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OVULATORY FAILURE IN MARES DURING BREEDING SEASONS

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ABSTRACT

In the years 2022 and 2023 we investigated the prevalence of the ovulatory failure in 133 mares of different breed, 3 to 24 years old, brought to the Equine clinic of the University of Veterinary Medicine and Pharmacy (UVMP) in Košice. Our research involved the monitoring of the reproduction cycle in these mares with the aim to determine the prevalence of ovulatory failure during the breeding season, focusing on certain specific parameters that were considered sufficiently relevant with respect to the development of such failure. These parameters included environmental temperature and the amount of daylight the mares were exposed to, attributable to the months of the breeding season. In addition, we also evaluated the size of pre-ovulatory follicles and the influence of age of the investigated mares. During the mentioned years, one of the ovulatory failures occurred in 17 out of 133 mares (12.78 %). Examination by ultrasonography (USG) and the subsequent analysis of the occurrence of failures in relation to the investigated parameters allowed us to conclude that the length of daylight, age, and environmental temperature were the most important factors. Our observations indicated that the 15–16 h category of daylight presented the highest risk of development of cycle disorders with the most rapid increase observed for HAF. With respect to age, the highest occurrence of ovulatory failures was observed in the category of 15–24 years. The external temperature in the range of 18.1–23.0 °C affected the most the development of cycle disorders. Again, the effect was most evident with respect to HAF. This resulted in our recommendations for horse breeders that they should manage reproduction of their mares in a way so that the receptive phase of the cycle does not occur in the months during which the daylight length and environmental temperatures support ovulatory failure. We also recommend that if the owner′s wish is to keep in the breeding the mares older than 15 years, regular thorough gynaecological examination of these animals should be ensured, aimed at early diagnosis of ovulatory failure.

Key words: failure; mare; ovulation

INTRODUCTION

Reproductive health and fertility of mares are the basic assumption not only for the development of new generations by equine breeding, but also for increasing the population of horses. Many factors affect the development of future offspring but one of the essential ones is the reproductive cycle of the mare and its correct functioning. Ovulation disorders may become a serious issue causing a frequent disorder of this cycle and thus unsuccessful fertilization.

The mean length of the oestrous cycle in the mare population during the physiological breeding season is approximately 21 days, but it can vary greatly (range 18–24 days) [11]. However, although the oestrus typically comprises 4–7 days of the cycle, its length is more variable, ranging from 4 to14 days or more [13]. In the beginning and at the end of the breeding season, the length of the oestrus may be 7 to 12 days, whereas, around the summer solstice, oestrus may last only 3 to 4 days. Therefore, the duration of the oestrus is the shortest during the peak of the ovulatory season [11].

The general seasonal pattern is that the incidence of ovulation is minimal or absent during the winter, transitionally increasing during the spring, maximal during the summer, and transitionally decreasing during the fall. The low incidence of ovulation for the winter months reflects anovulatory seasons of different lengths among individual mares combined with uninterrupted ovulation throughout the year by some mares.

Changing daylength is the primary controller of the follicle dynamics of seasonal reproductive rhythms [4, 10].

In most of the breeding programmes, the main aim of the breeder should include a thorough gynaecological veterinary care, serving as a basis for evaluation of potential performance of his mares. Today, ultrasonographic (USG) examination is a routine examination tool owing to which it is possible to accurately assess not only the phase of the oestrus cycle but, within the regular checking also detect the occurrence of ovulatory disorders, such as haemorrhagic anovulatory follicle, luteinisation anovulatory follicle, diestral follicle or ovarian haematoma.

Haemorrhagic anovulatory follicle (HAF) is a follicle that contains blood. By USG examination of the follicular fluid in the dominant follicle it is possible to observe at first the presence of diffuse echogenic spots and later echogenic fibrous filaments that may form an echogenic mass throughout the follicle [9]. Anechogenic structures – coagulums – are present among the fibres. The presence of HAF follicles causes fertility disorders because these haemorrhagic follicles fail to ovulate. Within 1–7 weeks, their spontaneous regression occurs [5].

