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Concentration of oxytocin at the time of ovulation in cattle with and without synchronised ovulation

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submitted by

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Contents

Zusammenfassung

Seit Jahrzehnten steigen die Anforderungen an die moderne Landwirtschaft, wodurch LandwirtInnen regelmäßig vor neue Herausforderungen gestellt werden. Auch in der Landwirtschaft spielt Wirtschaftlichkeit eine immer bedeutendere Rolle, dadurch steigen die Anforderungen an eine verlässliche und effiziente Brunsterkennung. Hormonprogramme zur Östrussynchronisation und zur künstlichen Besamung werden häufig eingesetzt und dementsprechend viel erforscht, da ein korrekter und gewissenhafter Einsatz von Hormonen in der Landwirtschaft höchste Relevanz besitzt. Dementsprechend stellt die Erweiterung des Wissens über Hormonprogramme und deren Auswirkung auf die Physiologie der Tiere bzw. auf reproduktive Vorgänge die Basis für viele Studien dar, welche in den letzten Jahrzehnten durchgeführt wurden. Mehrere Studien untersuchten hormonelle Unterschiede zwischen Kühen mit induzierter und natürlicher Brunst, vermehrt wurden allerdings typische Sexualhormone wie Progesteron oder Östrogene gemessen. Diese Studie wurde durchgeführt, um den Unterschied der Oxytocin- Cortisol- und Progesteronkonzentrationen bei Kühen mit und ohne Ovulationssynchronisation zu untersuchen. In der Regulation des hormonellen Zyklus bei Rindern spielt Oxytocin eine wichtige Rolle, zudem wurde es als Indikator für das Wohlbefinden von Kühen entdeckt. Weil Stress bekannter Weise eine wesentliche Rolle in der modernen Milchviehhaltung spielt, wurde die Cortisolkonzentration im Speichel der Tiere gemessen. Das typische Sinken der Progesteronkonzentration zum Zeitpunkt des Östrus wurde als Indikator für die richtige Brunsterkennung verwendet. Zwölf laktierende Milchkühe wurden in dem randomisierten Versuchsfeld in zwei Gruppen unterteilt. Tiere der Gruppe 1 erhielten keine Behandlung, hatten dementsprechend einen natürlichen Östrus und eine unbehandelte Brunst. Bei Tieren der Gruppe 2 wurde die Brunst durch ein Ovsynch Protokoll synchronisiert. Die Blut- und Speichelproben von jedem Tier der beiden Gruppen wurden täglich genommen, beginnend vier Tage vor der errechneten, nächsten Brunst, bis zwei Tage nach der Brunst. Die gemessenen Oxytocinkonzentrationen befanden sich zwischen 0,39ng/ml und 2,10ng/ml, kein signifikanter Unterschied zwischen Tieren mit natürlichem Östrus und synchronisierter Brunst war erkennbar. Ebenfalls konnte zwischen den beiden Versuchsgruppen kein signifikanter Unterschied in den Progesteronkonzentrationen festgestellt werden. Die Cortisolkonzentrationen beider Gruppen unterschieden sich ebenfalls nicht signifikant. Aus den Ergebnissen lässt sich schließen, dass eine Ovulationssynchronisation keine Auswirkung auf die hormonelle Situation oder das Wohlbefinden der Tiere hat.

Abstract

Dairy farms have faced many challenges and changes over the last decades. As economic efficiency became more important, the requirements for a reliable and accurate oestrus detection increased. According to this, hormone programmes to induce or synchronise the oestrus cycle of cows and to time the ovulation for artificial insemination became more relevant. Because the use of hormones in livestock animals is subjected to criticism by public, a correct and prudent handling of those programs is necessary. Thus, increasing the knowledge about the effects of hormone programmes on the animals' physiology and resulting reproductive and economic performance has been the goal of numerous studies since decades. In the past, several studies have shown the hormonal pattern of cows with induced oestrus compared to non-induced oestrus, but most of them focused on typical sexual hormones, such as progesterone and oestrogens. This study was designed to analyse the concentrations of oxytocin and cortisol in comparison with progesterone in cows with synchronised ovulation and natural oestrus. Oxytocin plays an important role in the oestrous cycle of cattle. Furthermore, it has been suggested as indicator for well-being. Since stress is an important issue in dairy farming cortisol concentration in saliva was measured. A drop in progesterone concentration was used as indicator for the onset of oestrus. In this randomized field trial, 12 lactating dairy cows were allocated to two groups. Animals in group 1 remained without treatment and had a natural oestrus and ovulation. The oestrus of animals in group 2 was synchronised with an Ovsynch protocol. Sampling of blood and saliva started four days before expected oestrus and was repeated daily until two days after oestrus. Oxytocin concentrations ranged from 0.39ng/ml to 2.10ng/ml, with no significant difference between cows with synchronised ovulation and natural oestrus. There were no significant differences in progesterone concentrations between the two groups. Also, the concentrations of cortisol showed no significant difference between cows with and without synchronised ovulation. In summary, we found no significant differences in hormone concentrations between the groups, indicating that the use of an Ovsynch protocol has no effect on well-being -related hormones.

