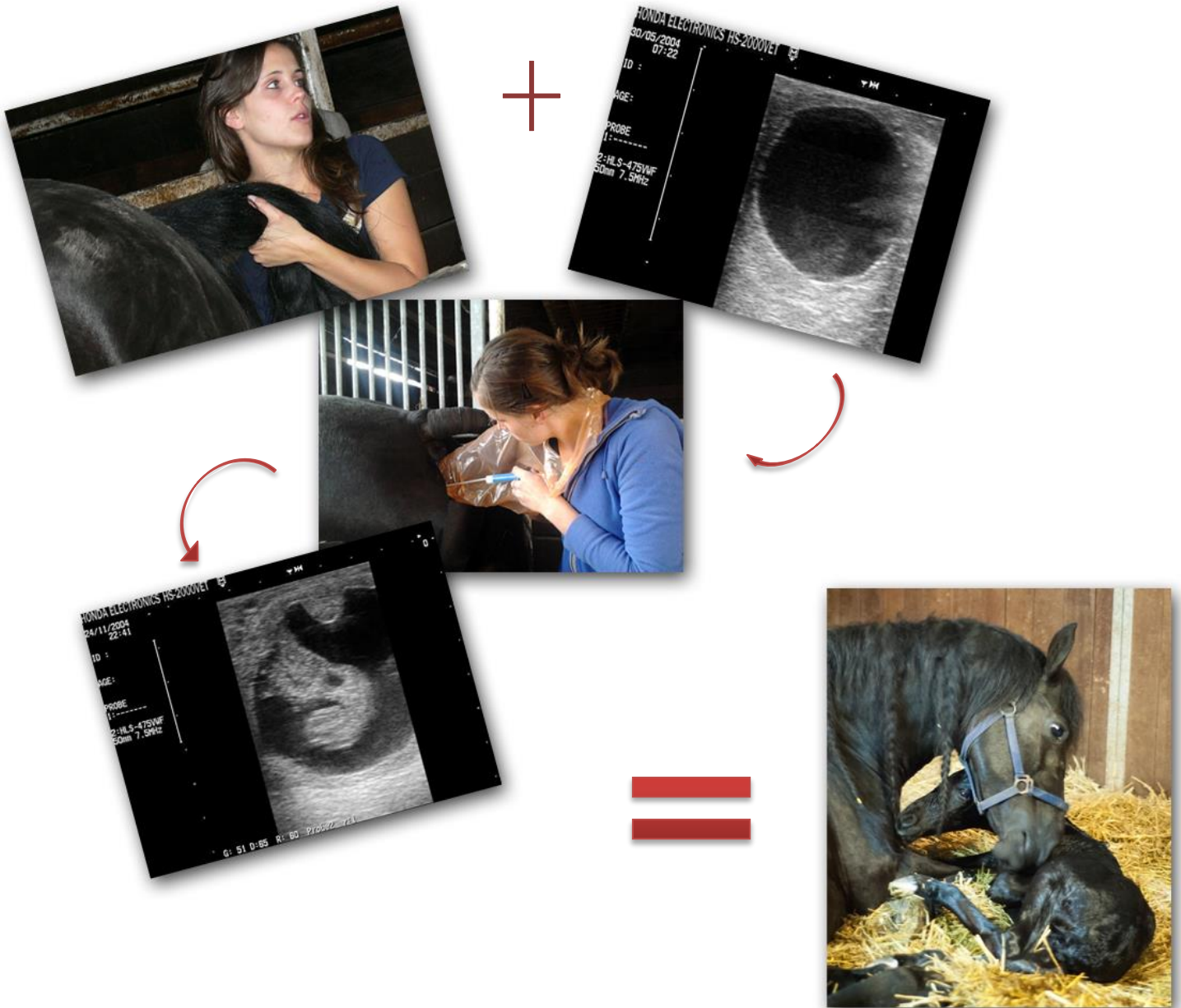# The oestrous cycle
in FRIESIAN mares

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Abstract

This study aimed to describe the characteristics of the oestrous cycle in the Friesian mare, and to produce a guideline for veterinarians responsible for reproductive management of mares from this breed. The description of the oestrous cycle included the following parameters: the interovulatory interval (IOI), follicle diameter, follicle softness, uterine tone, cervical tone, endometrial oedema and free fluid. Also analysed were the number of artificial inseminations (AI) per cycle, any hormones administered, the age and reproductive status of the mare and the season in which the mare was monitored. The data were collected from three different clinics or stud farms in the Netherlands and included 687 cycles from 340 mares, aged between 3 and 21 years. Multiple cycles in one part of season were averaged into a single representative cycle for each individual mare. Cycles that were altered by the use of hormones were grouped and analysed separately. In non-manipulated oestrous cycles, an IOI of 24.46 ± 0.32 days was recorded. The IOI was not significantly affected by season (spring, summer), reproductive status (barren, maiden or lactating) or mare age. The maximum follicle diameter was 5.18 ± 0.04 cm at approximately 48 hours prior to ovulation. Growth rate of the pre-ovulatory follicle was 0.242 ± 0.08 cm per day until day -1 (day 0 = day of ovulation). Cessation of follicle growth was a good indicator of impending ovulation within 24 hours. Uterine oedema began to increase on day -8 and peaked on day -6. In conclusion, the reproductive cycle of Friesian mares is characterized by a relatively long IOI, large follicle diameter and early peak in uterine oedema compared to other horse breeds. The signs of an impending ovulation within 48 hours, and therefore for AI with fresh or chilled semen, were: a softening pre-ovulatory follicle of >4.0-4.5 cm with a thick follicular wall, allied to decreasing uterine oedema.

1. Introduction

In the last quarter of the previous century, the reproductive management of mares developed enormously. One of the most important developments was the introduction of transrectal ultrasonography. Using the combined information from transrectal palpation and ultrasonography, oestrous cycle stage can be better evaluated and the optimal insemination time more accurately predicted. Ultrasonographic examination is used to determine the presence of a pre-ovulatory follicle and to evaluate the echo texture of