tissue, exposing the specimen notch (gray arrow). (d) The inner needle is followed by the outer cannula (white arrow), which cuts and traps ovarian tissue inside the notch.
9. Statistical analysis

Statistical processing of all data was done in SPSS Statistics (IBM Corporation) v.18.0 (TRIAL-I and TRIAL-V), v.23.0 (TRIAL-II), v.24.0 (TRIAL-III), or SAS (SAS Institute Inc.) v.9.4 (TRIAL-IV). All variables were checked for normal distribution. Logarithmic transformation of data was applied for farrowing duration and square-root transformation for placenta expulsion duration and last placenta expulsion to achieve normality. Descriptive data are presented as mean ± SD. Before a regression model was applied, an initial univariable screening was performed to identify potential confounders and predictors of the outcome. Based on the type of variable, either Pearson Chi-square tests, independent t-tests, ANOVAs, Spearman rank correlation coefficients or Pearson correlation coefficients were used. Details of the methods and results of the initial univariable screenings can be found in the original publications. Overview of the models, and outcome and explanatory variables used in the major statistical analyses is presented in Table 3.

In TRIAL-I, the outcome variable was defined as ‘pregnant’ or ‘not pregnant’. The explanatory variables were: parity, herd (SATELLITE-I vs. SATELLITE-II), housing (crate vs. pen) gestation length, farrowing duration, number of total, liveborn and stillborn piglets, backfat at farrowing, lactation length, number of weaned piglets, weaning-to-estrus interval, boar semen used at insemination and number of inseminations. Farrowing duration was transformed into a binary variable based on a 300 min threshold value. Sow and Batch were included as random factors. A logistic regression model was built and a backward stepwise elimination with a likelihood-ratio test was performed. When significant predictors were found, the odds ratio ± SE was calculated.

In TRIAL-II, the outcome variables related to placenta expulsion were: number of expelled placental parts, placenta expulsion duration, and first and last placental part expulsion. The following explanatory variables were: parity, gestation length, farrowing duration, and number of liveborn and stillborn piglets. Each of the outcome variables was tested in a distinct general linear model. Sow and batch were included as random factors and the use of oxytocin (yes/no) as a fixed effect. Furthermore, the quadratic term for farrowing duration was included, except for the ‘first placental part expulsion’ model, because we hypothesized a positive correlation between farrowing and placenta expulsion duration in sows with short farrowings, as found in the study of Van Rens and Van der Lende (2004), and a negative correlation between farrowing and placenta expulsion duration in sows with prolonged farrowings due to impaired placenta expulsion. Furthermore, because we hypothesized that there were differences in the correlation between farrowing duration and placenta expulsion between sows receiving and sows not receiving exogenous oxytocin during parturition, data were further divided according to the use of oxytocin and consequently two additional submodels for each outcome were created. Results are presented as least squares means ± SEM or estimates ± SE. Furthermore, for post hoc comparisons of means, Tukey’s HSD was used. Furthermore, we compared farrowing duration and other explanatory variables (parity, gestation length, and number of liveborn and stillborn piglets) between sows with no retained placentas, partially retained placentas and totally retained placentas. The effects were tested using a multinomial logistic regression model with ‘no retained placentas’ as the reference category.
In TRIAL-III, the outcome variables were uterine size and uterine fluid accumulation. Uterine fluid accumulation was defined as 'no fluid' or 'fluid'. Uterine size was transformed into a binary variable and defined as 'normal' or 'enlarged' based on the postpartum day of examination and the mean of all sows examined on that specific postpartum day. The explanatory variables were: parity, gestation length, number of live and stillborn piglets, application of obstetrical intervention (yes vs. no), farrowing duration, placental expulsion duration, placentae retention (defined as no expelled placental parts within the observation period of 24 h after expulsion of the last piglet), relative placental expulsion duration (placental expulsion duration relative to farrowing duration), and relative number of expelled placental parts (number of expelled placental parts relative to the number of total piglets born). These last two variables were used as indicators for impaired placental expulsion and transformed into binary variables based on observations done on TRIAL-II. Placental expulsion was defined as ‘normal’ if relative placental expulsion duration and relative number of expelled placental parts were higher than 10% and defined as ‘impaired’ if they were lower than 10%. Furthermore, farrowing duration was transformed into a categorical variable and defined as ‘short’ if farrowing was briefer than 400 min and defined as ‘prolonged’ if it exceeded 400 min. Altogether, two binary logistic regression models were created, one for each outcome variable. Sow and batch were included as random factors and use of oxytocin (yes vs. no) and antimicrobials (yes vs. no) during parturition or between parturition and postpartum day of ultrasound examination were fixed factors. A backward stepwise elimination with a likelihood-ratio test was performed. If an explanatory variable was a significant predictor, the odds ratio (± S.E.) was also calculated.

In TRIAL-IV, the outcome variables were: oxytocin concentration at breeding (divided into four categories: minutes -15 to -5; minutes 0 to 10, minutes 15 to 60, and minutes -15 to 60) and follicular development (follicular size at day 3, 4, and 5 after weaning as well as maximum follicular size during weaning-to-ovulation interval and size at first day of standing heat). As explanatory variables, farrowing duration and parity were used and transformed into categorical variables. Farrowing duration was defined as ‘short’ if it was shorter than 300 min and defined as ‘prolonged’ if it was longer than 300 min. Parity was defined as ‘young’ if it was three or lower and defined as ‘old’ if it was four or higher. Linear mixed models for repeated measures were used with a nested design where farrowing duration and parity were nested within herd (HERD-I vs. HERD-II). For post hoc comparisons, a Kenward-Rogers approximation was used.