Luteinization anovulation follicle (LAF). Similar to haemorrhagic anovulatory follicles, LAF follicles do not ovulate. Their presence may lengthen the interovulatory interval and thus affect the reproduction of mares. The USG image shows an anechogenic appearance of the follicle that is also associated with increased progesterone levels (above 1 $ng.ml^{-1}$) [6]. However, the luteal tissue responds well to administration of PGF2α with luteolytic effect. At the same time, progesterone level decreases and the mare returns to oestrus. The therapy is most effective when PGF2α is administered 7– 10 days following the diagnosis of [9].

Diestral follicle (DF) is the result of the presence of secondary follicular wave. Diestral follicle is destined to undergo ovulation, luteinisation or regression. In case of ovulation, the so called diestral ovulation is involved. When the process of diestral ovulation is not initiated before the process of luteolysis, the luteal phase is prolonged so is the overall cycle [1]. However, diestral ovulation can also result in formation of the so called persistent *corpus luteum* (CL). This CL fails to regress as is expected during the functional cycle after ovulation by the action of PGF2α. Persistent CL can be present for several months and the mare expresses no signs of oestrus while the progesterone level is increased above 1 $ng.ml^{-1}$. Such condition may be resolved by administration of one dose of PGF2α [9].

Examination for anovulatory follicles is the same as that for any other follicle and in dependence on the type of pathology, hormonal or surgical interventions can be used or the pathology can become extinct with time. Observations involve mainly growth progression or structural changes. It should be mentioned that the presence of anovulatory follicle or pathology in one ovary does not mean that normal ovulation cannot take place in other physiological preovulatory follicle.

The aim of this study was to point out to the importance of supervision of the reproductive cycle in mares as insufficient diagnosis before and after fertilisation can result in the situation that prevents the breeder to use the mare for breeding in the respective year and thus obtain no offspring. We aimed to evaluate the cycle of each examined mare, diagnose potential reproduction disorders and analyse the factors that may contribute to their development and, if possible, regulate their occurrence.

MATERIALS AND METHODS

Material

The experiment was carried out on 133 mares of various breeds, 3–24 years old, brought to the Equine clinic of the University of Veterinary Medicine and Pharmacy (UVMP) in Košice in years 2022 and 2023. The mares were examined during the breading season from April to September in both years.

The study was conducted with the farmers' consent and willingness to participate in the research.

The diagnosis and subsequent management took place at the clinic. In order to ensure optimum conditions and safety of animals as well as of examining and assistant personnel, the mares were placed in fixation cages.

USG examination was carried out using a SonoScape E1V (SonoScape Medical Corp., China) with rectal probe adjustable from 4 to 8 MHz. With respect to the character of examination, the inevitable aids (disposable rectal gloves, lubricants) were used to protect the veterinarian against contact with faeces and the rectal mucous membrane against damage. Samples of blood from *vena jugularis* were obtained for determination of progesterone level. The blood samples were transported and examined in RIA laboratory s.r.o. at Kukučínova 84/5 in Košice, Slovakia. Some mares were administered the preparation Chorulon (hCG – human Chorionic Gonadotropin, MSD Animal Health) for ovulation induction. The mares with progesterone level exceeding 1 ng.ml^{-1} were administered PGF2α for luteolysis of the luteal tissue.

Methods

The mares were first subjected to adspection and gynaecological examination.

We asked the owners whether they observed signs of the oestrus and subsequently we checked their claim. We observed the mares for vaginal discharge, "winking", frequent urination, reddish and swollen vulva, and if stallion or gelding were available we observed her willingness to mate and the immobility reflex.