1. Introduction

The role of oxytocin in the regulation of the oestrous cycle in ruminants, including cattle (Armstrong and Hansel 1959) has been the topic of numerous studies since decades. The administration of oxytocin resulted in a premature regression of the corpus luteum in cows (Armstrong and Hansel 1959). This luteolytic effect could be blocked by immunisation against oxytocin in ewes, which resulted in a prolonged luteal phase. Both, active and passive immunisation against oxytocin delayed luteal regression in ewes (Sheldrick et al. 1980, Schams et al. 1983,). The luteolytic effect of oxytocin was also related to prostaglandin, as a positive correlation between oxytocin and prostaglandin has been observed. The release of oxytocin from the corpus luteum in response to a luteolytic prostaglandin analogue suggested that there might be a positive feedback between the ovary and the uterus (Flint et al. 1990). It has been suggested that the activation of that feedback loop begins on the uterine side of the loop since concentrations of prostaglandin in the utero-ovarian venous effluent increased before any increase in concentrations of oxytocin were detectable during spontaneous luteolysis (Silvia et al. 1991). Oxytocin release according to a prostaglandin injection was demonstrated in a study where the amount of released oxytocin was dependent on the dosage of the prostaglandin administration (Skarzynski et al. 1997). Conversely, a study also showed an increase of the utero-ovarian prostaglandin concentration in response to an oxytocin administration (Sharma and Fitzpatrick 1974). This effect was less pronounced in pregnant cows or cows with late embryonic death than in non-pregnant cows or cows with early embryonic death (Kubo et al. 2018). In addition to the association between oxytocin and prostaglandin, a close relationship has also been demonstrated between the secretion of oxytocin and progesterone from the corpus luteum (Flint and Sheldrick 1983). A study analysing the secretion of hormones during the luteal phase of the oestrus cycle in cattle observed a parallel pulsatile secretion of oxytocin and progesterone during the mid-luteal phase and described a higher mean concentration of oxytocin in the vena cava than in jugular vein plasma (Walters et al. 1984). Another study showed an oxytocin-induced release of progesterone in a dose-dependent manner, and that a cell-to-cell contact seems to be very important for the luteal response to oxytocin (Miyamoto and Schams 1991). Experiments by Kotwica et al. (1991) revealed the ability of norepinephrin to stimulate the release of both oxytocin and progesterone (Kotwica et al. 1991). Oxytocin was suggested to have an auto- or paracrine function in the regulation of the reproductive process rather than a systemic function (Kotwica et al. 1991, Miyamoto and Schams 1991).

Furthermore, some studies have been published describing the origin, control of synthesis and dynamics of oxytocin concentrations during the oestrous cycle. The occurrence of high concentrations of oxytocin in the corpus luteum coupled with the presence of bovine neurophysin I confirmed that oxytocin is synthesised locally in the corpus luteum (Wathes et al. 1984). Moore et al. (1986) also described the corpus luteum as a major source for pulsatile peaks of oxytocin. Analysis of the oxytocin-associated neurophysin revealed a significant venous-arterial difference between the ovary and uterus but not across the ovary and the head (Moore et al. 1986). The dynamics of the oxytocin concentration during the oestrous cycle was described as follows: Oxytocin levels increase in the first two quarters of the oestrous cycle, show a decline or maintenance in the third quarter and are low at the end of the oestrus cycle (Wathes et al. 1984, Parkinson et al. 1992). Wathes et al. (1984) identified two groups of cows, one with high and one with low oxytocin concentrations during the oestrous cycle. The dramatic increase of specific oxytocin mRNA expression in luteinising tissue at the time of ovulation has been observed and has led to the conclusion that ovulation triggers oxytocin gene expression in the corpus luteum. In contrast no variation in hypothalamic oxytocin mRNA expression was found (Ivell et al. 1985).

First doubt about the direct luteolytic effect of oxytocin came up in 1983, when administration of cloprostenol, a prostaglandin analogue, to hysterectomised ewes resulted in luteal regression, which occurred as rapidly as in intact animals (Sheldrick and Flint 1983). Based on the knowledge of norepinephrin-induced release of ovarian oxytocin Kotwica and Skarzynski (1993) tried to influence the duration of the oestrus cycle. As norepinephrine indeed reduced the concentration of oxytocin in the corpus luteum but animals showed no difference in the duration of the oestrus cycle, the role of oxytocin in regulating the oestrus cycle was assumed to be more modulating than mandatory (Kotwica and Skarzynski 1993). Since the use of an oxytocin antagonist did not affect the duration of the oestrus cycle nor the time of luteolysis, the facilitating role of ovarian oxytocin was suggested again (Kotwica et al. 1997). Another study suggested that the contribution of oxytocin in cattle may be less than that supposed for the ewe, because in cattle there was no detectable difference between oxytocin-release after treatment with prostaglandin in the study group nor in the control group with spontaneous luteolysis (Shaw and Britt 2000).

As summarised above, the role of oxytocin in the regulation of oestrus and ovulation has been studied since decades. Another aspect of this hormone is its potential as an indicator for animal well-being, as recently reviewed by Rault et al. (2017). The authors of this critically review came to the conclusion that, although suggested by several authors, so far only studies

provided valid results about the correlation between oxytocin concentration and measurable well-being. Furthermore, methodologies vary a lot between studies and make it hard to compare the results.

Schweinzer et al. (2020) worked with two groups of cows, one with synchronised ovulation and one with natural oestrus to compare behavioural patterns. They worked with an ear-tag based accelerometer system to detect the oestrus and synchronised one group of the animals with an Ovsynch protocol. Behavioural patterns showed significant changes in cows with natural oestrus between non-oestrus days and the day of oestrus. This massive changes could not be detected in cows with induced ovulation (Schweinzer et al. 2020). Basing on the idea of the previously mentioned study of Schweinzer et al. (2020), the aim of our study was to compare hormone concentrations of cows with synchronised ovulation and natural oestrus. As oxytocin plays an important role in the oestrous cycle of the cattle, our intention was to find out whether the concentration of oxytocin differs in cattle with and without synchronised ovulation at the time of ovulation. The results might contribute to the discussion why hormone programs often have lower conception rates compared to cows that were inseminated at natural oestrus (Tenhagen et al. 2004). Therefore, we worked with two groups of cows, one with natural oestrus and one with induced ovulation using the Ovsynch protocol.

