

Intrauterine fluid accumulation during foal heat increases embryonic death

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Summary

This study aimed to verify if mare's age, body condition score, number of parturitions, month of parturition and intrauterine fluid (IUF) accumulation during foal heat and after breeding influence the embryonic death rate during foal heat. All parturitions were assisted and, if necessary, mares were submitted to surgical repair of the vulva 30 minutes after delivery. Only mares having normal parturition and without any impairment after delivery were included in the study. Mares were classified according to body condition on the 5th day post-partum and, from that day until breeding, they were examined each 24 hours by means of rectal palpation and ultra-sound to evaluate follicular growth, degree of uterine edema and presence or absence of IUF. An additional examination was conducted 36 to 48 hours after breeding to confirm ovulation and to detect IUF accumulation. Mares with IUF accumulation at the first post-breeding examination were immediately submitted to uterine flushes followed by oxytocin administration. Pregnancy was diagnosed on the 12th day (D12) after ovulation and examinations were repeated, at least once a week, until the 45th pregnancy day (D45). Ninety nine Thoroughbred mares with ages between 4 and 23 years, pregnant during foal heat, were studied. Embryonic death (ED) was considered as having occurred when pregnancy was interrupted between D12 and D45. ED was detected in 18 (18.2%) mares. Mare's age ($p=0.849$), body condition score ($p=0.07$), number of parturitions ($p=0.417$) and post-breeding IUF ($p=0.944$) did not differ between mares with and without ED. However, ED rates were significantly greater ($p=0.015$) in mares with IUF accumulation during foal-heat (30.5%) than in mares without IUF accumulation (11.1%).

Keywords: mare, foal heat, intrauterine fluid, embryonic death

Intrauterine Flüssigkeitsansammlung während der Fohlenrosse begünstigt embryonalen Fruchttod

Die vorgestellte Studie untersucht den Einfluss von Alter und Body Condition Score (BCS) der Stute, Anzahl und Zeitpunkt der Abfohlungen, sowie intrauterinen Flüssigkeitsansammlungen während der Fohlenrosse und nach der Belegung auf die Häufigkeit von embryonalem Fruchttod während der Fohlenrosse. Bei allen Abfohlungen wurde Geburtshilfe geleistet; bei Bedarf wurde 30 Minuten post partum eine chirurgische Versorgung der Vulva durchgeführt. Nur Stuten mit normalem Geburtsablauf und ohne Verletzungen wurden in die Studie aufgenommen. Die Einteilung der Tiere erfolgte anhand ihres BCS am fünften Tag post partum. Von diesem Zeitpunkt bis zur Belegung wurden alle 24 Stunden mittels Sonographie und rektaler Palpation Follikelwachstum, Grad des uterinen Ödems und mögliche intrauterine Flüssigkeitsansammlungen evaluiert. Eine zusätzliche Untersuchung wurde 36 bis 48 Stunden nach der Belegung zur Bestätigung der Ovulation und zum Nachweis möglicher intrauteriner Flüssigkeit durchgeführt. Stuten, die hierbei durch Ansammlung von intrauteriner Flüssigkeit auffielen, wurden sofort mit Uterusspülungen und anschließender Oxytocingabe behandelt. Eine Trächtigkeitsdiagnostik erfolgte am Tag 12 post ovulationem (D12); die Untersuchungen wurden mindestens wöchentlich bis zum 45. Trächtigkeitstag (D45) wiederholt. 99 Vollblutstuten im Alter von 4 bis 23 Jahren, welche während der Fohlenrosse erfolgreich belegt worden waren, wurden untersucht. In dieser Studie wurde embryonaler Fruchttod als Trächtigkeitsabbruch zwischen D12 und D45 definiert. Dieser konnte bei 18 Stuten (18,2 %) beobachtet werden. Das Alter ($p=0,849$) und der BCS ($p=0,07$) dieser Stuten, sowie die Anzahl der Abfohlungen ($p=0,417$) und intrauterine Flüssigkeitsansammlungen nach der Belegung ($p=0,944$) unterschieden sich nicht von Tieren mit ungestörter Trächtigkeit. Signifikant höher ($p=0,015$) war jedoch die Rate von embryonalem Fruchttod bei Stuten mit Ansammlungen von Flüssigkeit im Uterus während der Fohlenrosse (30,5 %) im Vergleich zu solchen ohne intrauterine Flüssigkeitsansammlungen in diesem Zeitraum (11,1 %).

