

# Association of several factors with embryonic death in mares in north-east Poland

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<sup>1</sup>Veterinary Practice prof. dr hab. Andrzej Raś, ul. I Dywizji Wojska Polskiego 15, 10-069 Olsztyn

<sup>2</sup>Department of Animal Reproduction with Clinic, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, ul. M. Oczapowskiego 14, 10-719 Olsztyn

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Raś A., Noryńska-Raś M., Klajna J.

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### Summary

The aim of this study was to investigate the impact of several factors on the occurrence of embryonic death in mares in north-east Poland. In the years 2018-2021 the pregnancy was diagnosed 12-19 days after mating in 3230 out of 4090 cold blooded mares (78.9%). During a re-examination carried out 45-60 days after mating, 288 mares (8.9%) were found not pregnant. The embryonic death rate was significantly higher in mares aged 14-23 years compared to younger ones (18.3% vs. 7.8%;  $p < 0.05$ ). Mares treated previously for endometritis lost significantly more embryos than healthy mares (16.7% vs. 7.9%;  $p < 0.05$ ). The embryonic loss rate was significant higher in mares with endometrial cysts than mares without endometrial cysts (16.9% vs. 8.5%;  $p < 0.05$ ). Mares mated during foal heat showed a significantly higher embryonic death rate than mares mated in later heats (22.8% vs. 7.8%;  $p < 0.05$ ). Mares with induction of ovulation lost significantly fewer pregnancies between both examinations than mares without induction of ovulation (5.7 vs. 9.4%;  $p < 0.05$ ). In conclusion, age, history of endometritis, endometrial cysts, and mating during foal heat were associated with increased embryonic mortality in mares in north-east Poland. The age of mares and mating in the foal heat exerted the greatest influence on the embryonic death rate. The ovulation induction with hCG had beneficial effect on the embryonic death rate compared to that associated with spontaneous ovulation.

**Keywords:** embryonic death, mares, age, history of endometritis, endometrial cysts, foal heat, induced ovulation

Embryonic death is a common problem in equine reproduction, causing significant economic losses. It is defined as loss of the embryo in mare during the first 40-50 days of gestation, before the process of organogenesis is completed (23). The term early embryonic death is widely used, but it is not entirely accurate, because embryos can be lost throughout the entire embryonic period. The embryonic death in mares might be defined as very early mortality (0-7 days), early mortality (7-24 days), and late embryonic mortality (24-50 days) (3). From a practical point of view, embryonic death in mares can be divided into two classes: before pregnancy diagnosis and thereafter. There is no practical method of diagnosing pregnancy prior to day 10-12 and it makes detection of embryonic loss between fertilization and day 10-12 difficult. The estimated incidence of embryonic loss before day 14 was 9% for normal mares and 62 to 73% for aged, subfertile mares (4). In practice, the diagnosis of embryonic death is based on the determination of pregnancy loss during a follow-up examination after a positive preg-

nancy diagnosis in the mare. The common method for diagnosing pregnancy and embryonic death in mares is transrectal ultrasonography performed 12-18 days after mating. The pregnancy loss is characterized by disappearance of embryonic vesicle between ultrasonographic examinations until day 20 or the absence of embryo in embryonic vesicle after day 20. With ultrasonography signs of impending embryonic death may be observed. They include irregular shape of an embryonic vesicle, an undersized vesicle, prolonged mobility of a vesicle beyond day 16, excessive endometrial edema, loss of embryonic heartbeat, increased echogenicity of fluid within the conceptus, and abnormal development of the embryonic membranes (12, 28). Using ultrasonography, estimates of embryonic death for normal mares range from 5-23% between 12 to 50 days of gestation (7, 10). In subfertile mares embryonic loss rate of 39% between days 15 and 50 was reported (29).

Many factors may contribute to embryonic loss in mares. They can be divided into intrinsic, extrinsic and

embryonic (4, 28). The most important intrinsic factors include clinical and subclinical endometrial diseases, luteal insufficiency, maternal age, mating during foal heat, time of mating or insemination, placement of embryo after nidation and maternal chromosomal abnormalities. External causes include stress for various reasons, but mainly related to pain, poor nutrition, weight loss, season, climate, rectal examination, and factors related to the stallion. Embryonic factors refer to chromosomal anomalies inherited or arising during meiotic division or fertilization.

The effect of breed on embryonic death is not clear. Some studies reported that breed had no effect on pregnancy loss (7). However, other studies indicate that heavy breeds lose fewer pregnancies than warm-blooded breeds (16). There are only few studies on embryonic death in mares in Poland, and these have focused on the pathogenesis of this phenomenon. Altered lymphocyte profiles and increased levels of serum amyloid A and haptoglobin in Arabian mares with embryonic death were reported (1, 15). No differences in endometrial bacteriology, cytology and histopathology between warmblood mares that had suffered from embryonic death and healthy mares in the diestrus phase were found (8).