the uterus, as well as the presence of abnormalities such as free fluid and/or endometrial cysts. These data together with information on uterine and cervical tone and the softness of the pre-ovulatory follicle, obtained by palpation, will help the veterinarian determine the optimal time for insemination with fresh or chilled semen (Samper, 1997; Stout, 2003). The general aim is to inseminate the mare between 0 and 48 hours prior to ovulation to offer a good chance of fertilization and to prevent multiple inseminations per cycle that, in problem mares, increases the risk of endometritis (Samper, 1997; Gastal et al., 2011). With frozen-thawed semen, the mare should be inseminated between 12 hours pre- and 15 hours post-ovulation (Katila et al., 1996; Newcombe et al., 2011) using an induced ovulation protocol. Knowledge about the signs of an impending ovulation could be helpful for the veterinarian to induce at the right time. It should be noted that each insemination will induce a post-

breeding 'physiological endometritis' and may lead to a pathological 'persistent post-breeding endometritis' which will reduce the likelihood of pregnancy if not adequately treated. By inseminating just once or twice, the risk of causing a persistent post-breeding endometritis can be reduced (Samper, 1997). The exact dynamics of the oestrous cycle differ between mares and cycles, however aspects of the cycle (e.g. maximum follicle diameter) are known to be quite repeatable within individual mares (Ginther et al., 2008a; Ginther et al., 2008b). There also seem to be breed differences in oestrous cycle characteristics. For example, in ponies oestrous lasts approximately two days longer than in horses, and the maximum diameter of a pre-ovulatory follicle is smaller (Gastal et al., 2008). The most useful information for reproductive fertility management of a mare is the information observed during previous reproductive cycles in that same animal. Mean values for the reproductive parameters for several other breeds have been reported (Table 1). Studies of Thoroughbred and Standardbred mares, report a mean cycle length of approximately 22 days with an average duration of oestrous of 5-7 days. The oestrous cycle has also been reported to be influenced by season, reproductive stage, age and breed of the mare (Heidler et al., 2004). The mean size of the pre-ovulatory follicle just prior to ovulation has been reported to be 40 mm, with a very large range of 30 to 70 mm (Bergfelt, 2007), and follicular growth is reported as approximately 3 mm a day until one day pre-ovulation (Aurich, 2011).