Schlüsselwörter: Stute, Fohlenrosse, intrauterine Flüssigkeit, embryonaler Fruchttod

Introduction

The mare shows an ovulatory, fertile estral cycle few days after parturition. This cycle, known as foal heat, begins 7 to 8 days after parturition and ovulation takes place between days 10 and 13. The fertility rate during this cycle is considered inferior to those occurring in subsequent cycles. Merkt and Günzel (1979) reported that mares bred during the foal heat showed pregnancy rates 10 to 30% lower than mares mated during subsequent cycles. In recent studies, anyway, similar pregnancy rates were found in the first post-partum and in other cycles (Mattos et al. 1995, Camillo et al. 2000).

Higher embryonic death rates (ED) have been reported in mares bred during the foal heat (Ball 1993). Kurtz Filho et al. (1999), studying 578 Thoroughbred mares, found ED rates of 30.3% and 11.5%, respectively, in mares bred during the foal heat and in subsequent heats. Similar data were reported by Bell and Bristol (1987), who found ED rates of 26% in mares bred during foal heat and of 16% in mares bred in other cycles. Several factors, such as the age of the mare, the period of the year, handle conditions and nutrition may influence the ED rate in the mare (Ball 1993).

The lower pregnancy rates and the higher ED rates during foal heat have been attributed, at least partially, to an incom-

plete uterine involution (Ball 1993). According to McKinnon et al. (1988), intrauterine fluid accumulation (IUF) during heat has a negative influence on the pregnancy rate in foal heat. Reilas (2000) did not observe a negative effect of IUF accumulation on the quality of embryos collected from mares bred during the foal heat. Schilela et al. (2001) did not find differences between the pregnancy rates of mares with and without fluid during heat, but they observed a significant reduction in the pregnancy rates of mares showing uterine fluid 36-48 hours after breeding. Although lower pregnancy rates in mares with IUF may be partially caused by embryo loss before the first pregnancy diagnosis (Ball 1993), no reports have been found about the effect of intrauterine fluid accumulation on the incidence of embryonic death.

The objective of this study was to compare the effect of intrauterine fluid accumulation during heat and after breeding, of mare's age, of the body condition status, of the number of parturitions and of the month of parturition on the embryonic death rate during foal heat.

Material and methods

The group of study was composed by 99 Thoroughbred mares, with positive pregnancy diagnosis 12-14 days after the first ovulation during the foal heat, kept in a reproduction management center in Southern Brazil, during the seasons of 1998, 1999, 2000 and 2001.

Mares were submitted to a 15 hour light/9 hour dark photoperiod/day with incandescent bulb (10W/m²) 45 to 60 days before parturition. At the moment of parturition, mares were classified, according to body condition, using a modified Henneke's score (Henneke et al. 1983, Malschitzky et al. 2001), in which the nine original categories were joined in pairs and the intermediate one, which showed the minimal body conditions for maximal reproductive efficiency, was maintained.

All births were assisted and, when necessary, perineum was repaired 30 minutes after foaling. Only mares having normal parturition and without any impairment after delivery were included in the study. Mares were kept on pasture and fed a concentrate mix twice daily.

From the 5th day post-partum on, mares were examined each 24 hours by means of rectal palpation and ultra-sound in order to evaluate follicular growth, degree of uterine edema and presence or absence of IUF. Examinations were repeated at maximal intervals of 48 hours, until a preovulatory follicle (>40mm) was detected. From that moment on, examinations were performed every 24 hours, until reduction in the degree of uterine edema was observed, and that moment was considered as the ideal mating time. An additional examination was conducted 36 to 48 hours after breeding to confirm ovulation and to evaluate uterine status.