Other focuses set were the control of the course of the oxytocin concentration around ovulation and the determination of a correlation between the concentration of oxytocin and progesterone in blood and cortisol in saliva. The course as well as the function of progesterone in the female reproduction is well known and the concentration of progesterone in variation in time represented a reference point for our study. Since stress plays an important role in dairy farming and reproduction, cortisol was measured primary to identify whether the cortisol concentration in synchronised cows differs from non-synchronised cows. The relationship between stress and the intensity of oestrus behaviour has been controversy discussed among researches. In a study by Stoebel and Moberg (1982), stressed cattle showed the same oestrus behaviour than not stressed ones, hence oestrus behaviour seemed to be unaffected by stress. Studies also suggested that stress is able to disrupt preovulatory LH surge (Stoebel and Moberg 1982) and showed an inhibitory role of cortisol on reproductive events (Stoebel and Moberg 1982). Management-related stress, such as transport or isolation affected oestrus behaviour in cattle and reduced the average length of oestrus (Ehnert and Moberg 1991). Another study found a relationship between stress and the disruption of oestrus behaviour, whereas cortisol levels reached its maximal concentration towards the time of ovulation (Lyimo et al. 2000). Conversely, the oestrous cycle of oestrogen-treated ovariectomised cows could

not be affected by a treatment with cortisol in a study by Cook et al. (1987). The result of this study led to the conclusion that an infusion of cortisol altered the ovarian function, but oestrous behaviour of the cows was stimulated by the treatment with oestrogen (Cook et al. 1987). Further studies revealed the important local influence of cortisol and suggested that formation and function of the corpus luteum might benefit from local cortisol release (Andersen 2002, Majewska et al. 2012). Measurement of cortisol in this study was conducted to compare the stress level of the two study groups apparent in saliva cortisol concentration, whether cattle with synchronised ovulation experienced more stress than the control group with natural oestrus.

The aim of this study was to test the hypothesis that the blood concentration of oxytocin differs between cows with and without synchronised ovulation.

2. Material and Methods

2.1. Animals and experimental design

This study was approved by the institutional ethics committee and the national authority according to §8 of Law for Animal Experiments, Tierversuchsgesetz- TVG (BMWFW-68.205/0004-WF/V/3b/2016).

This study was conducted at the VetFarm Kremesberg of the University of Veterinary Medicine Vienna, Austria, in 2020. Before calving, 12 dairy cows were randomly allocated into two groups. Group 1 (n=8) remained without treatment after the first detected oestrus, whereas group 2 (n=4) received an Ovsynch protocol, see chapter 3.3. To identify the time of the first oestrus, oestrus detection was performed by 3D accelerometer sensors (Smartbow®, MKW-electronics GmbH, Weibern, Austria), starting between day 28 and 30 after calving. Additionally, the cows were continuously monitored by cameras. Further information about the stage of the oestrus cycle were gained by rectal palpation and ultrasound examination of uterus and ovaries. These methods of oestrus detection in combination with the daily observation by the employees of the VetFarm were used to predict the next expected oestrus, i.e. the day of the first detected oestrus + 21 days for group 1. In group 2, the Ovsynch protocol was initiated 14 days after the first detected oestrus.

2.2. Sampling group 1

In group 1, blood and saliva sampling started four days before expected oestrus and was repeated in daily intervals until two days after oestrus (Fig. 1). As animals in group 1 remained without any treatment, results of their hormone analysis served as basis (day 0) for the comparison with animals in group 2.

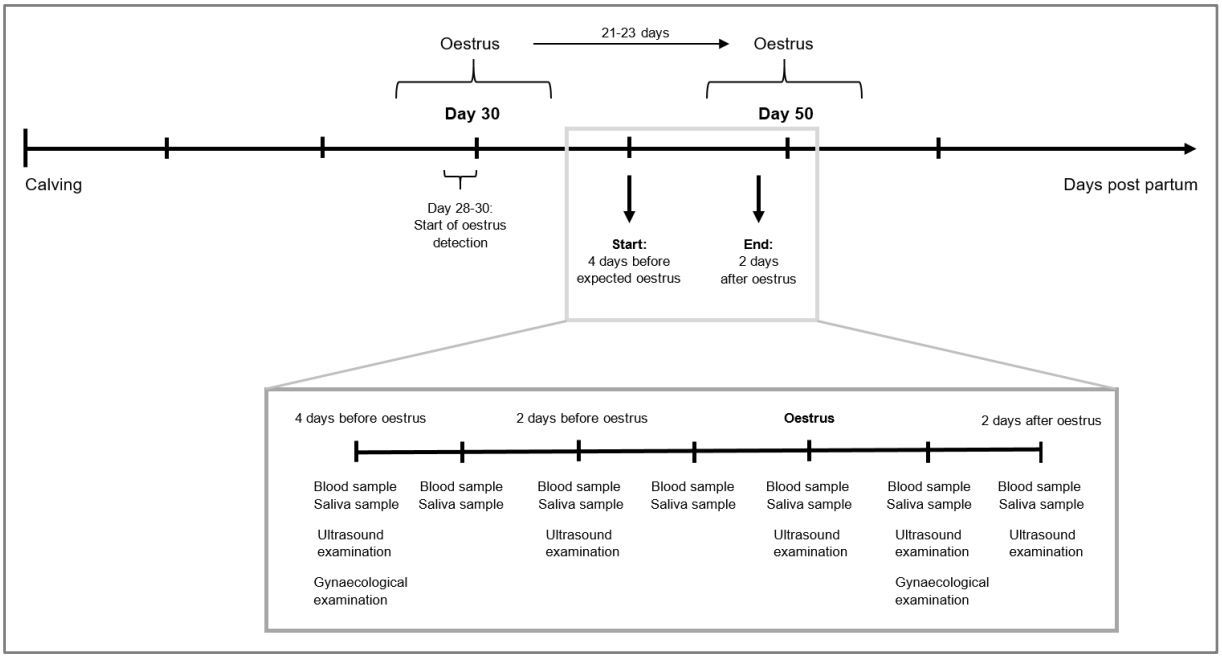

Figure 1: Sampling schedule in group 1

2.3. Sampling group 2

Animals in group 2 received an Ovsynch protocol, starting on day 14 after the first detected oestrus (Fig. 2). The cows received the first injection of 0.01 mg of Buserelin (2.5 ml i.m., Receptal®, Intervet International B.V., Kenilworth, NJ, USA) on starting day of the programme, 7 days later 0.5 mg of Cloprostenol-Natrium (2 ml i.m., Estrumate®, Intervet International B.V., Kenilworth, NJ, USA) and 48 hours later 0.01 mg of Buserelin. Buserelin is a synthetic analogue of Gonadorelin and is used for GnRH-like effects. Cloprostenol-Natrium is a synthetically produced prostaglandin $F_2\alpha$-analogue. In group 2 blood and saliva sampling started five days after the first GnRH administration, four days before the next expected oestrus and was repeated daily until two days after the final GnRH administration (Fig. 2).

