

## Effect of single vs multiple ovulations on oestrous behaviour and pregnancy rate in Thoroughbred mares

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A relatively high rate of multiple ovulation and resulting multiple pregnancies are one of main problems in Thoroughbred reproduction. The aim of the study was to examine the relationship between the intensity of oestrous behaviour and incidence of multiple (MO) vs single (SO) ovulations in 47 Thoroughbred mares. The frequency of five distinct receptive and 12 non-receptive behavioural traits was analysed. For quantitative analysis of sexual behaviour an 8-point behavioural score (BS) expressing increasing mare's sexual receptivity was used. MO occurred in 48.9% of ovarian cycles observed. Neither frequencies of receptive behaviours, nor BS differed significantly between MO and SO mares. Overall pregnancy rate per one ovulatory cycle was estimated as 40.4%. Pregnancy rate was higher in MO mares (52.2%) as compared to SO mares (29.2%); however, the difference was not proved significant.

**KEY WORDS:** horse / multiple ovulation / reproduction / sexual behaviour / Thoroughbred

Thoroughbred mares are known for their low reproductive efficiency [Byszewski and Gromnicka 1994]. The breed is mainly selected on the basis of race performance and canter speed. For this reason horses characterized by respective physical (good development of muscles, skeleton and respiratory system) and psychical (high reactivity) traits are preferred as breeding material. One-way selection focused on racing performance may, however, cause a simultaneous decrease of reproductive efficiency in Thoroughbreds.

Thoroughbred mares are known for a relatively high rate of multiple ovulation. Multiple ovulation may result in multiple pregnancy [Deskur 1985, Ginther 1983, Kulisa *et al.* 1999, Newcombe 1996], and consequently in early embryonic mortality, underdevelopment of foetuses, abortions or births of underdeveloped foals. However, in modern

Thoroughbred breeding systems, reproductive activity of mares is carefully monitored by frequent ultrasonic examinations of genital tract for ovulation or pregnancy diagnosis. Multiple pregnancies are monitored and, if necessary, one foetus is eliminated [Mattos *et al.* 1996]. From this point of view, multiple ovulation and pregnancy, if monitored, may increase overall reproductive efficiency in mares. Bhuvanakumar [1996] claimed that in India, throughout the period of intensive general use of ultrasound scanners enabling early diagnosis and management of multiple pregnancy, an increase had been achieved in reproductive indices of Thoroughbred mares.

In mares kept under stable conditions oestrus detecting is mainly based on the observations of mare's behaviour towards a teaser stallion. Therefore, in mares showing stronger heat and demonstrating low incidence of behavioural problems (e.g. silent oestrus), pregnancy rate should be higher, because of more chance to be mated in a due time.

Estrogens from maturing ovarian follicle evoke the sexual receptivity of a mare [Ginther 1992]. In the case of multiple ovulation the intensity of oestrus may depend on the amount of estrogens produced by the secretory area of more than one dominant follicle. The aim of the present investigation was to examine whether the multiple ovulation affects the sexual receptivity and following pregnancy rate in Thoroughbred mares as compared to single ovulation.

### Material and methods

Forty-seven Thoroughbred mares were used, at the age of 4-18 years. The data were collected in the years 1998 and 2000, during the peak of reproductive season (May) in a stud located in central Poland.

Mares' behavioural reactions towards a teasing stallion throughout oestrous cycle have been recorded using a video camera (PANASONIC, M-50, Japan) and analysed thereafter. Five receptive (Re) and 12 non-receptive (Nre) behaviours have been distinguished (below).

#### Receptive behaviours (Re)

- Re 1. Standing still
- Re 2. Raising tail
- Re 3. Winking
- Re 4. Urinating
- Re 5. Pelvis lowering

#### Non-receptive behaviours (Nre)

- Nre 6. Holding ears back
- Nre 7. Squealing
- Nre 8. Kicking attempts
- Nre 9. Kicking
- Nre 10. Clasp tail
- Nre 11. Biting
- Nre 12. Tail switching
- Nre 13. Pushing against teaser/  
bar
- Nre 14. Moving around
- Nre 15. Paving
- Nre 16. Escaping

Since different combinations of Re and Nre behaviours may be demonstrated by a mare towards the teasing stallion, for quantitative evaluation of sexual behaviour during follicular phase an 8-point behavioural score (BS) was used, expressing increasing mare's sexual receptivity (below).

**One point.** Nre behaviour (holding ears back, squealing, kicking attempts, tail switching, moving around). The mare attacks or kicks the teasing stallion.

**Two points.** Nre behaviour (holding ears back, squealing, kicking attempts, tail switching, moving around). No attack towards the teasing stallion.

**Three points.** Nre behaviour (holding ears back, squealing, kicking attempts, tail switching, moving around). The mare stands still.

**Four points.** The mare stands still indifferently. Neither Nre nor Re behaviour.

**Five points.** The mare shows Re behaviour (standing still, raising tail, winking) accompanied by some Nre behaviour (holding ears back, squealing, kicking attempts, tail switching).