Subsequently, we examined the internal reproductive organs by a rectal probe. According to the shape and size of follicles we were able to judge and confirm whether the animal was in heat. In the USG image, in each of the ovaries we detected the dominant follicle with assumption that one of them will ovulate. The approaching ovulation was suggested by damage to the integrity and thickening of wall of the follicle, presence of hyperechogenic particles inside the follicle and the size of endometrial folds of uterus. We evaluated their oedematization and presence of fluid among them. During the introductory examination we collected samples of blood for determination of progesterone that should be lower than 1 ng.ml^{-1} in mares in heat. Owing to regular checking of mares we had very accurate picture about development of size and shape of the follicles. The basic feature of the preovulatory follicle is the change of its shape as it turns from oval to pear-shaped. One can also observe reduction in follicle tension sensitivity of ovaries at transrectal palpation.

Although physiological cycle was detected in the majority of mares, there were also those who showed some ovulatory failures which could prevent the ovulation. We were able to diagnose the following: haemorrhagic anovulatory follicle (HAF), luteinization anovulatory follicle (LAF), and diestral follicle (DF).

RESULTS

Our study was based on the input data obtained from mares in both breeding seasons and the specific information that allowed us to determine potential correlation with the occurrence of ovulatory failures in the cycle. The specific information included mean monthly external temperature and mean daylight length in individual months of the breeding seasons. We also analysed the size of preovulatory follicles and the age of mares.

In order to obtain a clearer picture of the situation, some of the specific data were divided to the following categories:

External temperature – three categories: 8.0–13.0 °C (low); 13.1–18.0 °C (medium); 18.1–23.0 °C (high).

Daylight length – four categories: 12–13 h; 13–14 h; 14–15 h; 15–16 h.

Size of preovulatory follicles – seven categories: 3.0–3.5 cm; 3.6–4.0 cm; 4.1–4.5 cm; 4.6–5.0 cm; 5.1–5.5 cm; 5.6–6.0 cm; 6.1–6.5 cm.

Age of mares – three categories: A: < 6 years (young); B: 6–15 years (medium); C: > 15 years (old).

The input information obtained in the 2022–2023 breeding seasons showed that some of the ovulatory failures occurred in 17 of the 133 examined mares which comes to 12.78 %. In the 2022 breeding season we detected only HAF and LAF failures while in 2023 there were diagnosed HAF and DF.

Evaluation of results obtained in the 2022–2023 breeding seasons (Fig. 1) showed that the lowest number of failures was recorded at low temperatures. Increased number of HAF failures was detected in the temperature ranges 13.1–18.0 °C and 18.1–23.0 °C. In these ranges, 8 of the total of 17 failures (47.06 %) were HAF failures. The occurrence of HAF increased more remarkably with the increasing external temperature. Mild increase in LAF and DF occurrence was observed only in the 18.1–23.0 °C category. The lack of sufficient information prevented us from confirming an increasing trend in LAF and DF failures. Summarisation of our data indicated that higher external temperatures will result in higher risk of development of ovulatory failures, particularly of HAF.

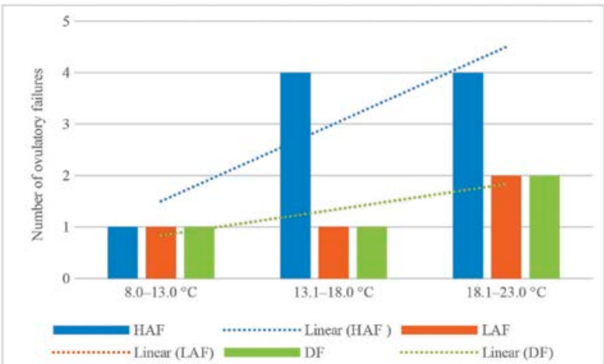

Fig. 1. Number of ovulatory failures in relation to mean monthly external temperatures during the breeding seasons of 2022–2023.

Evaluation of the effect of daylight on ovulatory failures in both years (Fig. 2) indicated that the number of failures increased considerably when the mean monthly daylight length was 15–16 hours. This figure showed that the most rapid increase was observed for HAF, therefore the 15–16 daylight hours present the highest risk particularly for this failure as 8 of 13 failures (61.54 %) detected in our study were caused by HAF. With respect to LAF and DF, it was difficult to estimate the trend of their occurrence and correlation with daylight length because their number was very low in comparison with HAF and can be equal to statistical deviation. However, according to our results, the influence of the first three daylight categories (12–13 h, 13–14 h, 14–15 h) on failures of the breeding cycle was from negligible to none.