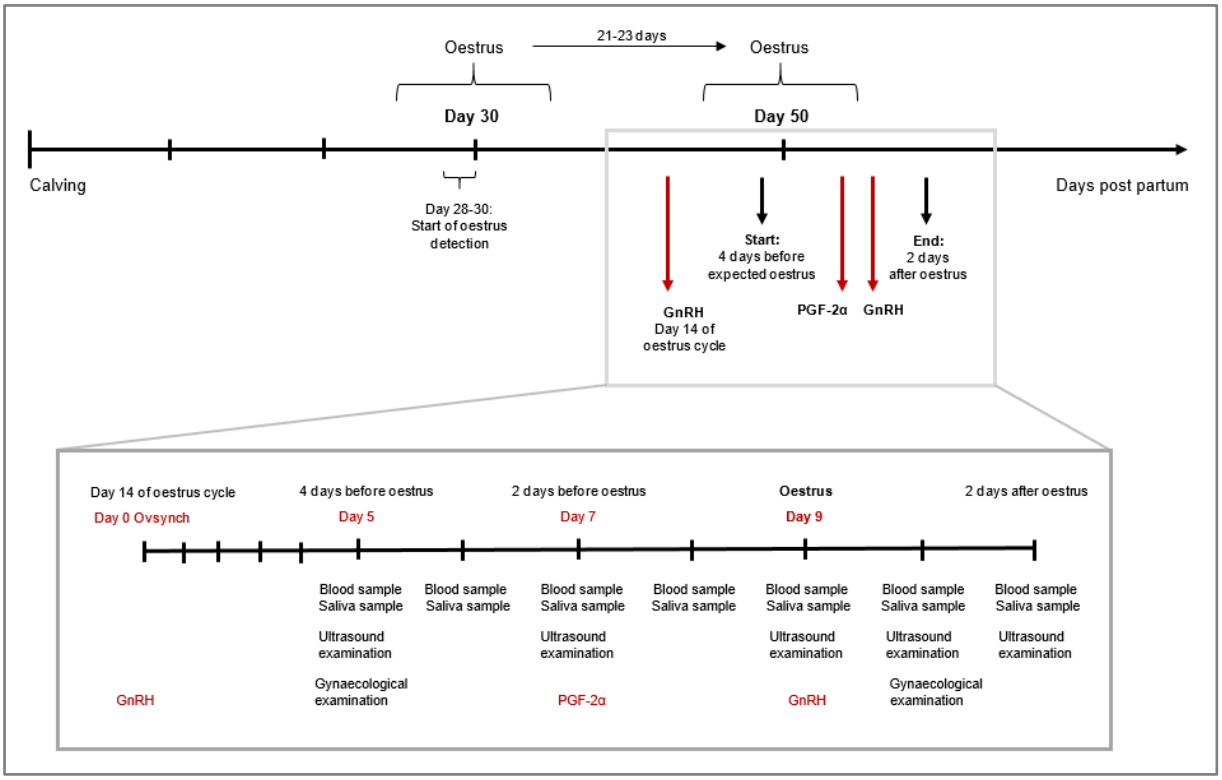

Figure 2: Ovsynch protocol and sampling schedule in group 2

2.4. Hormone analysis

For determination of oxytocin and progesterone concentrations, blood samples were taken from all cows, both group 1 and group 2. For every blood sample, 10 ml of blood was collected from the V. jugularis with a Vacuette®- system (Vacuette® Tube Premium, Greiner Bio-One GmbH, Kremsmünster, Austria) with 20G/ 0,9x38 needles. After collecting, samples were centrifuged at 2200 x g, at a temperature of 18°C for 10 min, cooled, and stored at -20°C until analysis in the laboratory.

Analysis of plasma oxytocin was performed by enzyme immunoassay without extraction (Demeditec Diagnostics, Kiel-Wellsee, Germany) as described (Melchert et al. 2019). The intra-assay coefficient of variation was 6.81%, the interassay coefficient of variation 9.70% and the minimal detectable concentration was 0.01ng/ml. The validated solid-phase immunoassay method (Progesterone ELISA kit, Enzo Life Sciences Inc., NY, USA) was used for the analysis of progesterone concentrations (Pothmann et al. 2015). The intra-assay coefficient of variation was 5.32%, the interassay coefficient of variation 10.00% and the minimal detectable concentration was 0.01ng/ml.

Saliva samples for cortisol measurement were conducted with commercialised tubes for saliva sample drawing and analysis for cortisol (Salivette® Cortisol, Sarstedt AG&Co., Nümbrecht-Rommelsdorf, Germany). A commercial enzyme immunoassay without extraction (Demeditec Dignostics, Kiel-Wellsee, Germany) was used for the analysis of cortisol concentrations (Nagel et al. 2016). The intra-assay coefficient of variation was 15.45%, the interassay coefficient of variation 6.76% and the minimal detectable concentration was 0.005ng/ml.