**Six points.** The mare shows Re behaviour (standing still, raising tail, winking). No Nre behaviours.

**Seven points.** The mare shows full Re behaviour (standing still, raising tail, winking, passing fluids, lowering pelvis) accompanied, however, by some Nre behaviours (holding ears back, squealing, kicking attempts, switching tail).

**Eight points.** The mare shows full Re behaviour (standing still, raising tail, winking, passing fluids, lowering pelvis). No Nre behaviours.

Visual observations were followed by daily ultrasonic examination of the mares' reproductive tracts using a linear-array, real-time scanner, equipped with a 5 MHz transducer (PIE-MEDICAL, Holland). Follicular development was monitored until ovulation, and then mares were classified into two groups: with multiple (MO) or single (SO) ovulation. As MO classified were synchronic and asynchronic unilateral double and bilateral double or triple ovulations within one follicular phase. A transrectal ultrasonic examination for pregnancy was performed on day 17 after ovulation.

Pregnancy rate (per cent) was calculated as the number of mares pregnant  $\times$  100 divided by the number of those that ovulated. For the reason of behavioural character of this work, we assumed that each mare that had ovulated had an opportunity to conceive during follicular phase. Mares that had not been mated because of behavioural problems, but had ovulated, had also been included in the calculation of pregnancy rate.

For statistical evaluation a one-way analysis of variance (GLM procedure) to determine the effect of the ovulation rate (MO, SO) on behavioural score (BS) was used. The remaining data (frequencies of distinct sexual behaviours, pregnancy rate) were analysed by a *Chi*-square test [SAS System 1992].

## **Results and discussion**

MO occurred in 23 out of 47 ovarian cycles observed (48,9%, figures not tabulated). The incidence of MO was higher than those found by Henry *et al.* [1982], Okólski

[1985] and Davies Morel and O'Sullivan [2001] who reported 27.2, 17.0 and 22.4%, respectively. Newcombe [1996] gave the mean MO rate of 33.2%. In one observed stud, however, the estimated MO rate appeared as high as 41%. According to Ginther [1992] in the experimental herd selected for a high ovulation rate, the MO reached 56%. Inconsistency between these results might have been caused by different methods of data gathering. The data cited have been averaged based on slaughterhouse material or stud records (compiled from rectal palpation and/or ultrasonic scanning). Means may also be affected by breed [Newcombe 1996], reproductive status [Ginther 1983], rate of inbreeding [Mahon and Cunningham 1982] and season [Deskur 1985]. Small number of mares and ovarian cycles could explain high incidence of MO in the present study.

Pregnancy was diagnosed in 19 mares (40.4%, figures not tabulated). Ginther [1983] reported pregnancy rates higher ( $P < 0.01$ ) for double (84%) than for single ovulations (54%). Also in this study the pregnancy rate in MO was higher (52.2%) than in SO mares (29.2%), but difference between groups was not found significant.

During follicular phase the mares included in this study showed receptive (Re) as well as non-receptive (Nre) behaviour. Frequency of receptive (Re1-Re5) behaviours were higher in MO than SO mares (Fig. 1). On the other hand, frequencies of Nre behaviours (except for Nre15, Nre16 and Nre17) as estimated by *Chi*-square test

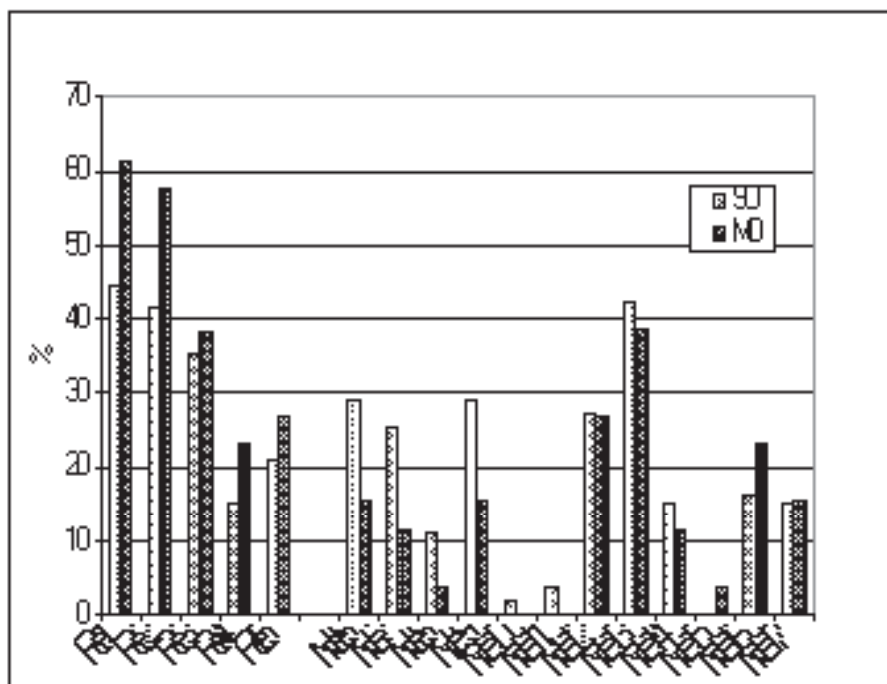


Fig. 1. Frequencies of receptive (Re) and non-receptive (Nre) behaviours in single (SO) and multiple (MO) ovulating Thoroughbred mares during follicular phase. No significant differences between SO and MO mares.