In TRIAL-Va, logistic regression analysis and ANOVA were performed to investigate differences in backfat, weight and parity between successfully and unsuccessfully sampled sows. A sow was defined as successfully sampled if at least one biopsy during the entire sampling period was successful. A biopsy was defined as successful if the first or second attempt was successful. An attempt was defined as successful if ovarian tissue was present in the biopsy needle notch.

In TRIAL-Vb, corpus luteum size at day 13 of pregnancy, and subsequent gestation length and litter size were compared between sows that underwent transvaginal ultrasound-guided biopsy and sows of the control group using an independent t-test. Furthermore, the pregnancy rate was compared using a Pearson Chi-square test.
Table 3. Overview of the models, and outcome and explanatory variables used in the major statistical analyses.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Model</th>
<th>Outcome</th>
<th>Major explanatory variable</th>
<th>Other explanatory variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Logistic regression model</td>
<td>Pregnancy (Yes / No)</td>
<td>Farrowing duration (normal / prolonged)</td>
<td>Gestation length</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>SATELLITE</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Litter size</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number of stillborn piglets</td>
</tr>
<tr>
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<td></td>
<td>Backfat at farrowing</td>
</tr>
<tr>
<td>II</td>
<td>Linear regression model</td>
<td></td>
<td>Farrowing duration (continuous)</td>
<td>Parity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lactation Length</td>
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<td>Multinomial logistic</td>
<td>Number of expelled placental part</td>
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<td>Weaned piglets</td>
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<td>regression model</td>
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<td>Weaning-to-estrus interval</td>
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<td>First and last placental part expulsion</td>
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<td>Boar used at insemination</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Number of inseminations</td>
</tr>
<tr>
<td>III</td>
<td>Logistic regression model</td>
<td>Partial retained placenta (Yes / No)</td>
<td>Farrowing duration (continuous)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total retained placenta (Yes / No)</td>
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<td></td>
</tr>
<tr>
<td>IV</td>
<td>Linear mixed models for</td>
<td>Uterine size (normal / increased)</td>
<td>Farrowing duration (normal / prolonged)</td>
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</tr>
<tr>
<td></td>
<td>repeated measures</td>
<td>Intrauterine fluid (Yes / No)</td>
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<td></td>
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<tr>
<td>Va</td>
<td>No model – descriptive</td>
<td>Follicular size at weaning and at day 3, 4, and 5 after weaning</td>
<td>Farrowing duration (normal / prolonged)</td>
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</tr>
<tr>
<td></td>
<td>statistics</td>
<td>Oxytocin concentrations at estrus before, during, and after boar</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>stimulation</td>
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<tr>
<td>Vb</td>
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<td>Biopsy (study group)</td>
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<td>analysis</td>
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<td>Corpora lutea size at day 10 and 13 of gestation</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gestation length</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Litter size</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 – Normal farrowing < 300 minutes and prolonged farrowing ≥ 300 minutes

2 – Expulsion of last placental part observed before the expulsion of the last piglet

3 – No expelled placental parts during the observation period

4 – Based on the postpartum day of examination and the mean of all sows examined on that specific postpartum day. Normal size < mean and enlarged size ≥ mean.

5 – Normal if relative placental expulsion duration and relative number of expelled placental parts were higher than 10% and impaired if they were lower than 10%.

6 – Young ≤ parity 3 and old > parity 3
Results
1. Effect of prolonged farrowing on subsequent pregnancy rate

In TRIAL-I, 148 sows were of parity, 4.4 ± 1.9 (mean ± SD), had a gestation length of 116 ± 1.2 d, a backfat at farrowing of 14.3 ± 3.6 mm, and a farrowing duration of 268 ± 142 min. There were 11.9 ± 2.9 liveborn piglets and 0.8 ± 1.3 stillborn piglets at birth. After a lactation of 31.6 ± 2.9 d 9.8 ± 1.6 piglets were weaned. 87% (n = 129) of the sows were ‘pregnant’ and 13% (n = 19) of the sows were ‘not pregnant’. In the final logistic regression model, duration of farrowing was significantly associated with the outcome (P < 0.01; Fig. 28). Sows with a farrowing duration of ≥ 300 min were 3.4 ± 1.8 (Odds ratio ± SE; P = 0.027) more likely to be ‘not pregnant’.

Fig. 28: Sows that failed to become pregnant at first insemination after weaning had a previous farrowing 100 minutes longer than sows that become pregnant.
2. Effect of prolonged farrowing on placenta expulsion and retained placentae

In TRIAL-II, 142 parturitions of 101 sows in 10 batches were recorded: 66 sows had a single parturition, 29 sows had two consecutive parturitions and 6 sows had three consecutive parturitions. An overview is provided in Table 4.

Use of exogenous oxytocin towards the end of farrowing (used in 44 out of 142 parturitions) increased the number of expelled placental parts (3.8 ± 0.2 vs. 2.9 ± 0.3; LSmean ± SEM; P = 0.035) and decreased the placenta expulsion duration (172 ± 44 vs. 328 ± 26 min; P = 0.011) and the time of last placental part expulsion (148 ± 48 vs. 300 ± 24 min; P = 0.025).

If oxytocin was not used, farrowing duration obeyed a quadratic relationship with the number of expelled placental parts (P = 0.001; Fig. 29), placenta expulsion duration (P = 0.002; Fig. 30) and time of last placental part expulsion (P = 0.024; Fig. 31). Nevertheless, when the two observations with FAR < 100 min and the five observations with FAR > 800 min were excluded from the model, the quadratic term for farrowing duration was not significantly correlated with the placenta expulsion duration but only the linear term of farrowing duration (0.7 ± 0.2; B ± SE; P = 0.005).