2.5. Statistical analysis

Statistical analysis for this study were performed with SPSS Statistics 23 (IBM Cooperation, New York, USA). Data were illustrated using the graphic app Prism® (GraphPad Software, San Diego, USA) and presented as mean ± standard error mean (SEM).
Normal distribution was tested by using the Kolmogorow-Smirnow-test and the Shapiro-Wild-test. Results showed a normal distribution of oxytocin, progesterone and cortisol data. Oxytocin, progesterone and cortisol concentrations were compared between group 1 and group 2 by analysis of variance with repeated measurements (ANOVA). The variation of data in time was utilised as main effect. The variation of blood concentration of oxytocin, progesterone and cortisol in time was analysed separately within the groups (time = within subject factor) as well as variation of hormone concentrations in time between the two groups (time = between subject factor). By using Pearson's correlation coefficient statistical relationship between data of blood concentration of oxytocin and data of concentration of progesterone and cortisol, respectively was calculated as well as statistical relationship between data of blood concentration of progesterone and cortisol.

3. Results

3.1. Oxytocin

Results of oxytocin concentrations in group 1 and group 2 are illustrated in Figure 3 as mean ± SEM. Time had no significant effect on oxytocin concentration (P = 0.306). The course of oxytocin in variation in time comparing the two groups was significantly different with P < 0.05 (P = 0.033), whereas the group had no significant effect on concentrations of oxytocin (P = 0.729). No significant correlation was found between oxytocin and progesterone (r = 0.031) nor between oxytocin and cortisol concentrations (r = -0.091).

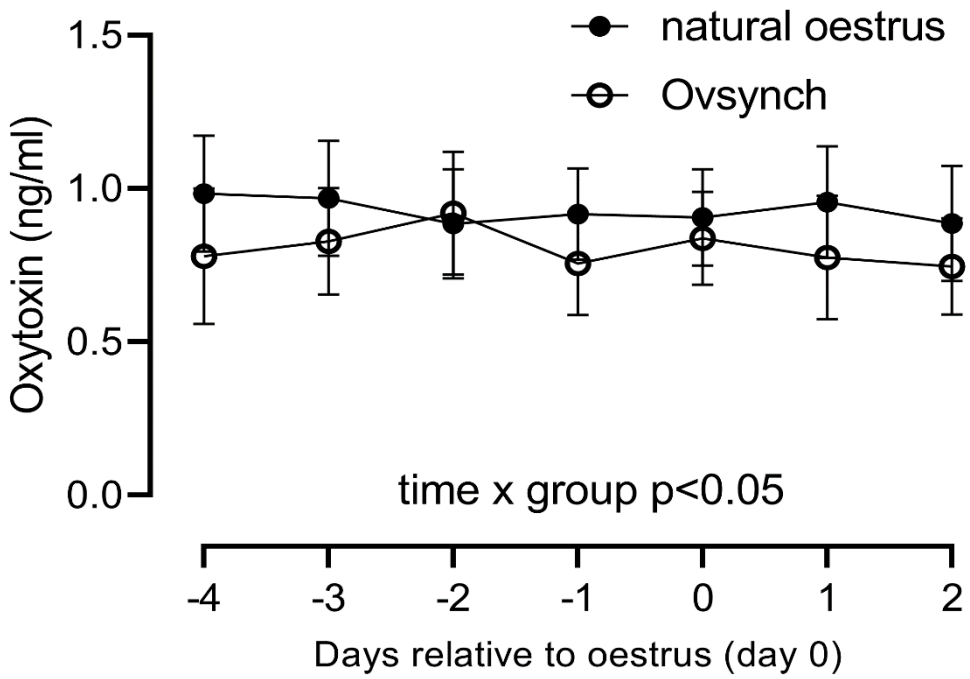

Figure 3: Concentrations of oxytocin in group 1 and group 2 (mean ± SEM)

3.2. Progesterone

Results of progesterone concentrations are illustrated in Figure 4 as mean ± SEM. Time had a significant effect on the progesterone concentrations with $P < 0.01$. Both time*group interaction and group had no significant effect on concentrations of progesterone ($P > 0.05$). No significant correlation was found between progesterone and cortisol concentrations ($r = 0.071$).

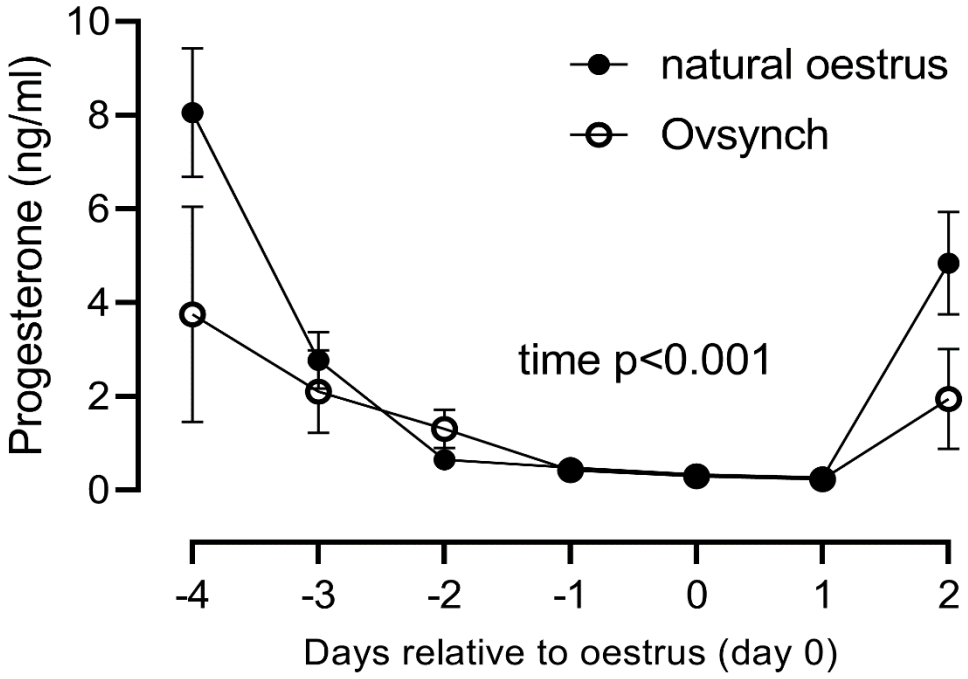

Figure 4: Concentrations of progesterone in group 1 and group 2 (mean ± SEM)

3.3. Cortisol

Cortisol concentrations are illustrated in Figure 5 as mean ± SEM. There was no significant effect detectable on the course of cortisol, neither time nor time*group or the group presented results of $P < 0.05$ at the t-test. With $P = 0.052$ for the effect of the time on the course of cortisol a trend was observed, but both the time*group interaction ($P = 0.321$) and the group had no significant effect on the cortisol concentration ($P = 0.561$).