Breed	Interovulatory interval (days)	Pre-ovulatory follicle (cm)
Appaloosa[a]		4.4
Arabian[b]	22.3	4.03 ± 0.1
Breton horse[c]	20.3 ± 0.7	4.54 ± 0.1
Caspian[d]	22.1 ± 0.4	4.26 ± 0.1
Finnhorse[e]		4.3 ± 0.4
Large ponies[c]	23.9 ± 0.5	4.07 ± 0.1
Lipizzaner lactating[f]	21.2 ± 1.8	
Lipizzaner non-lactating[f]	22.8 ± 1.4	
Miniature ponies[c]	23.3 ± 0.9	3.83 ± 0.1
Thoroughbred[g]		3.99 ± 0.5
Trotter/riding horse[b]		4.15 ± 0.6
Quarter horse[b]	21.4	4.33 ± 0.1
Standardbred[b]		4.54 ± 0.1

Table 1. Mean interovulatory interval and follicle diameter in different horse breeds.
[a]Ginther, 1992; [b]Dimmick et al., 1993; [C]Gastal et al., 2008; [d]Shizari et al, 2002; [e]Koskinen, 1991; [f]Heidler et al., 2004; [g]Morel et al., 2010

2. Aim of the study

The aim of this study was to describe the basic reproductive cycle parameters in the Friesian mare, and thereby assist veterinarians in optimizing reproductive management in this breed. Veterinarians lacking experience with Friesian mares often find it difficult to determine the optimal time for induction of ovulation and/or insemination. In this respect, Friesian mares seem to have slightly different oestrous cycle dynamics than other common horse breeds. The cycle length is thought to be longer, the pre-ovulatory follicle larger and uterine oedema to follow a different time-course. Dutch veterinarians with years of experience in the reproductive management of Friesian mares are familiar with the larger pre-ovulatory follicle diameter (Stout, 2003). In other countries, veterinarians often have less experience with Friesians and the added challenge of dealing primarily with frozen-thawed semen which requires a higher degree of accuracy and certainty in terms of the timing of ovulation induction and insemination. Therefore providing basic information about findings during palpation and transrectal ultrasonography of the reproductive tract in the period leading up to ovulation in this breed will be very useful.

3. Materials and methods

The data were collected from 3 locations in the Netherlands, namely; the stud farm 'De Nieuwe Heuvel' in Lunteren, the equine veterinary clinic 'De Graafschap Dierenartsen' in Vorden and the Department of Equine Sciences, Utrecht. Oestrous cycle data from Friesian mares during the breeding seasons 2009, 2010 and 2011 were included. The mares were examined every other day or every day until ovulation occurred. The collected data consisted of 687 cycles derived from 340 different horses, ranging in age from 3 to 21 years with a mean age of 6.43 ± 0.21. Ovulation was determined by the disappearance of a follicle observed during a previous examination and replacement by a corpus haemorrhagicum (CH) or corpus luteum (CL). Data were collected from day 8 prior to ovulation (day -8) until the day of ovulation (day 0) to create a detailed overview of the oestrous phase of the cycle. The following parameters were evaluated for each cycle:

- diameter of the follicle,
- softness of the follicle,
- uterine and cervical tone ,
- intensity of uterine oedema,
- presence of free fluid in the uterus,
- age of the mare (3-6 years, 7-12 years, >12 years),
- reproductive status (maiden, lactating or barren),
- stage of the season (1 January - 31 March, 1 April - 30 June, 1 July - 30 September),
- interovulatory interval (IOI),
- number of inseminations per cycle,
- pre-ovulatory follicles: uni- or bilateral,
- use of hormones: Prostaglandin F2-alpha (PGF2α) and human chorionic gonadotropin (hCG).

Reproductive cycles in which hormones were administered were filtered and analysed separately. Finally, the detection of pregnancy on days 14-18 and days 28-37 after insemination were included.

3.1 Parameters

3.1.1. Interovulatory interval
The IOI was determined as the number of days between ovulation in two consecutive oestrous periods. Ovulation was deemed to have occurred when the pre-ovulatory follicle that was seen on the previous day had disappeared and was replaced by a CH or early CL.

3.1.2. Number of inseminations
Mares were inseminated when the examining veterinarian felt that combination of relevant parameters indicated impending ovulation.

These usually included the presence of a follicle of at least 40 mm that may have begun to soften, reduced tone of the uterus and cervixand visible uterine oedema that may already have begun to decline in intensity. Mares were also teased daily to confirm behavioural heat. Insemination was repeated every other day until ovulation to ensure that fertile sperm were present in the oviduct at the time of ovulation. Previous studies have indicated that pregnancy rates are good when a 48 hour insemination interval is maintained but drop markedly when insemination is performed only every 72 hours (63.8% versus 34.0%, respectively) (Brinsko et al., 2011).

3.1.3. Diameter of the follicle

The diameter of the follicle was measured during each ultrasound scan and the average of width and height of the antrum was noted (Fig. 1). When more than one pre-ovulatory follicle (>35 mm) was visible, all were measured and recorded.