If oxytocin was used, number of expelled placental parts was positively associated with number of liveborn piglets (0.2 ± 0.1; B ± SE; P = 0.002) and was affected by parity. 5th parity sows expelled more placental parts (4.3 ± 0.4; LSmean ± SEM) than 4th (3.2 ± 0.3; P = 0.024) and 3rd parity sows (2.7 ± 0.4; P = 0.008). Furthermore, placenta expulsion duration was positively associated with number of liveborn piglets (18 ± 8 min; B ± SE; P = 0.025). First placental part expulsion was negatively correlated with farrowing duration (0.3 ± 0.1; P = 0.001; Fig. 32) and there was no difference between sows that received oxytocin and sows that did not.

Table 4: Descriptive statistics for all sows included in TRIAL-II.
Figure 29. Relationship between farrowing duration and the number of expelled placental parts in sows receiving no oxytocin during parturition. Error bars indicate a single SE.

Figure 30. Relationship between the farrowing duration and the placenta expulsion duration in sows receiving no oxytocin during parturition. Error bars indicate a single SE.
Figure 31. Relationship between the farrowing duration and the expulsion of the last placental part relative to the birth of the last piglet in sows receiving no oxytocin. Error bars indicate a single SE.

Figure 32. Relationship between the farrowing duration and the expulsion of the first placental part relative to the birth of the last piglet. Error bars indicate a single SE.
Four sows experienced total (no expulsion of placental parts) and four sows partial retained placentae (no expulsion of placental parts after birth of the last piglet). Their farrowing durations were longer ($1009 \pm 275$ and $734 \pm 136$ min with $P = 0.004$ and $P = 0.021$, respectively) compared with those of sows that had no retained placentas ($369 \pm 202$ min; LSmean ± SD). In 3 sows with total retained placentae, hyperechoic structures within the lumen of the uterus and/or the endometrium were detected (Fig. 33).

Figure 33. Ultrasound image of one sow with total retained placentae at day 3 after farrowing. Ultrasound findings are dense structures inside the uterine lumen and endometrium. A – Uterine horn; B – Uterine lumen and endometrium; C – Enlarged blood vessels.
3. **Effect of prolonged farrowing on postpartum uterine size and intrauterine fluid**

In TRIAL-III, the uterine size at the 2\textsuperscript{nd} postpartum (p.p.) day was 4.19 ± 1.23 cm\(^2\) (mean ± SD; n/N = 8/99); at the 3\textsuperscript{rd} p.p. day 4.05 ± 1.05 cm\(^2\) (24/99); at the 4\textsuperscript{th} p.p. day 3.96 ± 0.89 cm\(^2\) (25/99); at the 5\textsuperscript{th} p.p. day 3.75 ± 0.99 cm\(^2\) (20/99); at the 6\textsuperscript{th} p.p. day 3.73 ± 0.44 cm\(^2\) (13/99); and at the 7\textsuperscript{th} p.p. day 3.53 ± 0.86 cm\(^2\) (9/99). 55 sows had a ‘normal’ and 44 sows had an ‘enlarged’ uterine size (Fig. 34). 15 had accumulation of intrauterine fluid (Fig. 35). 13 out of 15 sows with uterine fluid accumulation had also increased uterine size.

Results for the final binary logistic regression models are presented in Table 5 and 6.

![Ultrasound image of one sow with enlarged uterus at day 3 after farrowing. A – Enlarged uterine horn; B – Enlarged blood vessels.](image-url)
Table 5. Significant predictors of the final binary logistic regression model with ‘normal’ and ‘enlarged’ uterine size as binary outcome variable.

### Enlarged uterine size
(in 44/99 sows)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>n/N</th>
<th>%</th>
<th>B</th>
<th>SE</th>
<th>Wald χ²</th>
<th>Odds</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal farrowing*</td>
<td>9/44</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prolonged farrowing</td>
<td>35/55</td>
<td>65</td>
<td>2.037</td>
<td>0.565</td>
<td>13.012</td>
<td>7.671</td>
<td>0.001</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2*</td>
<td>1/11</td>
<td>9</td>
<td></td>
<td></td>
<td>9.595</td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>3</td>
<td>10/19</td>
<td>53</td>
<td>2.404</td>
<td>1.274</td>
<td>3.563</td>
<td>11.070</td>
<td>0.059</td>
</tr>
<tr>
<td>4</td>
<td>12/35</td>
<td>34</td>
<td>1.867</td>
<td>1.235</td>
<td>2.286</td>
<td>6.466</td>
<td>0.131</td>
</tr>
<tr>
<td>5</td>
<td>21/34</td>
<td>61</td>
<td>3.107</td>
<td>1.217</td>
<td>6.520</td>
<td>22.352</td>
<td>0.011</td>
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<tr>
<td>No obstetrical intervention*</td>
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<td>39</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Obstetrical intervention</td>
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<td>70</td>
<td>1.457</td>
<td>0.696</td>
<td>4.381</td>
<td>4.293</td>
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<td>No oxytocin administration*</td>
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<td>38</td>
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</tr>
<tr>
<td>Oxytocin administration</td>
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<td>4.721</td>
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<tr>
<td>Stillborn piglets</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0*</td>
<td>17/45</td>
<td>38</td>
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<tr>
<td>1</td>
<td>12/33</td>
<td>36</td>
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<td>0.074</td>
<td>0.848</td>
<td>0.786</td>
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<tr>
<td>≥ 2</td>
<td>15/21</td>
<td>71</td>
<td>1.353</td>
<td>0.697</td>
<td>3.769</td>
<td>3.871</td>
<td>0.052</td>
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</tbody>
</table>

* - reference category
Figure 35. Ultrasound image of one sow with uterine fluid accumulation at day 3 after farrowing. A – Uterine horn with fluid accumulation.
Table 6. Significant predictors of the final binary logistic regression model with ‘no intrauterine fluid’ and ‘uterine fluid’ as binary outcome variable.