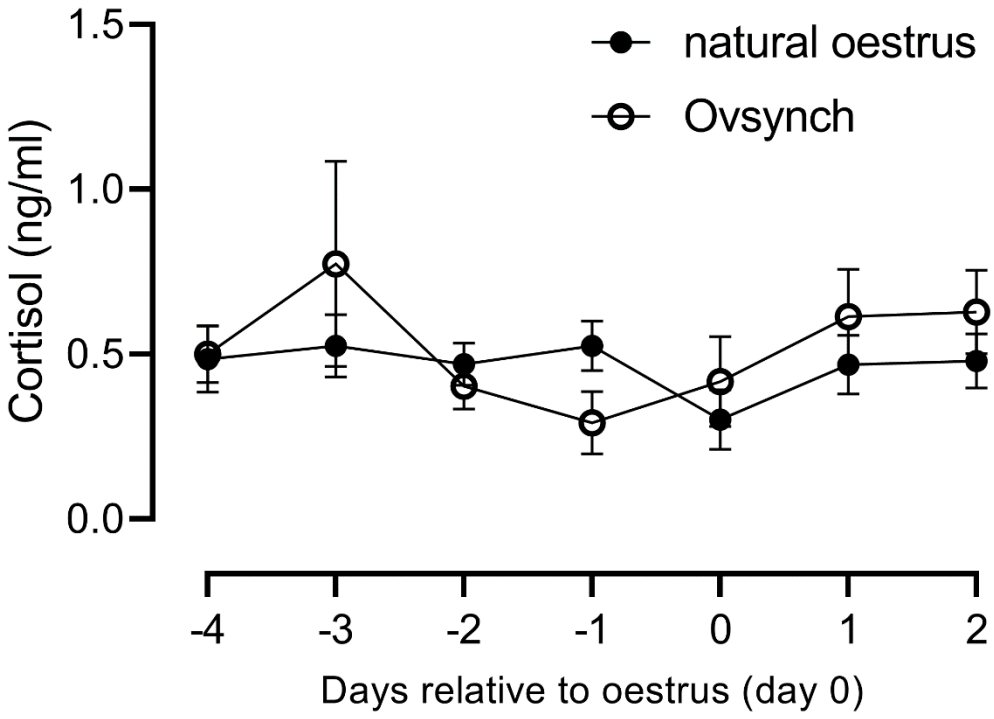

Figure 5: Concentrations of cortisol in group 1 and group 2 (mean ± SEM)

4. Discussion

The present results showed a significant difference in concentration of oxytocin in variation in time between the two groups, i.e. natural oestrus vs Ovsynch, but results also showed that there was no effect of group or time on the measured concentrations. This result has to be interpreted with caution. All oxytocin concentrations measured in our study were between 0.39 ng/ml and 2.10 ng/ml. Parkinson et al. (1992) recorded comparably low concentrations until three days post ovulation. Studies showed that oxytocin concentrations are at its minimum around the time of ovulation (Parkinson et al. 1992, Wathes et al. 1984). We assumed that oxytocin is released in a pulsatile manner in the hypophysis and leads to extenuated levels of oxytocin concentrations in periphery blood. According to that, a main limitation of our study seemed to be the blood sampling, which was performed only once per day. For a reliable and comprehensible documentation of the variation of oxytocin and to detect circadian variations (Parkinson et al. 1992), blood sampling in a more frequent manner would have been necessary. In a retrospective view, hormone measurements and analysis over the entire oestrus cycle and with an increased sampling frequency around oestrus could have made the study more valid. We, however, decided for this short sampling period and measurements because we wanted to focus on the peri-oestrus phase and ovulation. Focusing on the oestrus, the time from four days before oestrus until two days after ovulation seemed to be an adequate period for measuring. In further studies, we will analyse hormones throughout the oestrus cycle to gain more information about the physiological variations over time.

Another critical point to mention was the effect of group sizes on the average concentration of oxytocin. Unfortunately, incomplete data sets due to missing blood samples led to smaller group sizes than planned. The very low oxytocin concentrations and the reduced data for the statistical analysis as well as the small sizes of the two study groups reduced the validity of our study result. For the interpretation of the difference in concentration of oxytocin in variation in time between the two study groups, all the previous mentioned facts had to be considered. The effect of the time as well as the effect of the group showed no significant difference in oxytocin concentration between the two study groups. Since both, the effect of the group and the effect of the time had no significant impact on the concentration of oxytocin, scrutinising the significance of the difference of the oxytocin concentration between animals with synchronised ovulation and natural oestrus seemed to be justified. We recommend the idea of a facilitating role of oxytocin in the regulation of the oestrus and ovulation in cattle (Kotwica et al. 1997), as concentrations of oxytocin of cows with and without synchronised ovulation are

comparable. Furthermore, we recommended the idea of Shaw and Britt (2000), who suggested a low contribution of oxytocin at the regulation of the oestrus of cattle although our results had to be interpreted with caution.

Data of progesterone showed a course of the hormone in variation in time as expected, concentrations as well as the course of the hormone were comprehensible in both groups. With this result of progesterone analysis, we found a good reference point for the exact start of oestrus and the time of ovulation. There was no difference in progesterone concentration of cattle with or without synchronised ovulation, as the effect of group*time for progesterone was not significant.