To date, there have been no studies on occurrence of embryonic death in mares in Poland. The aim of this study was to investigate the impact of several factors on the occurrence of embryonic death in cold blooded mares in North-east Poland.

### Material and methods

A total of 4090 coldblooded mares were ultrasonographically examined for pregnancy in the years 2018-2021. The horses belonged to private breeders in north-east Poland. The age of mares ranged from 3 to 23 years. The examinations were performed at the request of the owners as part of a routine veterinary practice and there was no need to obtain the consent of the local ethics committee. The owners agreed to the use of data for scientific purposes. All mares were in good or very good body condition, weighing 700-900 kg, regularly dewormed, and naturally bred by licensed stallions (mating every 48 hours until the end of heat). In 423 mares ovulation induction was performed by intravenous administration of 1500 IU hCG (Chorulon®-Intervet International B.V, Netherlands). Pregnancy diagnosis was made 12-18 days after the last mating using Honda 1500 device with a 5-7.5 MHz probe. Re-examination was performed 45-60 days after mating. Mares that showed the presence of an embryonic vesicle in the first examination and were found not to be pregnant in the second examination were considered as having embryonic death. The effect of age (3-13 years vs. 14-23 years), previous treatment for endometritis, endometrial cysts, mating during foal heat, and hCG-induced ovulation on occurrence of embryonic death was analyzed. The results were statistically analyzed using the chi-square test using GraphPadPrism version 10 (GraphPad Software, San Diego, CA, USA). The level of significance was set at  $p < 0.05$ .

### Results and discussion

The pregnancy was diagnosed 12-18 days after mating in 3230 out of 4090 mares (78.9%). During a re-examination carried out 45-60 days after mating, 288 mares (8.9%) were found not pregnant (Tab. 1). The embryonic death rate ranged from 8.5% to 9.6% between the years and the differences were not significant ( $p > 0.05$ ) (Fig. 1).

The embryonic death rate was significantly higher in mares aged 14-23 years compared to younger ones (18.3% vs. 7.8%;  $p < 0.05$ ). Mares treated previously for endometritis lost significantly more embryos than healthy mares (16.7% vs. 7.9%;  $p < 0.05$ ). Similarly, the embryonic loss rate was significantly higher in mares with endometrial cysts than mares without endometrial cysts (16.9% vs. 8.5%,  $p < 0.05$ ). Mares mated during foal heat showed significantly higher

Tab. 1. Average pregnancy and embryonic death rates in mares

Pregnancy rate n/n (%)	Embryonic death rate n/n (%)
3230/4090 (78.9%)	288/3230 (8.9%)

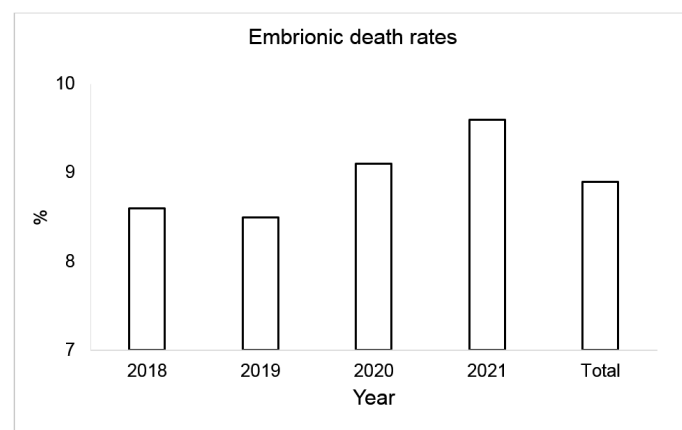


Fig. 1. Embryonic death rates in mares between 2018 and 2021

Tab. 2. The effects of age, history of endometritis, endometrial cysts, mating during foal heat and hCG-induced ovulation on embryonic death rates in mares

Group of mares	Embryonic death rate	
	n/n	%
Aged 3-13 years	223/2874	7.8% <sup>b</sup>
Aged 14-23 years	65/356	18.3% <sup>a</sup>
With history of endometritis	64/383	16.7% <sup>a</sup>
Without history of endometritis	224/2847	7.8% <sup>b</sup>
With endometrial cysts	29/172	17.9% <sup>a</sup>
Without endometrial cysts	259/3058	9.1% <sup>b</sup>
Mating in foal heat	351/1539	22.8% <sup>a</sup>
Mating in later heats	132/1691	7.8% <sup>b</sup>
hCG-induced ovulation	351/1539	5.7% <sup>a</sup>
Spontaneous ovulation	132/1691	9.4% <sup>b</sup>

Explanations: Different superscript letters indicate statistical significance among groups at  $p < 0.05$

embryonic death rate than mares mated in later heats (22.8% vs. 7.8%;  $p < 0.05$ ). Mares with induction of ovulation lost significantly fewer pregnancies between both examinations than mares without induction of ovulation (5.7 vs. 9.4%;  $p < 0.05$ ) (Tab. 2).