**Intrauterine fluid**

**(in 15/99 sows)**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>n/N</th>
<th>%</th>
<th>B</th>
<th>SE</th>
<th>Wald χ²</th>
<th>Odds</th>
<th>Sig.</th>
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<td></td>
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<tr>
<td>Prolonged farrowing</td>
<td>13/55</td>
<td>24</td>
<td>1.740</td>
<td>0.843</td>
<td>4.255</td>
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**Stillborn piglets**

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<th>%</th>
<th>B</th>
<th>SE</th>
<th>Wald χ²</th>
<th>Odds</th>
<th>Sig.</th>
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<td>1</td>
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<td>0.603</td>
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<tr>
<td>≥ 2</td>
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<td>2.616</td>
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**No obstetrical intervention***

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<th>%</th>
<th>B</th>
<th>SE</th>
<th>Wald χ²</th>
<th>Odds</th>
<th>Sig.</th>
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<td>0.802</td>
<td>5.027</td>
<td>6.042</td>
<td>0.025</td>
</tr>
</tbody>
</table>

**Normal placental expulsion***

<table>
<thead>
<tr>
<th></th>
<th>n/N</th>
<th>%</th>
<th>B</th>
<th>SE</th>
<th>Wald χ²</th>
<th>Odds</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impaired placental expulsion</td>
<td>4/6</td>
<td>67</td>
<td>3.293</td>
<td>1.638</td>
<td>4.040</td>
<td>26.928</td>
<td>0.044</td>
</tr>
</tbody>
</table>

* - reference category
4. Effect of prolonged farrowing on follicular development after weaning and oxytocin release at subsequent estrus

In TRIAL-IV, farrowing duration was 358 ± 235 min. Sows with ‘short’ farrowing duration (n = 14) had 159 ± 29 min and sows with ‘prolonged’ farrowing duration (n= 16) had 533 ± 190 min. ‘Young’ sows (n = 14) had a parity of 2.5 ± 0.8 and ‘old’ sows (n = 16) had a parity of 6.4 ± 2.3.

At subsequent estrus, oxytocin concentrations were higher during boar presence compared with before and after (P < 0.05, Fig. 36). Furthermore, during the boar presence, there was an effect of farrowing duration on oxytocin concentrations (P < 0.01; Fig. 36; Table 7). Sows with ‘short’ farrowing duration had lower oxytocin concentrations compared with sows with ‘prolonged’ farrowing duration. Post hoc comparison showed that in ‘young’ sows in particular, oxytocin concentrations during and after boar presence were higher in sows with ‘prolonged’ farrowing duration compared with sows with ‘short’ farrowing duration (P < 0.001 and P < 0.01, respectively). On the other hand, oxytocin concentrations of ‘old’ sows were not affected by previous farrowing duration (P > 0.10, for all).

Figure 36. LS means and SE of plasma OT concentrations of oestrous sows in boar presence are plotted by SHORT-YOUNG (n = 9), SHORT-OLD (n = 5), LONG-YOUNG (n = 5) and LONG-OLD (n = 11) groups, according to farrowing duration and parity classes. The red shading indicates 10 min of boar presence.
Table 7. The effects of farrowing duration and parity on the plasma oxytocin concentrations at subsequent estrus before, during, and after boar exposure as well as during the whole sampling period.

<table>
<thead>
<tr>
<th>Period</th>
<th>Young Normal (n = 9)</th>
<th>Young Prolonged (n = 5)</th>
<th>Old Normal (n = 5)</th>
<th>Old Prolonged (n = 11)</th>
<th>FD</th>
<th>Parity</th>
<th>FD*parity</th>
</tr>
</thead>
<tbody>
<tr>
<td>-15 to -5 min</td>
<td>13.8 ± 1.5</td>
<td>14.8 ± 1.7</td>
<td>13.7 ± 1.7</td>
<td>13.3 ± 1.6</td>
<td>0.79</td>
<td>0.76</td>
<td>0.48</td>
</tr>
<tr>
<td>0 to 10 min</td>
<td>18.6 ± 7.6</td>
<td>28.9 ± 7.8</td>
<td>24.2 ± 7.8</td>
<td>27.6 ± 7.7</td>
<td>&lt; 0.01</td>
<td>0.84</td>
<td>0.13</td>
</tr>
<tr>
<td>15 to 60 min</td>
<td>13.7 ± 2.5</td>
<td>16.8 ± 2.6</td>
<td>16.6 ± 2.7</td>
<td>16.2 ± 2.6</td>
<td>0.58</td>
<td>0.79</td>
<td>0.07</td>
</tr>
<tr>
<td>-15 to 60 min</td>
<td>16.9 ± 2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.7 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.8 ± 2.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>18.8 ± 2.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.44</td>
<td>0.91</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1 – Values represent LS means ± SE of plasma oxytocin concentrations of the sows (total n = 30).
2 – Repeated measures carried out on the values over four periods: 1) -15, -10 and -5 min before boar presence, 2) 0, 1, 2, 3, 4, 6, 8 and 10 min during 10 min of boar presence, 3) 15, 20, 25, 30, 40, 50 and 60 min after boar presence, and 4) total period.
3 – FD (farrowing duration) was divided into two classes: 1) SHORT: between 103 and 192 min, and 2) LONG: between 326 and 878 min.
4 – Parity was divided into two classes: 1) YOUNG: between 1 and 3, and 2) OLD: between 4 and 11.
<sup>a,b</sup> Different letters indicate that variables were significantly different at P < 0.05.
The follicular size at day 4 after weaning tended to be larger in the sows with ‘prolonged’ farrowing duration compared with sows with 'short' farrowing duration (P = 0.06, Table 8), but no other differences were established.

Table 8. The effects of farrowing duration and parity on follicular development and weaning to oestrus intervals of subsequent oestrous sows.