Mares with IUF at the examination conducted 36 to 48 hours after mating were immediately submitted to, at least, three successive uterine flushes (2L saline each) followed by oxytocin administration (20 IU, IV). Any kind of treatment to remove IUF before breeding has been administrated to the studied mares.

Pregnancy was diagnosed on the 12th day after ovulation using a real-time mode B ultra-sound scanner fitted with a 5 MHz linear transducer. When an embryonic vesicle was detected, examinations were repeated, at least once a week, until the 45th pregnancy day. Embryonic death was considered as having occurred when pregnancy was interrupted between day 12 and day 45. There were no cases of twin pregnancies in this group of mares.

Results were analyzed using Analysis of Variance and randomization test, comparing mares with and without embryonic death according to age, body condition, number of parturitions, month of parturition, presence of intrauterine fluid during heat and 36-48 hours after breeding. Criteria of probability superior to pre-established α ($\alpha = 0.05$) were used in order to accept null hypothesis. Analysis was done using MULTIV 2000 software (Pillar 2000).

Results

Among the 99 mares studied, 18 (18.2%) showed embryonic death, 10 of them (55,5%) until day 20 and 8 of them (45.5%) between days 20 and 45. Incidence of embryonic death in mares with and without IUF during heat and 36-48 hours after breeding is shown in Tables 1 and 2.

Presence of IUF during heat influenced significantly ($p=0.015$) the incidence of embryonic death (Tab. 1), but did

Tab 1 Incidence of ED in Thoroughbred mares with and without IUF during foal heat. (a,b) ($p=0.015$) different characters indicate significant difference

Inzidenz von ED bei Vollblutstuten mit und ohne IUF in der Fohlenrosse. Die unterschiedlichen Ziffern bezeichnen signifikante Unterschiede

IUF	ED		
	n	n	%
Present	36	11 ^a	30.5
Absent	63	7 ^b	11.1

Tab 2 Incidence of ED in Thoroughbred mares with and without IUF 36-48 hours after breeding in foal heat. (a,a) ($p=0.944$) no significant difference

Inzidenz von ED bei Vollblutstuten mit und ohne IUF 36-48 Stunden nach dem Belegen in der Fohlenrosse. Keine signifikanten Unterschiede

IUF	ED		
	n	n	%
Present	16	3 ^a	18.75
Absent	83	15 ^a	18.07

Tab 3 Effect of average age, average corporal status, average number of parturitions and month of parturition on the occurrence, or not, of embryonic death (ED) in Thoroughbred mares with positive diagnosis for pregnancy during the foal heat. Same characters (a,b,c,d) in rows indicate no significant difference (respectively $p=0.85$; $p=0.07$; $p=0.42$ and $p=0.19$)

Einfluss von Alter, mittlerer Gelbkörperstatus, mittlere Geburtenrate und Geburtsmonat beim Auftreten oder Nichtauftreten von ED bei Vollblutstuten mit positiver Trächtigkeitsdiagnose in der Fohlenrosse. Gleiche Ziffern bezeichnen keine signifikanten Unterschiede

Characteristic	Without ED	With ED
Age (years)	10.2 ^a	9.94 ^a
Corporal status	3.8 ^b	4.3 ^b
Number of parturitions	3.6 ^c	4 ^c
Month of parturition	8.54 ^d	8.89 ^d

not influence ($p=0.944$) the incidence of embryonic death in mares with IUF accumulation 36 to 48 hours after breeding.

The effect of different characteristics, such as age, corporal status, number of parturitions and month of parturition on the occurrence of embryonic death is shown in Table 3.