3.1.4. Softness of the follicle

The turgidity of the follicle wall (softness) was recorded during palpation and classified from 1 to 4: hard (1), very soft (4).
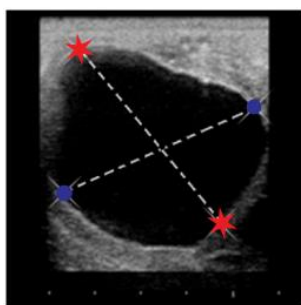
Fig. 1. A large pre-ovulatory follicle. *Red stars and blue dots showing measurements of height and width, respectively.*

3.1.5. Uterine and cervical tone

The tone of the uterus and cervix were recorded during palpation. The uterine tone was classified on a scale of 0-3, soft (0) to firm (3).

The cervical tone was assessed on the basis of cervical width as palpated per rectum and ranged from 1 finger to 4 fingers wide.

3.1.6. Oedema

The echo texture of the uterus was scored during each ultrasound scan and classified on a scale of 0 to 5. The quantity of oedema was classified as no oedema (0), slight oedema (1), moderate oedema (2), obvious oedema (3), intense oedema (4) and excessive oedema (5) (Fig. 2). If the echotexture was homogenous, no oedema was present in the uterus. When no oedema was visible and uterine tone was firm, the cervix closed (1 finger) and a CL was visible on one or other ovary, the mare was classified as being in dioestrous (Fig. 2a).

3.1.7. Free fluid

The amount of free fluid was classified from no fluid (0), a very small fluid 'stripe' (1), < 1cm of fluid (2), 1-3 cm fluid (3) and >3 cm fluid (4). (Fig. 3).
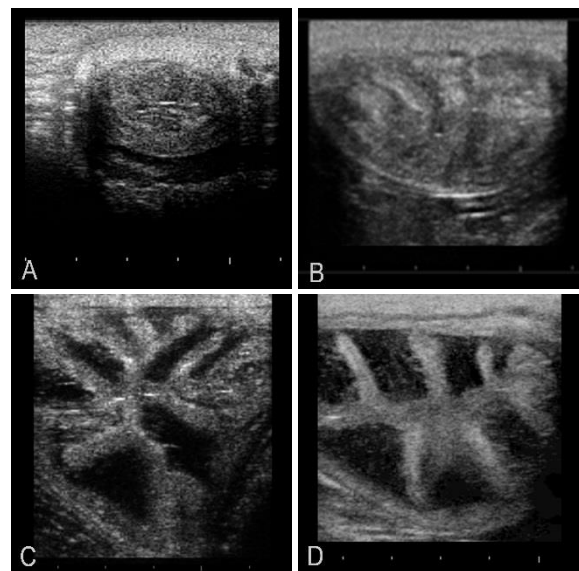
Fig. 2. The ultrasonographic echo texture of the uterus: amount of oedema and classification. *A, Dioestrous: no oedema (-). B, slight oedema (+-). C, obvious oedema (++), D, excessive oedema (++++)*
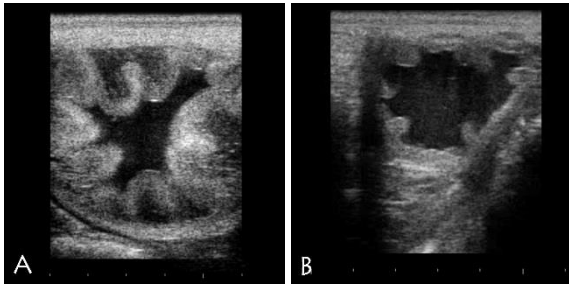
Fig. 3. Ultrasonographic images of visible free fluid in the uterus. *A, uterus with 1-3 cm fluid. B, uterus with >3cm fluid.*