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Old</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHORT (n = 9)</td>
<td>LONG (n = 5)</td>
<td></td>
</tr>
<tr>
<td>Days³</td>
<td></td>
<td></td>
<td>FD¹</td>
</tr>
<tr>
<td>WOI</td>
<td>5.0 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>Maximum</td>
<td>4.0 ± 0.4</td>
<td>4.4 ± 0.5</td>
<td>4.7 ± 0.5</td>
</tr>
<tr>
<td>Diameters, mm⁴</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>4.5 ± 0.4</td>
<td>5.0 ± 0.6</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>Day 4</td>
<td>5.5 ± 0.3</td>
<td>5.9 ± 0.5</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>Day 5</td>
<td>5.5 ± 0.3</td>
<td>5.8 ± 0.3</td>
<td>6.1 ± 0.3</td>
</tr>
<tr>
<td>Oestrus</td>
<td>6.0 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Maximum</td>
<td>5.7 ± 0.2</td>
<td>5.6 ± 0.3</td>
<td>6.2 ± 0.3</td>
</tr>
</tbody>
</table>

¹ – FD (farrowing duration) was divided into two groups: 1) SHORT: between 103 and 192 min, and 2) LONG: between 326 and 878 min.

² – Parity was divided into two groups: 1) YOUNG: between 1 and 3, and 2) OLD: between 4 and 11.

³ – Values represent LS means ± SE of the days for 1) weaning-to-oestrus interval, and 2) when the 5 largest follicles reached their maximum size after weaning until ovulation.

⁴ – Values represent LS means ± SE of the mean diameters of the five largest follicles measured 1) on day 3, 2) on day 4 and 3) on day 5 after weaning until ovulation, 4) at oestrus in which the sow showed a standing response to the back test, and 5) when the follicles reached their maximum size after weaning until ovulation.
Furthermore, the follicular size at fifth day after weaning and the maximum follicular size between weaning and ovulation were correlated with oxytocin concentrations at estrus before, during and after boar presence (rs = 0.23, P < 0.0001 and rs = 0.38, P < 0.0001, respectively) (Fig. 37).

Figure 37. Average plasma oxytocin concentrations of the sows during the total sampling period, i.e. -15 to +60 min from beginning of 10 min of boar presence, and follicle diameters measured at five days after weaning and when the follicles reached their maximum size after weaning until ovulation are indicated with Spearman correlation coefficients (rs) (n = 30).
5. Transvaginal ultrasound-guided biopsy of corpora lutea

In TRIAL-Va, 18 out of 24 biopsies were successful (75%). In TRIAL-Vb, 20 out of 52 biopsies were successful (38%). Thus, samples were obtained in 50% of all biopsies. All sows in TRIAL-Va were successfully sampled whereas only 20 sows (77%) in TRIAL-Vb. Overall, compared with unsuccessfully sampled sows (n = 6), successfully sampled sows (n = 24) were older (parity 5.0 ± 2.8 vs. 2.2 ± 0.5), heavier (290 ± 26 vs. 244 ± 27 kg) and had thicker backfat (11.4 ± 2.7 vs. 6.4 ± 2.5 mm; P < 0.05 for all).

In TRIAL-Va, no abnormal cyclic changes during the sampling period were found (Table 9) and no major pathological changes after the sampling period (Fig. 38) were found in the ovary. The only pathological gross findings noted were small focal blood clots and small multifocal scars.

Figure 38. Macroscopic inspection of the ovaries of one sow after six consecutive biopsies taken during three consecutive estrus cycles. Pathological changes found were (a) small multifocal scars (arrows) and (b) small focal blood clots (bolt arrow).
Table 9. Mean sizes and ranges of follicles and/or corpora lutea present in the ovaries before each transvaginal ultrasound-guided biopsy.

<table>
<thead>
<tr>
<th>Day of TUB</th>
<th>Area corpora lutea (cm²)</th>
<th>Diameter follicles (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cycle – Day 9</td>
<td>0.93 (0.90 – 0.99)</td>
<td>2.6 (2.2 – 3.2)</td>
</tr>
<tr>
<td>1. Cycle – Day 15</td>
<td>0.73 (0.69 – 0.78)</td>
<td>6.6 (5.9 – 7.2)</td>
</tr>
<tr>
<td>2. Cycle – Day 9</td>
<td>0.94 (0.89 – 1.00)</td>
<td>2.7 (2.4 – 3.1)</td>
</tr>
<tr>
<td>2. Cycle – Day 15</td>
<td>0.73 (0.71 – 0.76)</td>
<td>6.5 (5.7 – 7.2)</td>
</tr>
<tr>
<td>3. Cycle – Day 9</td>
<td>0.95 (0.93 – 0.96)</td>
<td>2.9 (2.6 – 3.4)</td>
</tr>
<tr>
<td>3. Cycle – day 15</td>
<td>0.72 (0.70 – 0.75)</td>
<td>6.3 (5.9 – 6.8)</td>
</tr>
</tbody>
</table>

In TRIAL-Vb, there were no significant differences (P > 0.05) between the successfully sampled sows (n = 20) and the control group (n = 26) in terms of corpora lutea size at day 13 (0.89 ± 0.10 vs 0.92 ± 0.11 cm²), pregnancy rate (95% vs 96%), gestation length (115 ± 1 vs 115 ± 1 days) and the total number of piglets born (12.7 ± 2.5 vs 13.3 ± 2.8).

Seventeen samples were subjected to RNA extraction and assessment of RNA quality and quantity. Three samples (15%) were excluded from analysis because they contained almost no RNA. Across the remaining samples, the RNA concentration was 520 ± 160 μg per sample and the RIN was 8.5 ± 1.9 (range 6.7 – 10). RNA extracted from 14 samples (82%) had a RIN >8.