No differences were found between the two groups of mares in what concerns to any of the characteristics studied. The average age of the mares was 10.1 years and the average corporal status was 4.05. Mares with embryonic death showed a tendency to better corporal status, but the difference was not significant ($p=0.07$). There was no difference in the number of parturitions (average 3.8 parturitions/mare) and in the month in which parturition occurred. Parturitions occurred more frequently in the end of August and in the beginning of September. The reproductive season did not influence the incidence of embryonic death. No significant difference was found ($p=0.217$) in the incidence of ED in the four years studied.

Discussion

ED rates found in this study were inferior to those observed by Bell and Bristol (1987) and Kurtz Filho et al. (1999), and similar to those observed by Mattos et al. (1995). The average age of the mares (10.1 years), in this study, probably reduced the incidence of ED during the foal heat, since younger mares show a faster uterine involution (Mattos et al. 1995), as well as superior fertility rates than older ones (Schoon et al. 1999). On the other side, the differences in ED found may be related to distinct management conditions, or to different experimental designs.

Presence of IUF during the foal heat influenced the ED rate. From 36 mares with IUF during the foal heat, 11 (30.5%) had ED. This result is almost three times greater than that of the mares without IUF during estrous. The presence of IUF during the foal heat probably indicates an incomplete uterine involution (Ginther 1992). Anyway, presence of IUF during the foal heat did not stop sperm transport, fertilization and onset of pregnancy, what confirms Reilas' (2000) findings, who did not observe any negative effect on the quality of embryos collected from mares with IUF during the foal heat. Although the uterine involution was delayed, pregnancy onset is probably due to the low rate of IUF 36-48 hours after breeding. Embryonic deaths may be consequent to an extended period of embryo exposure to an unhealthy environment. Results found in the present experiment agree with those observed by Malschitzky et al. (2002), who found that the presence of IUF during the foal heat did not affect pregnancy rate detected on day 12 in mares without treatment or submitted to uterine flushes 6 to 8 hours after breeding. However, they observed that embryonic loss rates, until day 42, were superior in untreated mares (control group) than in treated ones.

The presence of IUF in uterine lumen 36 to 48 hours after breeding did not influence on ED rate. Anyway, presence of IUF post-breeding affected ($p=0.01$) the pregnancy rate on day 12, as also previously reported (Schilela et al. 2001, Malschitzky et al. 2002). The number of mares achieving pregnancy with IUF after breeding was extremely low, despite the

treatments done. Post-breeding IUF is probably an inflammatory process provoked by a puerperal endometritis, which promotes an endometrial prostaglandin release, which leads to luteolysis. Koskinen and Katila (1987) found only one difference between mares getting and not getting pregnant during the foal heat, that is, a greater neutrophile surge in the endometrial deeper sheets. Post-breeding uterine flushes may have eliminated inflammatory products in some mares, allowing pregnancy continuation.

Other factors studied, such as age, period of the year, reproductive season, corporal status and number of parturitions, although associated to ED during foal heat by some authors (Ball 1993, Mattos et al. 1995), were not significantly different in mares with and without ED in the present study. The average age of the mares, in this study, was 10 years. Although there is a linear reduction in fertility with aging, the problem seems to be aggravated from the age of 15 years on, specially due to degenerative injuries (Ricketts and Alonso 1991). The average number of parturitions, which is a determining factor in the surge of vascular degenerative injuries (Schoon et al. 1997), was 3.8, probably due to the low age of most part of the animals. Most parturitions took place in the end of August and in the beginning of September, both corresponding to the beginning of the breeding season. The use of artificial photoperiod 45 – 60 days before parturition may have excluded the seasonal effects on fertility (Malschitzky et al. 2001). Both groups showed similar average corporal status, that is, above the minimal conditions for having a good reproductive performance.

The relatively low embryonic death rate, the fact that more than half the cases of embryonic death had occurred until the 20th pregnancy day (what corresponds to one cycle) and the observation that breeding during the foal heat does not impair fertility in subsequent cycles (Lieux 1980) reinforce the use of breeding during the foal heat as a routine procedure. It was concluded that the presence of IUF during foal heat increases embryonic death rate.

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