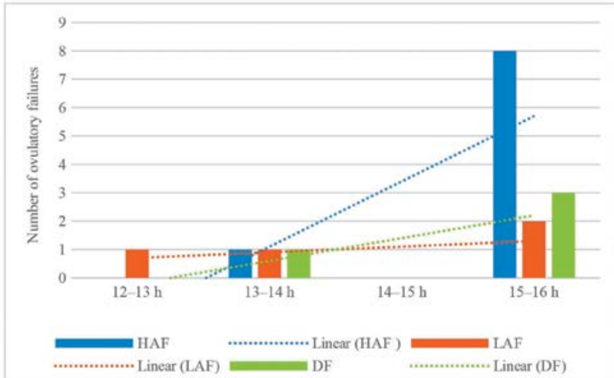

Fig. 2. Number of ovulatory failures in relation to mean monthly daylight length during the breeding seasons of 2022–2023

Our investigations showed that the failures can occur almost in every month of the breeding season. With respect to the number of failures it was evident that that higher occurrence was recorded in May and July when higher occurrence of HAF was confirmed. This can be related again to the higher daylight period (15–16 h). While the mean external temperature in May reached 16 °C in 2022 and 15.1 °C in 2023, in July the temperature was higher, reaching 22.2 °C and 21.1°C in years 2022 and 2023, respectively. They corresponded to the medium and high temperature categories (Fig. 1) that both posed a risk with respect to HAF failures. As far as LAF and DF failures were concerned, the trend of their occurrence and correlation with external temperature and daylight length was difficult to predict as their occurrence compared to HAF was very low and could correspond to standard deviation. The obtained data indicate that the influence of months can be discussed only in relation to mean external temperature or daylight length and not only of the months themselves.

Our data from two breeding seasons (Fig. 4) showed almost no relationship between the size of the preovulatory follicle and the occurrence of failures in the breeding cycle as the failures occurred within each size category. Minimal increase in HAF failures was observed at size range 6.1–6.5 cm that could suggest some role of follicle size in the occurrence of HAF failures.

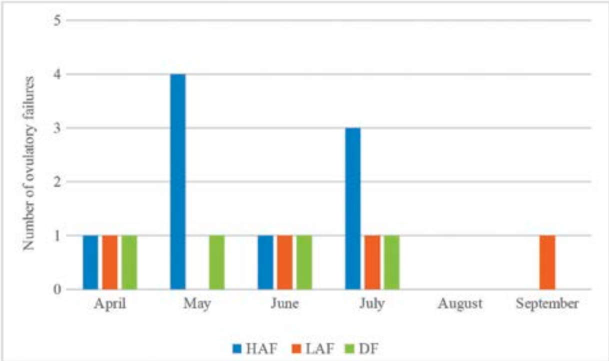

Fig. 3. Number of ovulatory failures in relation to months of the breeding seasons of 2022–2023

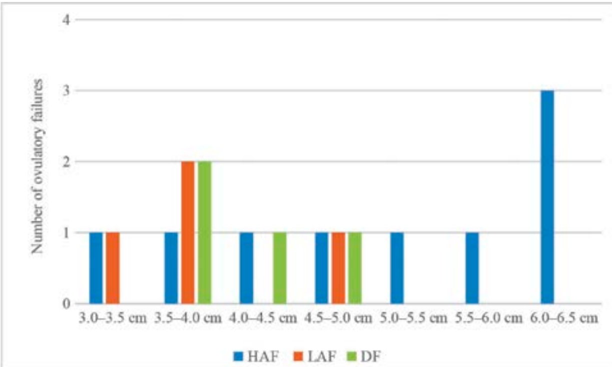

Fig. 4. Number of ovulatory failures in relation to the size of preovulatory follicle during the breeding seasons of 2022–2023

The results presented in Fig. 5 show that the age of the investigated mares was a substantial factor affecting occurrence of ovulatory failures that reached the highest level in the category of 15–24 years. However, it should be mentioned that this influence concerned only HAF that showed a marked increase with increasing age. In the category of old mares, HAF was diagnosed in 6 of 9 affected mares (66.67 %). More data are needed to draw conclusion about the effect of age on LAF and DF disorders.