The histological composition of 18 samples was assessed. The shape of the samples was round and they were yellow–red in color; the samples were ~1 mm in diameter (Fig. 39a). The histological composition was heterogeneous and contained luteal cells (Fig. 39b), follicular cells, such as granulosa cells (Fig. 39c), stromal cells (Fig. 39d) and occasionally a small number of blood cells and cells from the vagina wall.
Figure 39. Histological examination of a sample obtained on day 15 of the estrus cycle. (a) The sample is approximately 1 mm in diameter. (b–d) The histological composition of the sample is heterogeneous and contains luteal cells (b) and follicular cells, such as granulosa cells (c) and stromal cells (d).
Discussion
1. Parturition and subsequent uterine health and fertility

This study demonstrated, for the first time, an association between the length of farrowing and the subsequent pregnancy rate. Furthermore, this study demonstrated, also for the first time, that retained placentae can occur in sows. In general, the results indicated that prolonged farrowing has a negative effect on postpartum uterine health. The use of exogenous at the end of parturition, on the other hand, seems to support placental expulsion and subsequent uterine health.

On average the last placenta was expelled within 4.5 h after birth of the last piglet and at latest after 15.5 h, in overall agreement with the general opinion that the placenta is expelled within 4 h, but may take up to 12 h (Taverne and Noakes, 2009). However, only about 50% of the sows expelled their placenta within 4 h, which indicates that longer placenta expulsion durations are not unusual. Overall, the duration of placenta expulsion depended mainly on the use of exogenous oxytocin. If oxytocin was used, more placental parts were expelled faster. That indicates that there a direct link between farrowing duration and placental expulsion since both prolonged farrowing (Oliviero et al., 2008; Castrén et al., 1993) and prolonged placental expulsion (in this study) were associated with lack of oxytocin. If oxytocin was not used, farrowing duration and placenta expulsion duration were significantly quadratically associated. However, when sows with extremely short and long farrowing duration (< 100 and > 800 min) were excluded from the analysis, farrowing duration and placenta duration were linearly correlated, as in the study of Rens and Van der Lende (2004). Thus, it can be confirmed that in sows and gilts the placenta duration increases with increasing farrowing duration. Furthermore, this study showed that placenta duration was very short when farrowing duration was longer than 800 min, which could be a sign of impaired placenta expulsion and therefore placenta retention.

Furthermore, the same phenomenon was recorded for the number of expelled placental parts, which depended entirely on the use of oxytocin. Only if oxytocin was used did placental parts depend on the number of liveborn piglets. This is in agreement with the finding of Van Rens and Van der Lende (2004). In contrast, when oxytocin was not used, the number of liveborn piglets was not correlated and farrowing duration was quadratically correlated with the number of expelled placental parts. This is another indication that sows with prolonged farrowing might have experienced impaired placenta expulsion due to lower oxytocin concentrations. A third indication is that the expulsion of the last placental part relative to the last piglet was also influenced by oxytocin. In contrast, the onset of placenta expulsion seems to be entirely associated with duration of farrowing. In sows with short farrowing, placental expulsion started after the birth of the last piglet, whereas in sows with prolonged farrowing, placental expulsion started before the birth of the last piglet. This observation is in agreement with the report of Van Rens and Van der Lende (2004). Additionally, we observed delayed expulsion of the first placenta in older sows that received no oxytocin, which could also be related to lower oxytocin concentrations in older sows and is supported by the finding of Oliviero et al. (2008), who showed that older sows housed in farrowing crates had lower oxytocin concentrations.
Sows with partially retained placentas (no placenta expulsion after the expulsion of the last piglet) and sows with totally retained placentas (no placenta expulsion) had extremely long farrowing durations, of about 11 h and 17 h, respectively. These sows accounted for 3 - 6% of all sows and none received oxytocin during parturition. This finding has not been reported earlier for gilts or sows. In other species, causes of retained placenta are generally considered to be factors that interfere with normal maturation and/or expulsion of the placenta (Parkinson, 2009; Paccamonti and Pycock, 2009). Birth before maturation can be caused by, induced parturition or stress, for example. In the present study, none of the parturitions were induced and stress levels were similar for all sows because all sows experienced the same farrowing environment and management. Furthermore, we observed no effect of the gestation length on the placenta expulsion, which would indicate premature parturition. Thus, it is more likely that the retained placentas were rather caused by factors interfering with normal placenta expulsion. Impaired expulsion of the placenta can be due to a physical obstruction, such as retained piglet or cervical closure, or inadequate uterine contractions (Parkinson, 2009; Paccamonti and Pycock, 2009). No retained piglets were detected in sows with retained placentas. On the other hand, cervical involution starts already six hours after start of the second phase of parturition (Waldmann, 2009). The cervix will be still open but loss of elasticity, stretchability may complicate expulsion of the placentae. Nevertheless, most likely inadequate uterine contractility caused the impaired placenta expulsion.

The findings that the farrowing duration, number of stillborn piglets and number of obstetrical interventions were negatively associated with the occurrence of intrauterine fluid, and therefore puerperal metritis, is in agreement with the findings of Bostedt et al. (1998) in gilts, where 85.9% of gilts with consequent puerperal illness had farrowing durations of longer than 6 h, whereas 78.8% of control gilts of the same age finished parturition in less than 3 h. Other significant factors affecting the incidence of feverish puerperal illness were frequency of obstetrical intervention and stillbirth rate. The frequency of obstetrical intervention measured in the group of patients was 27%, but only 9.5% in the group of control sows. Thus, risk factors for puerperal metritis in gilts and sows seem to be similar and may be generalized for all female pigs. Furthermore, retention of placentae has not yet been associated with puerperal metritis in gilts but was evident in the present study. The reason why retained placentae increase the risk for uterine infection is not clear but may be due to retained placenta increasing bacterial contamination and providing a suitable medium for bacterial growth within the uterus, as mentioned by Maes et al. (1998). He also reported that prolonged farrowing increases the bacterial contamination within the uterus probably due to either prolonged opening of the cervix, and therefore prolonged reduction of the physical barrier against contamination, and/or reduced reduction in uterine contractions (Castrén et al., 1993; Oliviero et al., 2008), and therefore reduced uterine clearance.
Increased uterine contamination may also have been the reason why obstetrical intervention increased the risk for uterine infection. It is likely that during the obstetrical interventions additional contamination of the birth canal occurs, especially when the genital area of the sow is not washed with an antiseptic solution before intervention, as was the case in the present study. In conclusion, it can be said that prolonged farrowing most likely increases the odds for puerperal metritis to develop and therefore postpartum dysgalactia (PDS). It can be supported by evidence that prolonged parturition and PDS have similar risk factors. Risk factors for PDS were reviewed by Maes et al. (2010) and risk factors for prolonged farrowing by Peltoniemi and Oliviero (2015). Common risk factors are constipation, low dietary fiber, high body condition and restricted movement inside farrowing crates.