3.1.8. Hormonal intervention

Induction of oestrous and/or ovulation using exogenous hormones were examined for the time lapse between treatment and ovulation. PGF2α analogues were used for initiating oestrous in mares presented in dioestrous and to synchronise mares for embryo transfer. PGF2α initiates luteolysis which results in a rapid fall in circulating progesterone concentrations and allows the mare to return to oestrous. PGF2α is only fully effective when a mature CL is present (> 5 days after ovulation). Mares are normally expected to ovulate within 8-12 days of treatment with a PGF2α analogue (Aurich, 2011). Ovulation can be induced using hCG (Chorulon®, MSD Animal Health, New York, USA) or a GnRH agonist. Two forms of GnRH agonists are available: a deslorelin implant (Ovuplant®, Peptech Animal Health, Christchurch, New Zealand) or injectable deslorelin or buserelin, with the latter described for administration either as a single large bolus of Sucromate® (Thorn Bioscience, Louisville, USA) or multiple smaller doses of Receptal® (Intervet, Boxmeer, Netherlands). These hormones are helpful when inseminating mares with frozen or chilled transported semen since they help ensure insemination at the optimal time, while minimizing number of examinations for ovulation. In general, a number of criteria are described for when hCG should be administered to reliably induce ovulation: The mare must be in heat, have a follicle of >35 mm and endometrial oedema (Ferris et al., 2012).

However, Friesian mares have been reported to respond poorly to an ovulating agent when the dominant follicle was <50 mm in diameter (McKinnon et al, 2011). Both hCG and GnRH agonists will induce ovulation at around 36-48h after injection in 85% of the mares (McKinnon et al, 2011). The mean interval between hCG treatment and ovulation has been reported as 35.9 ± 3.8 hours (Sullivan et al., 1973, McKinnon et al, 1997) as compared to 40.7 ± 3.2 hours for the deslorelin implant (McKinnon et al., 1997) and 1.98 days after 2.2 mg deslorelin administration (McKinnon et al., 2011).

3.2. Statistics

To be able to describe the oestrous cycle of the Friesian mare, all data was collected in an Excel sheet and filtered for hormone intervention before export to SPSS Statistics 20. Three groups where formed: cycles during which no hormones were used, cycles in which oestrous was induced using PGF2α and cycles in which ovulation was induced with hCG. Multiple cycles within one part of season (January-March, April-June, July-September) for a single mare and year were converted into an average 'representative' cycle, to create independent data without losing specific season stage information. All data were tested for normality by examining the skewness and kurtosis to identify which tests were most suitable. The mean ± S.E.M and 95% confidence intervals (95%CI) were calculated for each parameter on each day of oestrous. The Bonferroni *post hoc* test was used to identify differences between groups. Significant level was set at P <0.05.

4. Results

Data were checked for normality and all parameters were normally distributed. The results are based on 264 independent representative oestrous cycles. The mean IOI was 24.46 ± 0.32 days, although the range for

this parameter was large: from 17 to 33 days in cycles without hormone interference.

The IOI was not affected by factors such as reproductive status, stage of season or mare age. The mean number of inseminations per cycle was 1.97 ± 0.06 and there was no correlation between number of inseminations and pregnancy rate. The follicle diameter showed considerable variations between the different days prior to ovulation; 0-24 hours pre-ovulation, the mean follicle diameter was 4.99 ± 0.06 cm. Size at 24-48 hours before ovulation was 5.18 ± 0.04 cm. No correlation between follicle diameter and reproductive status, stage of season or mare age was evident. During oestrous, an almost linear growth curve for follicular diameter was visible in which the follicle grew by 0.242 ± 0.08 cm per day until day -2 (Fig. 4). From that moment on it stopped growing or even reduced in size. The follicle softened progressively towards ovulation. It was soft to very soft on day -1. The tone of the uterus was relatively constant throughout oestrous. It was mostly soft to rather soft and softened even more during the 5 days prior to ovulation. It was softest on day -2. The tone of the cervix during oestrous showed little variation, it was rather soft and 3 fingers to 4 fingers wide. The intensity of endometrial oedema reached a peak on day -6, at obvious to intense, and gradually decreased each subsequent day towards ovulation. During or just after ovulation, oedema score decreased rapidly such that no or only slight oedema remained. The amount of endometrial free fluid showed no clear time-course and appeared to be independent of day of oestrous cycle. When mares had large amounts of intrauterine free fluid, oxytocin was administered. Most fluid was cleared after a single dose of oxytocin (20 I.E.). The means ± S.E.M. of all parameters on each day of oestrous are shown in Figure 5.