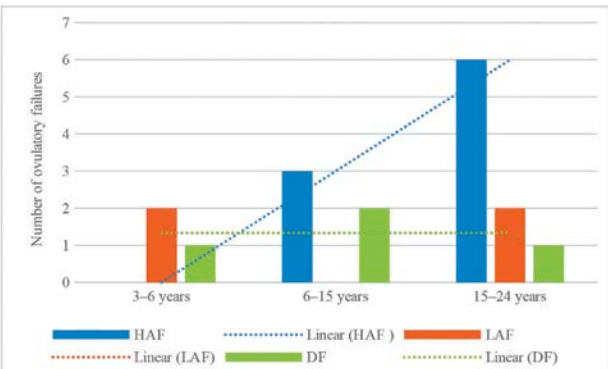

Fig. 5. Number of ovulatory failures in relation to age of mares during the breeding seasons of 2022–2023

DISCUSSION

Many factors are concerned in the causation of infertility in equines, and abnormal ovarian function is generally regarded as the most important of all [15].

The aim of our study was to identify the factors that affect the development of ovulatory failures. We focused on the relationship between occurrence of ovulatory failures and specific data that seemed to be sufficiently relevant so as to cause such failures.

Our study showed that the most important parameter was the daylight length. We observed that in the daylight category of 15–16 hours there was an evident increase in ovulatory failures, particularly with respect to HAF. It was interesting that outside this daylight range minimal presence of anovulatory follicles was observed. Because the mentioned photoperiods were recorded in different months, their incidence can be interconnected with the months of the breeding season, i. e. May and July.

The influence of light on the hypothalamus-hypophysis-ovarian axis in terms of reducing melatonin levels in the spring, period results in starting-up the reproduction cycle. A common practice in intensive farming is the artificial lengthening of the photoperiod even to the level of 16 hours of daylight that is as a rule initiated already in December and results in early onset of ovarian activity and also losing of the winter coat [8]. However, our observations indicated that the 15–16 h category of daylight presented the highest risk of development of cycle disorders. Other authors [17] described in their study a very effective way of suppression of melatonin involving the use of low-wavelength blue light (468 nm), directed to one or both eyes.

Another, not less important factor affecting the internal environment of the reproductive apparatus of mares is their age. Our study revealed that category C, i.e. mares older than 15 years of age, showed increased tendency toward ovulatory failures. The HAF failure was affected most by the age. Some authors [1] investigated the influence of age on the oestrous cycle in mares and arrived to the conclusion that the interovulatory interval and follicular phase were longer in older animals, the size of the preovulatory follicle in them tended to be smaller, and deviations in follicles appeared later than in younger mares. Interesting was the observation of these authors that DF was detected earlier in younger mares than in older ones. This was observed also in our study as we diagnosed 4 cases of DF in 2023 and of them 3 cases (75 %) were young and medium old mares (A and B categories). C o n t r i et al. [2] conducted a study on donkeys and reported that larger follicles tended to ovulate earlier but did not differentiate between the age. Another study [14] focused on the influence of age determined that the number of ovulatory cycles was lower in older mares compared to the younger ones.

Another important parameter was external temperature. Our results showed that high temperature in the range of 18.1–23.0 °C affected the most the development of cycle disorders. Again, the effect was most evident with respect to HAF that was affected also by temperature range of 13.1–18.0 °C. A study conducted in Slovakia [16] determined that increased ambient temperature resulted in shortening of the oestrus. The same effect was exerted by the length of daylight. Other authors [7] reported that the onset of oestrus was closely related to the minimum and maximum ambient temperature. They implied that the transition from the spring interim period up to the onset of reproductive activity tends to be longer as it is affected by colder weather.