Furthermore, there was no difference in stress-level for cattle with synchronised ovulation or natural oestrus. Results showed that there were no significant differences in concentrations of cortisol between animals in both groups. We interpreted these results that the synchronisation of ovulation does not lead to detectable stress, although the oestrus behaviour of cattle monitored by an accelerometer-system as in our study showed differences between an Ovsynch group and cows with natural oestrus (Schweinzer et al. 2020). Our results are similar to previous data that described oestrus as unaffected by stress (Stoebel and Moberg 1982, Cook et al. 1987). While management-related stress, such as transport or isolation affected oestrus behaviour in cattle and reduced the average length of oestrus (Ehnert and Moberg 1991), the manipulation of the oestrus by using the Ovsynch protocol in our study did not lead to detectable stress. Therefore, the use of hormone programmes like the Ovsynch protocol was not comparable with side effects of management-related stress. As stress always plays an essential role regarding fertility in dairy farming, the information of synchronisation of ovulation leading no significant stress to cattle is an important one in our point of view. Further studies with a sufficient number of animals could analyse the variations in oxytocin and cortisol levels in cows exposed to defined stress factors and in defined situations.

It has been discussed controversially whether oxytocin can be used as an indicator for animal behaviour and welfare (Rault et al. 2017). Our study provides only few contributions to this discussion because our hypothesis, that synchronisation of ovulation results in measurable stress, was not confirmed. Thus, it remains unclear if oxytocin was not eligible as indicator or if there was no difference in welfare between the cows at all.

To sum up, because of the above mentioned limitation in study design, our study can only be regarded as a pilot study. Future studies with a larger number of animals and a more frequent sampling schedule over the entire oestrus cycle could contribute to a better

understanding about the effects of oestrus synchronisation and other stress factors on the concentrations of oxytocin, its eligibility as indicator for well-being and its effect on fertility.

5. Conclusion

Hormone programmes to control oestrus and ovulation of cows are available since decades and in use in different combination of hormones on dairy farms to improve artificial insemination success and fertility outcomes. The aim of this study was to analyse the concentration of oxytocin, progesterone and cortisol in cattle at the time of oestrus and ovulation and compare them between cows with synchronised ovulation and natural oestrus. Our results indicate that there was no difference in oxytocin concentrations between the two groups, and, thus, synchronisation has no effect on oxytocin concentrations. Concentrations of progesterone changed significantly in variation in time in both groups as expected, and there were no significant difference detectable comparing progesterone concentrations between the two study groups. There were also no significant differences in cortisol concentrations between the two groups, what led to the conclusion, that synchronisation of the oestrus caused no detectable stress to the cows.

This study has to be regarded as a pilot study with limited statistical power and results have to be interpreted with caution.

6. References

(1) Andersen CY. 2002. Possible new mechanism of cortisol action in female reproductive organs: physiological implications of the free hormone hypothesis. The Journal of endocrinology, 173 (2): 211–217. DOI 10.1677/joe.0.1730211.

(2) Armstrong DT, Hansel W. 1959. Alteration of the bovine estrous cycle with oxytocin. Journal of Dairy Science, 42 (3): 533–542. DOI 10.3168/jds.S0022-0302(59)90607-1.

(3) Cook DL, Winters TA, Horstman LA, Allrich RD. 1987. Influence of cortisol and dexamethasone on estrous behavior of estradiol-treated ovariectomized cows and heifers. Journal of Dairy Science, 70 (1): 181–185. DOI 10.3168/jds.S0022-0302(87)79992-5.

(4) Ehnert K, Moberg GP. 1991. Disruption of estrous behavior in ewes by dexamethasone or management-related stress. Journal of animal science, 69 (7): 2988–2994. DOI 10.2527/1991.6972988x.

(5) Flint A, Sheldrick EL, McCann TJ, Jones D. 1990. Luteal oxytocin: characteristics and control of synchronous episodes of oxytocin and PGF2α secretion at luteolysis in ruminants. Domestic Animal Endocrinology, 7 (2): 111–124. DOI 10.1016/0739-7240(90)90018-U.

(6) Flint AP, Sheldrick EL. 1983. Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. Journal of reproduction and fertility, 67 (1): 215–225. DOI 10.1530/jrf.0.0670215.

(7) Ivell R, Brackett KH, Fields MJ, Richter D. 1985. Ovulation triggers oxytocin gene expression in the bovine ovary. FEBS Letters, 190 (2): 263–267. DOI 10.1016/0014-5793(85)81296-5.

(8) Kotwica J, Skarynski D, Jaroszewski J, Kotwica G. 1991. Effect of norepinephrine on the release of progesterone and ovarian oxytocin in cattle. Animal Reproduction Science, 26 (3-4): 179–191. DOI 10.1016/0378-4320(91)90045-2.

(9) Kotwica J, Skarzynski D. 1993. Influence of oxytocin removal from the corpus luteum on secretory function and duration of the oestrous cycle in cattle. Journal of reproduction and fertility, 97 (2): 411–417. DOI 10.1530/jrf.0.0970411.

(10) Kotwica J, Skarzynski D, Bogacki M, Melin P, Starostka B. 1997. The use of an oxytocin antagonist to study the function of ovarian oxytocin during luteolysis in cattle. Theriogenology, 48 (8): 1287–1299. DOI 10.1016/S0093-691X(97)00371-3.

(11) Kubo T, Iga K, Fukuju N, Kizaki K, Osawa T, Izaike Y, Takahashi T. 2018. Different prostaglandin F2 α secretion in response to oxytocin injection between pregnant and non-pregnant cows: effect of the day of oxytocin challenge test for determining the difference. Animal science journal = Nihon chikusan Gakkaiho, 89 (2): 332–339. DOI 10.1111/asj.12952.

(12) Lyimo ZC, Nielen M, Ouweltjes W, Kruip T, van Eerdenburg F. 2000. Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. Theriogenology, 53 (9): 1783–1795. DOI 10.1016/S0093-691X(00)00314-9.

(13) Majewska M, Lee HY, Tasaki Y, Acosta TJ, Szostek AZ, Siemieniuch M, Okuda K, Skarynski DJ. 2012. Is cortisol a modulator of interferon tau action in the endometrium during early pregnancy in cattle? Journal of reproductive immunology, 93 (2): 82–93. DOI 10.1016/j.jri.2012.01.004.