**Table 1.** Sexual behaviour score (BS) and pregnancy rate in single-ovulating (SO) and multiple-ovulating (MO) Thoroughbred mares

| Item               | SO mares |                    |                | MO mares |                    |                |
|--------------------|----------|--------------------|----------------|----------|--------------------|----------------|
|                    | n        | least squares mean | standard error | n        | least squares mean | standard error |
| BS (points)        | 24       | 3.97               | 0.21           | 23       | 4.52               | 0.52           |
| Pregnancy rate (%) | 7/24     | 29.2               |                | 12/23    | 52.2               |                |

No significant differences between groups.

were higher in SO mares. Differences in frequencies between groups were not found significant. Behavioural score (BS) was higher in MO (4.52) than in SO mares (3.97) – Table 1, but the difference was not found significant.

Weak or equivocal signs of oestrus and resulting low oestrus detection rate, may be one of reasons of unsatisfactory reproduction in horses. It has been shown that breed of the mare was an important factor influencing sexual behaviour. Mares of the primitive breed, selected for ability to survive in natural conditions, expressed more intense sexual receptivity and higher pregnancy rate than Thoroughbreds [Górecka and Jezierski 2002]. Overt and strong receptive behaviour in a mare facilitates detection of optimal breeding time and enables successful natural mating, which is the only way of mating accepted by Thoroughbred Stud Book regulations.

Although in this study sexual behaviours of MO did not differ significantly from those of SO mares, a tendency to exhibit more intense receptive behaviour was observed in MO group. It can be hypothesised that intrinsic and extrinsic factors affecting follicular development and sexual behaviour act, to some extent, independently. This may result in better oestrus detection by the observer and thus the MO mares may have more chance to be mated than the SO mares.

The use of ultrasonography in equine reproduction enables early diagnosis of twin pregnancy. In such cases, one embryo is often eliminated to ensure a normal development of the retained singleton [Ginther 1992, Mattos *et al.* 1996]. A tendency to multiple ovulations is supposed to be inheritable [Kulisa *et al.* 1999]. A significant relationship between MOs in successive cycles was demonstrated by Davies Morel and O'Sullivan [2001] who showed that the incidence of MO in a cycle coincided significantly with that in the next cycle. The reduction of number of foetuses may consequently increase the overall reproduction indices [Ricketts and Young 1990], as such pregnancies have a chance to terminate with birth of a sound and well developed foal. However, a filly born from 'twin-manipulated' pregnancy may be a potential carrier of genes responsible for multiple ovulation in successive generations. It has been stated [Pawlak *et al.* 2000] that over a period of 66 years, the highest incidence of multiple pregnancies in Thoroughbred mares in Poland was ascertained in the last two decades (1975-1995). Higher incidence of multiple pregnancy could have been caused by the increase in frequency of genes responsible for multiple ovulation in the Thoroughbred population.

That could be the result of applying ultrasound technique for pregnancy diagnosis and monitoring during last years.

Taking into account that ovulation rate did not affected intensity of sexual behaviour during follicular phase in the mare, and did not affected the pregnancy rate, elimination of multiple ovulating mares from the breeding stock seems justified. It is assumed that breeding only single-ovulating mares may result in improvement of the reproductive efficiency of Thoroughbreds.

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## Wpływ owulacji mnogiej na zachowanie płciowe i zażrebień klaczy pełnej krwi angielskiej

### Streszczenie

Owulacje i ciąży mnogie są jedną z głównych przyczyn problemów w rozrodzie koni pełnej krwi angielskiej. Celem pracy było określenie powiązań między intensywnością zachowań rujowych, ułatwiających wykrycie rui a występowaniem owulacji mnogich i procentem żrebności. Materiał stanowiło 47 klaczy. Analizie poddano frekwencje pięciu receptywnych (Re) i 12 niereceptywnych (Nre) zachowań płciowych klaczy wobec ogiera. Dla liczbowego oszacowania zachowania płciowego wprowadzono współczynnik zachowań płciowych (BS), wyrażający w skali 8-punktowej rosnącą akceptację ogiera przez klacz.

Owulacja mnoga wystąpiła w 48,9% badanych cykli płciowych. Nie wykazano istotnych różnic we frekwencji poszczególnych zachowań płciowych ani w wielkości BS między klaczami z owulacją mnogą (MO) a pojedynczą (SO). Żrebność klaczy po kryciu w badanych cyklach płciowych wynosiła 40,4% i była wyższa u klaczy MO (52,2%) niż u klaczy SO (29,2%); nie udowodniono istotnych różnic między grupami.