In this study, the average pregnancy rate in Polish coldblooded mares of 78.9% fell within the normal range of 75-90% (24). The embryonic death rate between 12-18 and 45-60 days after mating was 8.9%. The reported incidence of embryonic loss prior to day 60 of gestation in different breeds has ranged from 2.6% to 24.0%, with a weighted-mean across studies of 8.6% (28). In thoroughbred mares the embryonic death rate was 7.5% in England (2) and 12% in South Korea (31). The average rate of pregnancy loss in different horse breeds in France was 8.9%, with no significant differences between breeds (7). In another study, the embryonic death rate in mares in France was 9.1% and was lower in coldblooded mares than in warm blooded mares (16).

The results of our study confirmed that the age is an important factor in embryonic loss in mares, as the embryonic death rate was significantly higher in mares aged 14-23 years than in younger mares. The incidence of embryonic loss between days 12 and 40 was between 10% to 15% for young mares, and 20% to 30% for aged mares (30). The embryonic death rate was higher in mares aged 9 to 13 years and was more than twice the rate in younger mares (21). Several other studies indicate significant increases in embryonic loss rates with increased maternal age (5, 22, 31). Older mares had greater risk of embryonic death due to decreased oocyte quality and degenerative changes in endometrium (5, 21).

Clinical and subclinical endometritis are major causes of impaired fertility in mares (17). In our study, mares previously treated for endometritis showed a higher embryonic death rate than mares without treatment for endometritis. Similarly, significantly higher early embryonic loss rate was reported in mares with a history of endometritis compared to mares without history of endometritis (30). Apparently in some cases, the inflammation process may persist after clinical cure of clinical endometritis as subclinical endometritis without clinical signs, causing changes in the uterine environment and leading to embryonic loss. In mares with embryonic death, an increase in the concentration of acute phase proteins (serum-amyloid A and haptoglobin) was found, suggesting subclinical endometritis (14).

An important factor in the occurrence of embryonic loss in mares are non-infectious abnormalities of the endometrium, such as endometrial cysts (25). In the present study, mares with endometrial cysts had a higher embryonic death rate than healthy mares. The adverse effect of endometrial cysts on increased embryonic death rate was described in several reports (1, 7, 20, 26). The cysts might impair intrauterine mi-

gration of the embryo and deprive of adequate nutrient exchange (28). The incidence of endometrial cysts increases with mare age (9, 25).

Mares are often bred during the first postpartum estrus so called foal heat. The effect of being bred in foal heat on embryonic losses is unclear. Several studies indicate increased embryonic loss of mares bred in the foal heat compared with mares bred at later estrous periods (2, 20, 21, 31). In line with these studies, we found a higher embryonic death rate in mares bred during foal heat than at subsequent heats. The relationship was found between the presence of intrauterine fluid at foal heat and a greater risk of embryonic loss (18, 19). However, other studies reported no difference in embryonic loss rates between mares bred during foal heat and those bred on subsequent cycles (11, 30).

Induction of ovulation with hCG is commonly used to facilitate the management of broodmares and to synchronize ovulation more closely with mating of the mare (6). However, the effects of ovulation induction with hCG on embryonic death were inconsistent. Higher pregnancy losses after induction of ovulation with hCG in thoroughbred mares in Chile were reported (32). In contrast, the ovulation induction with hCG or buserelin appeared to be associated with lower early pregnancy loss rate between 15-42 days in thoroughbred mares in England (2). In another study, ovulation induction with hCG did not affect the maintenance of pregnancy in mares (27). Following hCG injection for ovulation induction, an increase in plasma progesterone concentration and embryo size in early pregnant mares were found (13). In our study the embryonic loss rate in mares with hCG-induced ovulation was lower than in mares with spontaneous ovulation. The differences between studies may be related to variations in dosage, route of administration, and follicle size.

In conclusion, the study showed that the embryonic death rate in Polish cold blooded mares was similar to that reported in other countries for different breeds. Age, history of endometritis, endometrial cysts, and mating during foal heat were associated with increased embryonic mortality in mares in North-east Poland. The age of mares and mating in the foal heat exerted the greatest influence on the embryonic death rate. The ovulation induction with hCG had beneficial effect on the embryonic death rate compared to that associated with spontaneous ovulation.

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Corresponding author: Julia Klajna, DVM, M. Oczapowskiego 14, 10-719 Olsztyn, Poland; e-mail: julia.klajna@uwm.edu.pl