4.1. Hormonal intervention

Results are based on 37 cycles in the PG group and 118 in the hCG group. The mean number of days between administering PGF2α and ovulation was 9.18 ± 0.34. No differences were found for any of the other measured parameters between a spontaneous oestrous and one induced with PGF2α. Following the use of hCG, a smaller diameter of the pre-ovulatory follicle was observed, with a maximum diameter of 5.11 ± 0.12 cm on day -2. A trend was seen for smaller follicle diameter on day -1 compared to the group that did not receive exogenous hormones (4.99 ± 0.06 cm vs 4.48 ± 0.25 cm, P=0.58). Endometrial oedema peaked on day -4. The other parameters did not differ from mares not treated with hCG.
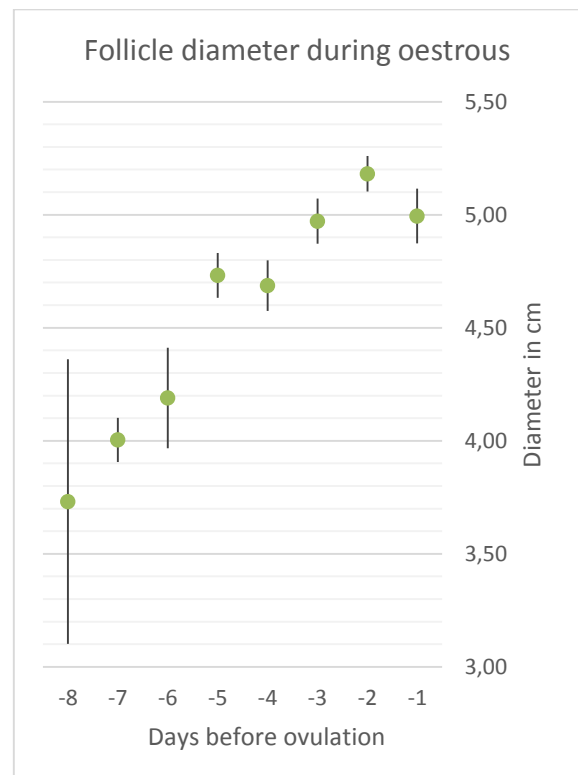
Fig. 4. Mean follicle diameter and 95%CI for each day of oestrous
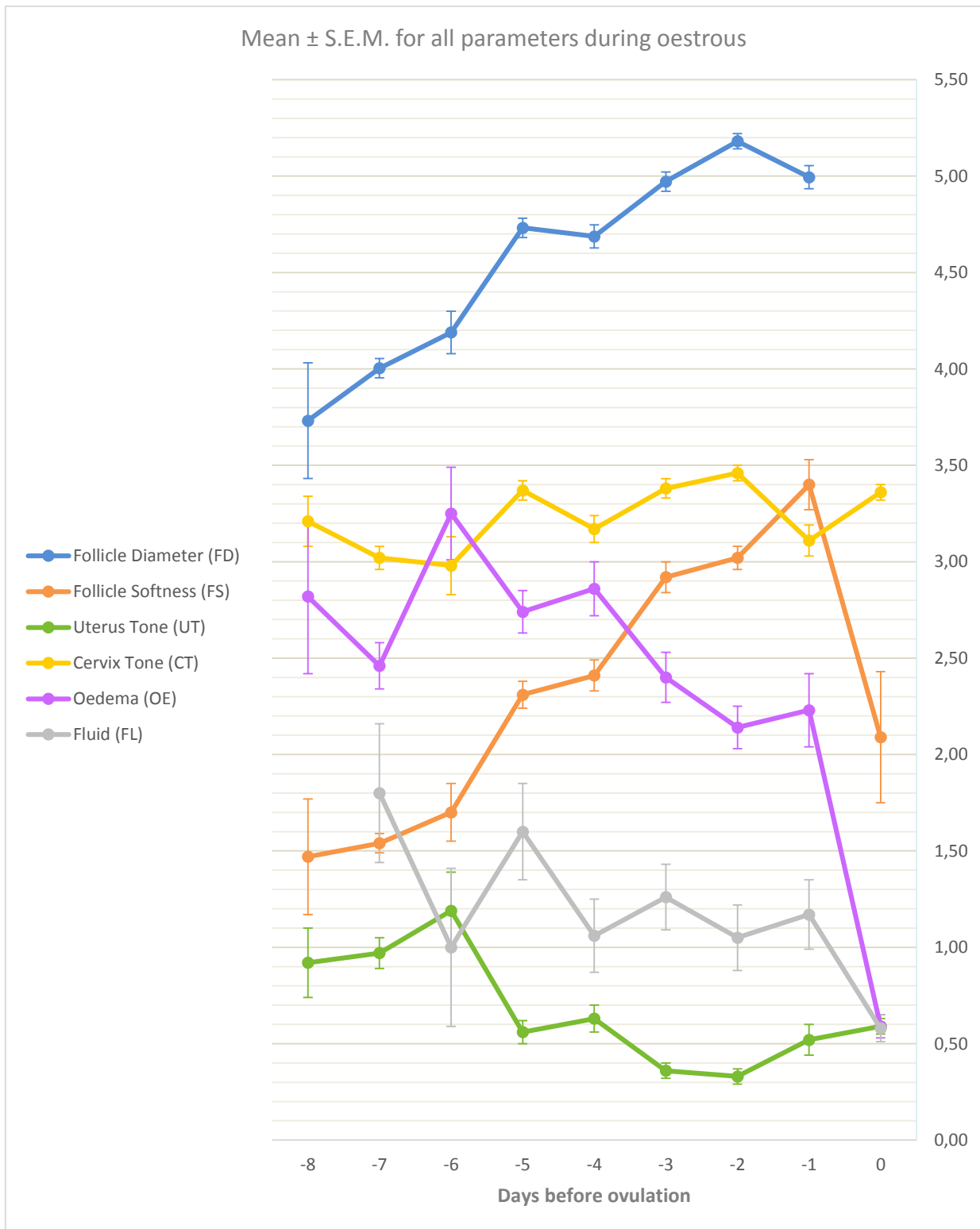
Fig. 5. Mean ± S.E.M. for all parameters on each day of oestrous.
FD = Follicle diameter in centimetres. FS = Follicle softness was graded from hard (1) to very soft (4). UT = Uterine tone was graded from soft (0) to firm (3). CT = Cervical tone was quantified from 1 finger wide (1) to 4 fingers wide (4). OE = Oedema was classified from no oedema (0), to excessive oedema (5). FL = The amount of free fluid was classified from no fluid (0) to >3 cm fluid (4).