Furthermore, the present study showed that farrowing duration could increase the time for uterine involution because sows with prolonged farrowing have larger uterine size at the beginning of involution. Considering that farrowing duration has increased substantially during recent decades, it is possible that uterine involution takes longer in modern hyperprolific sow lines than it has been reported earlier (Palmer et al., 1965; Kudlac and Groch, 1979; Busch, 2007). In addition to farrowing duration, all other factors affecting intraterine fluid were also found to increase uterine size. The reason may be that an inflamed uterus is usually enlarged (Kauffold et al., 2005). Thus, puerperal metritis may be one of the major causes of delayed uterine involution. On the other hand, the administration of oxytocin supported uterine involution. The reason may be that oxytocin supports uterine contractions and therefore uterine clearance. Considering that cervical involution and closure occur rapidly within a week, supported uterine clearance before that may lead to the uterus needing to resorb less compared with the case for sows that do not receive oxytocin.

Nevertheless, the present study also showed that postpartum ultrasound may be a practical and adequate tool to assess uterine involution. Busch (2007) investigated whether the visual study of the degree of edematization or the formation and length of the vulva could be used as an indicator for the point in time of uterine involution, but they did not show them to be appropriate parameters. Nevertheless, whether determination of the mean sectional area with the aid of ultrasound is an appropriate parameter remains to be investigated. The present study showed that there are large differences between sows and follow-up studies are needed on sows with ultrasound until involution is completed. The present study also shows that at least parity, probably also body size, should be taken into consideration because parity significantly affected uterine size. On the other hand, many authors disagree about the importance of the size of the uterus at the end of involution and whether uterine size is a good indicator (Kauffold et al., 2005). Nevertheless, ultrasound examination of the uterus at the beginning of the puerperium may represent a practical tool to anticipate the length of uterine involution and therefore could be used in reproductive management of the sow.
How prolonged parturition may negatively affect subsequent fertility was investigated, but the explanation for the connection remains unclear and further research is required. Contrary to the hypothesis, sows with prolonged farrowing did not have impaired follicular development after weaning and reduced oxytocin release during boar stimulation at subsequent estrus. In contrast, sows, especially young sows, with prolonged farrowing had slightly larger follicles and released more oxytocin compared with sows that farrowed normally. There are two possible explanations. One is that, as with cows, impaired uterine health, such as a uterine bacterial infection, and/or the associated inflammation, suppresses postpartum pituitary LH secretion and therefore perturbs postpartum ovarian follicular growth and ovulation (Peter et al., 1988; Sheldon et al., 2002; Opsomer et al., 2000). Follicles of sows with suppressed LH secretion will have larger pre-ovulatory follicles than sows with normal LH secretion because pre-ovulatory follicles may experience impaired luteinization of the follicular cells. Furthermore, sows with suppressed LH secretion will either experience ovulation failure or ovulate later than sows with normal LH-secretion. Ovulation failure will result in regression of follicles or development of follicle cysts. Late ovulation will cause release of reduced quality oocytes. On the other hand, in this study, the time between weaning and ovulation did not differ significantly for sows with normal and prolonged farrowing. Therefore, it is unlikely that there were significant differences in LH secretion between normal and prolonged-farrowing sows and therefore timing of ovulation. Another explanation why sows with prolonged farrowing had larger follicles after weaning could be lactation weight loss, the major factor affecting follicular growth after weaning (Soede and Kemp, 2015). If weight loss is high, follicular growth is low. Thus, in this study, sows with prolonged farrowing would have hypothetically lost less weight compared with those that farrowed normally because they had larger follicles. High weight loss is mainly caused by either low energy intake (reduced feed intake) or by high energy output (increased milk production). Since it is possible that postpartum dysgalactia (PDS) is the link between prolonged farrowing and subsequent subfertility it is thinkable that sows with PDS loose less weight because one of the major symptoms of PDS is reduced milk production. On the hand, this explanation is contrary to that of Tummaruk et al. (2013) and Hoy (2006). Tummaruk et al. (2013) showed that sows with prolonged farrowing had reduced feed intake for a longer time after farrowing than sows that farrowed normally. Thus, sows with reduced feed intake should experience higher lactation weight loss, in line with the findings of Hoy (2006) who reported that sows with PDS had a higher rate of anestrus after weaning or a higher rate of late onset of estrus which are typical signs for high lactation weight loss (Soede and Kemp, 2015).