The last investigated parameter was the size of the preovulatory follicle. On the basis of this parameter, we tried to determine whether it is possible to predict ovulatory failure after the preovulatory follicle reached certain size. Our observations showed that the ovulatory failure can occur at any size of the preovulatory follicle. R u a et al. [12] investigated the conditions of the ambient environment, and reported that neither external temperature nor precipitations affected the size of preovulatory follicles and the influence of daylight length was also low. Another study [3] confirmed a significant difference in the size of preovulatory follicle in young and old mares. The size of follicles in mares older than 19 years was considerably smaller and the authors also implied that viability of oocytes decreases with increasing age which can result in ovulation of smaller follicles or their maturation before ovulation.

Evaluation of results obtained in both breeding seasons allowed us to draw conclusions concerning the investigated issues and present useful recommendations to breeders with the aim to ensure the best possible reproductive performance of their mares.

Of the total number of 133 mares investigated in years 2022 and 2023 one of the ovulatory failures affected 17 of them which amounts to 12.78 % incidence.

As far as the particular disorders occurred in the 2 investigated years, the following failures were detected:

The most frequent was the HAF failure which was detected in 9 out of 17 affected mares (incidence 52.94 %). However, when taking into account of the total number of investigated mares (133), HAF was detected in 6.77 % of the animals.

LAF occurred in 4 out of the 17 affected mares (23.53 %), but only 3.01 % of the 133 investigated mares developed this failure.

DF was also detected in 4 out of the 17 affected mares (23.53 %) and the incidence was the same as that of LAF (3.01 %) when taking into account 133 investigated mares.

With respect to the specific parameters, results obtained in two years of investigation can be summarised as follows:

Daylight length affected considerably the occurrence of ovulatory disorders. Its effect was the highest in the category of 15–16 h which was confirmed by increasing occurrence of failure with increasing daylight length. At this photoperiod, failures were detected in 13 out of the 17 affected mares (76. 47 %), or in 9.77 % of mares out of 133 examined.

The age of mares was an important factor, affecting development of ovulatory failures. The most affected were the old mares (15–24 age category) in which the number of failures increased the most with increasing age. In this category, failure was detected in 9 of the17 affected mares (52.94 %), or 6.77 % of mares out of 133 examined developed the failure.

Temperature also plays a role in oestrous cycle disorders. Higher number of failures was also observed in the high temperature category in the range of 18.1–23.0 °C indicating increased occurrence of failures with increasing ambient temperature. In the mentioned category, failures were detected in 8 of the 17 affected mares (47.06 %), or 6.02 % incidence in 133 investigated mares.

The size of preovulatory follicles had almost no effect on the occurrence of failures in the oestrous cycle as the ovulatory failures were detected in all follicle-size categories with minimal deviations. Higher number of data is required for better assessment of the influence of this parameter.

Despite the fact that we evaluated data gathered over the period of two years and the investigated parameters differed in their importance, it is necessary to stress that more accurate evaluation of trends of occurrence of individual ovulatory failures in relation to the relevant parameters requires further investigations in future breeding seasons or obtain many additional data.

CONCLUSIONS

The incidence of ovulatory failures that was recorded in years 2022 and 2023 was not negligible but not too high, which was a positive finding. Higher number of such failures would result in more serious problems with equine fertility and considerable impact on maintaining and increasing population of horses. Despite the low number of the diagnosed failures they still can represent a troublesome obstacle for horse breeders. Therefore, it is important to enlighten the breeders, call their attention to risk factors that can cause the failure and provide useful recommendations that can help the breeders to prevent breeding problems. Our results allowed us to conclude that daylight length and temperature are the factors with the highest influence on occurrence of ovulatory failures. Therefore, our advice to equine breeders is to avoid or become more cautious with mating of mares in May and July when the investigated parameters reached levels that supported development of the monitored failures. We can also recommend that the breeders who strive to ensure successful reproduction of mares older than 15 years make certain that regular thorough gynaecological check-up of mares is ensured not only before but also after mating so the potential cycle disorder can be diagnosed early enough and the mating can be repeated in the respective year.

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