5. Discussion

The present study evaluated the reproductive cycle of Friesian mares, to help optimize reproductive management in this breed. The IOI in Friesian horses had a mean of 24.46 ± 0.32 days. The mean IOI in Thoroughbred and Standardbred horses has been reported as 22 days, and in ponies approximately 24 days. The cycle of the Friesian mare was thus approximately 2 days longer than in Thoroughbreds. This supports a relationship between breed and the length of the IOI, as noted by Aurich (2011). The length of the IOI was not affected by reproductive status, stage of season or mare age. In contrast to the results of the current study, a relationship between reproductive status and IOI was found in a study on Lipizzaner mares (Heidler et al, 2004); specifically, lactating mares had a significantly shorter IOI than non-lactating mares (21.2 ± 1.8 versus 22.8 ± 1.4 days). Carnevale et al. (1993) and Ginther et al. (2009) reported a relationship between age and IOI; older mares had a longer cycle due to lower FSH and LH concentrations resulting in a slower growth of the developing follicle. The reason that correlations with age or season were not found in the current study could be related to the manner of grouping data for age (3-6 years, 7-12 years, >12 years) and stage of season (1 January - 31 March, 1 April - 30 June, 1 July – 30 September). Increasing sample size and grouping differently could yield different conclusions. The follicle diameter increased from day -8 to day -2 with a mean growth rate of 0.242 ± 0.08 cm a day. By contrast, Pierson (1985) and Aurich (2011) reported a faster dominant follicle growth rate in their studies of respectively 0.27 cm and 0.30 cm a day. The follicle stopped growing or even showed a decrease in size on day -1. The slower follicle growth and bigger pre-ovulatory follicle size in Friesian mares presumably contributes to the longer IOI in this breed. The plateau or decrease in follicle growth just prior to ovulation has also been reported in studies on other breeds (Palmer, 1980; Ginther et al., 1992; Aurich, 2011) and postulated to be the result of LH reaching a critical level and having a negative effect on follicular Estradiol-17β production and formation of LH receptors on granulosa cells (Ginther et al., 2008a, 2008b). In this study, the mean maximum diameter of the follicle was 5.18 ± 0.04 cm on day -2. The mean diameter of the follicle just before ovulation (day -1) was 4.99 ± 0.06 cm. In other studies the diameter of the follicle on day -1 was 4.51 ± 0.05 cm (Pierson, 1985) and 4.0 cm (Ginther et al., 2008a). This demonstrates that the pre-ovulatory follicle of the Friesian mare is larger than in many other horse breeds such as Quarter horses and Thoroughbreds. No correlation between follicle diameter within 0-48 hours pre-ovulation and reproductive status, stage of season or age was found. By contrast, Morel et al. (2010) reported that follicle diameter was influenced by mare age (largest follicle 3.89 ± 0.56 cm in 2-4 year olds and smallest in >19 year old mares at 3.33 ± 0.47 cm) and season (largest from 1st-14th of February at 4.42 ± 0.39 cm and smallest from 15th-31st of August at 3.37 ± 0.49 cm). No significant difference was found in our study for the maximum diameter of the follicle in cycles with or without the use of PGF2α. However, the use of hCG resulted in smaller pre-ovulatory follicle diameters: 4.48 ± 0.25 cm on day -1 and 5.11 ± 0.12 cm on day -2. This is in agreement with the study of McKinnon et al (2011) and seems to be reasonable, given that hCG can be injected before the endogenous pre-ovulatory hCG surge (Miro et al., 2004). Certainly, follicle diameter is not the only parameter that helps predicting the optimal time to administer hCG. In practice, hCG is generally administered when all other parameters indicate clear oestrous, and in most Friesian mares the follicle at that time will be larger than 4 cm. It follows that hCG should be administered in Friesians at a larger follicle size than in other breeds to ensure efficacy.