A schematic presentation of the proposed mechanisms playing a role in the aetiology of puerperal diseases in sows is presented in Figure 40.
Figure 40. A schematic presentation of the proposed mechanisms playing a role in the aetiology of puerperal diseases in sows.
2. Ultrasound-guided biopsy collection of luteal tissue

The present study shows that it is possible to conduct a transvaginal ultrasound-guided biopsy (TUB) in pigs. The success rate, defined as the proportion of successful biopsies, of 50% is lower compared with that for cows (92%; Kot et al., 1999) and horses (68%; Beg et al., 2005). One explanation could be the smaller size of this species, and therefore narrower vaginal and rectal diameters, which make it harder to guide the ovary and biopsy needle towards each other. This effect is also seen in the correlation between success rate and sow factors. The older (after 3rd farrowing) and bigger (weight > 250 kg or backfat > 6 mm or both) the sow was, the more successful the biopsy. Furthermore, it seems that the success rate can be improved by increasing the time between two consecutive biopsies. In the first part of the study, when the success rate was 75%, 10.5 d was the average time between biopsies, whereas in the second part, when success rate was 38%, it was only 3 d. One explanation could be the swelling of the rectal wall due to the manipulation of the ovary. We noticed that with only 3 d between two biopsies the rectal wall remained swollen, which makes it hard to find, take, and pull the ovary against the vaginal wall. There seems to be no impact on the reproductive performance, such as cyclicity and pregnancy status, of the sow.

Our findings suggest that the four sows, included in the first part of the study, cycled normally during the sampling period of three consecutive cycles because they had normal sized functional bodies on each sampling day (Soede et al., 2011). Furthermore, we found no differences in the reproductive performance of the pregnant sows. The findings of small multifocal blood clots at the ovaries, during post-mortem investigation of the reproductive tract, and the reddish appearance of the biopsies, indicate that mild focal bleeding occurred. However, the bleedings appear to have no effect on reproductive performance. The size of the sample, 1 mm in diameter, is very small compared with that for cows (11.7 mm long; Kot et al., 1999) and horses (Beg et al., 2005). One explanation for why the sample is so much smaller could be the smaller size of the corpus luteum (10 mm; Langendijk and Peltoniemi, 2013) compared with that for cows (20-30 mm; Quintal-Franco et al., 1999) and horses (20-35mm; Allen 2001). Any minor deviation of the biopsy needle from the midline of the corpus luteum could potentially lead to a biopsy of significantly reduced size. That probably explains also the heterogeneous composition of the biopsy. If the biopsy needle hits the corpus luteum at the periphery rather than in the center, it is possible also that other cells, such as stroma cells, of the ovary are sampled. However, if the samples are intended to be used for RNA studies, such as real time qPCR and other gene expression studies, it is important to realize that the sample contains heterogeneous tissue. In these circumstances it might be beneficial to make use of methods that aim at individual cells, i.e. laser microdissection or immunohistochemistry. Still, it is possible to conduct gene expression studies. A RNA integrity number (RIN) of higher than 8 shows that RNA of sufficient quantity and quality can be collected. The RIN ranges from 1 to 10, where 1 indicates that RNA is completely degraded and 10 that RNA is completely intact (Schroeder et al., 2006). A RIN of higher than 8 is generally regarded as perfect total RNA and samples can be used for downstream applications (Fleige and Pfaffl, 2006).
Conclusions, practical implications and future research
1. Conclusions

1. Prolonged farrowing negatively affects subsequent fertility and sows with prolonged farrowing may require attention after farrowing.

2. Ultrasound examination of the uterus after farrowing is a practical and reliable tool to assess whether uterine health is compromised.

3. Prolonged farrowing impairs placenta expulsion and can lead to retained placentae.

4. Administration of exogenous oxytocin improves placenta expulsion and decreases uterine size in the first week postpartum and therefore may support uterine involution.

5. Prolonged farrowing and retained placentae, as well as obstetrical intervention and more than two stillborn piglets, increase the risk for intrauterine fluid accumulation and increased uterine size in the first week postpartum.

6. It is possible to collect luteal tissue of sows in vivo for research purposes using a transvaginal ultrasound-guided biopsy method.
2. Practical implications

1. Proper farrowing management and prevention of prolonged farrowing is important in order to preserve subsequent fertility and should be implemented on-farm.

2. Sows with a farrowing longer than 600 min are at risk of having retained placentae and developing puerperal metritis and therefore may need administration of exogenous oxytocin after the birth of the last piglet.

3. Obstetrical intervention of the birth canal during farrowing should only be done after proper obstetrical examination and in a hygienic and gentle manner.

4. Sows receiving obstetrical intervention and/or having more than two stillborn piglets may also be at risk of developing puerperal metritis and therefore may require administration of exogenous oxytocin after the birth of the last piglet.

5. A postpartum ultrasound examination of the uterus, e.g. three days after farrowing, is indicated for those sows at risk of developing puerperal metritis so as to be able to diagnose and treat sows with puerperal metritis.
3. Future research

1. How prolonged farrowing affects subsequent fertility needs further research.

2. How prolonged farrowing affects uterine involution needs clarification. Thus, uterine involution should be followed-up until the end and compared between sows with normal and prolonged farrowing.

3. How incomplete uterine involution at weaning and puerperal metritis affect subsequent fertility needs further attention. For instance, in other species, puerperal metritis or incomplete uterine involution can interrupt the subsequent pre-ovulatory LH surge. It should be investigated if that is the case also in sows and if it could be a reason for reduced subsequent fertility.

4. Sows with prolonged farrowing had larger follicles during the weaning-to-ovulation interval. One of the major factors affecting follicular size and growth is lactation weight loss. Thus, the relationship between farrowing duration and lactation weight loss should be investigated.

5. Sows with prolonged farrowing, i.e. a farrowing longer than 300 min, may need further classification. For instance, those sows may need to be further divided into those with a farrowing of between 300 and 600 min and those with a farrowing duration longer than 600 min. Because sows with a farrowing longer than 600 min are at increased risk of retained placentae, it is possible that those sows are at a higher risk of becoming clinically diseased, whereas sows with a farrowing of between 300 and 600 min may be only sub-clinically affected. There may be differences in the effect on subsequent fertility for clinically and subclinically diseased sows.
References


Original articles