(14) Melchert M, Aurich C, Aurich J, Gautier C, Nagel C. 2019. External stress increases sympathoadrenal activity and prolongs the expulsive phase of foaling in pony mares. Theriogenology, 128: 110–115. DOI 10.1016/j.theriogenology.2019.02.006.

(15) Miyamoto A, Schams D. 1991. Oxytocin stimulates progesterone release from microdialyzed bovine corpus luteum in vitro. Biology of reproduction, 44 (6): 1163–1170. DOI 10.1095/biolreprod44.6.1163.

(16) Moore LG, Choy VJ, Elliot RL, Watkins WB. 1986. Evidence for the pulsatile release of PGF-2 alpha inducing the release of ovarian oxytocin during luteolysis in the ewe. Journal of reproduction and fertility, 76 (1): 159–166. DOI 10.1530/jrf.0.0760159.

(17) Nagel C, Trenk L, Aurich C, Ille N, Pichler M, Drillich M, Pohl W, Aurich J. 2016. Sympathoadrenal balance and physiological stress response in cattle at spontaneous and PGF2α-induced calving. Theriogenology, 85 (5): 979–985. DOI 10.1016/j.theriogenology.2015.11.009.

(18) Parkinson TJ, Wathes DC, Jenner LJ, Lamming GE. 1992. Plasma and luteal concentrations of oxytocin in cyclic and early-pregnant cattle. Journal of reproduction and fertility, 94 (1): 161–167. DOI 10.1530/jrf.0.0940161.

(19) Pothmann H, Prunner I, Wagener K, Jaureguiberry M, La Sota RL de, Erber R, Aurich C, Ehling-Schulz M, Drillich M. 2015. The prevalence of subclinical endometritis and intrauterine infections in repeat breeder cows. Theriogenology, 83 (8): 1249–1253. DOI 10.1016/j.theriogenology.2015.01.013.

(20) Rault JL, van den Munkhof M, Buisman-Pijlman FTA. 2017. Oxytocin as an indicator of psychological and social well-being in domesticated animals: A Critical Review. Frontiers in psychology, 8: 1521. DOI 10.3389/fpsyg.2017.01521.

(21) Schams D, Prokopp S, Barth D. 1983. The effect of active and passive immunization against oxytocin on ovarian cyclicity in ewes. Acta Endocrinologica, 103 (3): 337–344. DOI 10.1530/acta.0.1030337.

(22) Schweinzer V, Gusterer E, Kanz P, Krieger S, Süss D, Lidauer L, Berger A, Kickinger F, Öhlschuster M, Auer W, Drillich M, Iwersen M. 2020. Comparison of behavioral patterns of dairy cows with natural estrus and induced ovulation detected by an ear-tag based accelerometer. Theriogenology, 157: 33–41. DOI 10.1016/j.theriogenology.2020.05.050.

(23) Sharma SC, Fitzpatrick RJ. 1974. Effect of oestradiol — 17β and oxytocin treatment on prostaglandin F alpha release in the anoestrous ewe. Prostaglandins, 6 (2): 97–105. DOI 10.1016/0090-6980(74)90021-5.

(24) Shaw DW, Britt JH. 2000. In vivo oxytocin release from microdialyzed bovine corpora lutea during spontaneous and prostaglandin-induced regression. Biology of reproduction, 62 (3): 726–730. DOI 10.1095/biolreprod62.3.726.

(25) Sheldrick EL, Flint AP. 1983. Regression of the corpora lutea in sheep in response to cloprostenol is not affected by loss of luteal oxytocin after hysterectomy. Journal of reproduction and fertility, 68 (1): 155–160. DOI 10.1530/jrf.0.0680155.

(26) Sheldrick EL, Mitchell MD, Flint AP. 1980. Delayed luteal regression in ewes immunized against oxytocin. Journal of reproduction and fertility, 59 (1): 37–42. DOI 10.1530/jrf.0.0590037.

(27) Silvia WJ, Lewis GS, McCracken JA, Thatcher WW, Wilson L. 1991. Hormonal regulation of uterine secretion of prostaglandin F2 alpha during luteolysis in ruminants. Biology of reproduction, 45 (5): 655–663. DOI 10.1095/biolreprod45.5.655.

(28) Skarzynski DJ, Bogacki M, Kotwica J. 1997. Changes in ovarian oxytocin secretion as an indicator of corpus luteum response to prostaglandin F2α treatment in cattle. Theriogenology, 48 (5): 733–742. DOI 10.1016/S0093-691X(97)00297-5.

(29) Stoebel DP, Moberg GP. 1982. Effect of adrenocorticotropin and cortisol on luteinizing hormone surge and estrous behavior of cows. Journal of Dairy Science, 65 (6): 1016–1024. DOI 10.3168/jds.S0022-0302(82)82303-5.

(30) Stoebel DP, Moberg GP. 1982. Repeated acute stress during the follicular phase and luteinizing hormone surge of dairy heifers. Journal of Dairy Science, 65 (1): 92–96. DOI 10.3168/jds.S0022-0302(82)82157-7.

(31) Tenhagen BA, Drillich M, Surholt R, Heuwieser W. 2004. Comparison of timed AI after synchronized ovulation to AI at estrus: Reproductive and Economic Considerations. Journal of Dairy Science, 87 (1): 85–94. DOI 10.3168/jds.S0022-0302(04)73145-8.

(32) Walters DL, Schams D, Schallenberger E. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow. Journal of reproduction and fertility, 71 (2): 479–491. DOI 10.1530/jrf.0.0710479.

(33) Wathes DC, Swann RW, Pickering BT. 1984. Variations in oxytocin, vasopressin and neurophysin concentrations in the bovine ovary during the oestrous cycle and pregnancy. Journal of reproduction and fertility, 71 (2): 551–557. DOI 10.1530/jrf.0.0710551.