The pre-ovulatory follicle slowly begins to soften towards ovulation and is softest on day -1 with a mean softness score of 3.40 ± 0.13, which indicates a soft (3) to very soft (4) follicle, this was in accordance with Pierson (1985). IU oedema decreased to 2.23 ± 0.19 on day -1 and was almost gone by ovulation, at 0.59 ± 0.06. The peak on day -6 differs with the peak found in other studies where oedema began on day -7 or -8 and peaked on day -2/-3 (Ginther, 1984; Hayes et al., 1985; Pierson, 1985; Squires et al., 1988; & McKinnon et al., 1997). This difference could be explained by the extended follicular phase in the Friesian breed. The fast decrease in amount of endometrial oedema from day -2 to ovulation is in agreement with other studies. According to Samper (1997) moderate or slight oedema in combination with a large follicle is a good indicator for impending ovulation. Another finding of Samper's study was an accelerated decrease in uterine oedema after using hCG, which was also seen in this study. The amount of free fluid in the uterus bore no relation to the timing of ovulation, because the presence and amount of fluid was independent of stage of oestrous. The presence of free uterine fluid is influenced by uterine drainage (cervical and lymphatic), insemination and the reaction of the endometrium to insemination. Mares inseminated having intense uterine oedema often accumulate fluid (Samper, 1997). The mean number of days between injecting PGF2α and ovulation was 9.18 ± 0.34. A previous study reported the interval between administering PGF2α and the onset of oestrous as 3-4 days with ovulation usually occurring within 8-12 days (Aurich, 2011); this agrees with the findings of our study. One fact that needs to be born in mind is that follicle diameter was the only objective parameter measured in this study. Follicle diameter can be measured quite accurately and repeatedly. When a mare had more than one follicle of >35 mm, only the largest was noted. This could have an impact on the mean diameter of the pre-ovulatory follicles, given that a study by Ginther et al. (2008a) indicated a smaller diameter of the largest follicle in double ovulations compared to single ovulations. The other parameters measured (e.g. uterine tone) are subjective and dependent on the subjective interpretation by the veterinarian and the way the ultrasonographic examination or palpation is performed. Differences in estimation may result from experience. Similarly, the classification of the amount of oedema leaves room for operator interpretation influenced by experience. The boundaries between the classification groups are not entirely clear and some variation is therefore possible. Most mares in this study were checked every other day, so when the mare was inseminated and ovulation was determined 48 hours later by scanning, the mare could have ovulated soon after insemination, or just before the next scan.

6. Conclusion

The oestrous cycle of the Friesian mare is subtly different to that in some other common horse breeds. The IOI is on average two days longer compared to light horse breeds, and more comparable to the longer cycle length in ponies (IOI of 24 days), with a mean of 24.46 ± 0.32 days. The IOI is not dependent on reproductive status, stage of season or age of the mare. The mean maximum follicle diameter was larger than in breeds like Quarter horses and Thoroughbreds at 5.18 ± 0.04 cm at 48 hours prior to ovulation. Uterine oedema began to increase on day -8 and reached a peak on day -6, which is about three days earlier than in other horse breeds. A soft pre-ovulatory follicle of >4.0-4.5 cm with a thickened follicular wall that is beginning to deform, decreasing uterine oedema and a soft uterus and cervix seem to be the indicators of impending ovulation within 48 hours.

Acknowledgements

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