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Comparative analysis of various technologies of breedings of horses

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Abstract. The article presents the results of studies to assess the effect of natural mating and artificial insemination with frozen semen on the duration of the service period in mares. The research is aimed at determining the optimal technology to improve the reproductive qualities of horses. The pregnancy rate of 716 breeding mares in 1093 estrus cycles after natural mating (NM) (534 mares, 850 cycles) or artificial insemination with frozen semen (AIfs) (182 mares, 243 cycles) at the foal heat and three subsequent cycles, at the end of the year, per one cycle and the level of pregnancy lost were calculated. The results showed that in the NM program the most part of mares (>80 %) were covered at the first cycle with the lowest level of pregnancy (44.7 %) and the highest level of embryonic losses (12.6 %). In subsequent cycles, the efficiency of NM significantly ($p < 0.001$) increased (up to 80–90 % pregnancy rate at the fourth cycle), and the percentage of embryonic death decreased to 0 %. In the AIfs group of mares, obviously with more careful monitoring of uterus condition after foaling, only 30 % of them were selected for insemination with frozen semen at the foal heat with results similar to the pregnancy level of NM at the first cycle (43–52 %). It was concluded that NM and AIfs at the foal heat in mares are advisable and can provide the normal pregnancy rate if a qualified control of the genital tract condition is ensured after parturition.

1. Introduction

Economics of horse breeding focuses on getting one foal per year from each breeding mare. The period of pregnancy in mares is longer than that in other farm animal species, and it lasts for 11 months. That is, in order to foal annually, the mare should concept within 1 month after parturition. This, in the absence of pathologies, is facilitated by a number of physiological features of the horse: easy passage of the placenta without damage to the endometrium, the rapid process of clearance and involution of the uterus, the rapid onset of postpartum estrus, called "foal heat".

According to statistics, the first estrus in mares occurs on day 7–10 after foaling with a variability of 5–20 days [1–5]. Usually the first estrus is characterized by the normal development of the follicle and ovulation, which occurs in the majority of mares (97 %) before 20 days. 43 % of them ovulate by day 9 and 93 % – by day 15 after foaling [3]. It was also noted that the onset of the first postpartum estrus cycle may be delayed if the mare foaled early in the breeding season [2, 3, 6, 7].

A temporary inflammatory reaction in uterus after parturition and contractile activity of the myometrium due to the sucking of the udder by the foal, contribute to the rapid release of the uterus from debris and contaminants (lochia) and to its involution. In the absence of complications of pregnancy and parturition, these processes generally finish within a week. However, a histological



analysis of the endometrium shows that the uterus in mares that have successfully foaled return to normal condition, on average, by the 15th day after parturition [8–10]. Therefore, the mating of mares with ovulation before the 10th day is not recommended [4]. A complete contraction of the uterus to the normal (unpregnant) size is occurred, approximately, on the 21st day after foaling with fluctuations from day 15 to day 25 [5].

The results of numerous studies indicate that conception rate of mares at foal heat is 10–20 % lower than in any subsequent estrus cycle [3, 7, 11, 12]. No relationship was found between size of the uterus at the time of the first ovulation and the level of mare's pregnancy rate. However, a direct relationship has been established between the presence of fluid in the uterus and decrease of that conception rate of mares in postpartum heat, since the fluid can have a spermicidal effect and at the same time can be a suitable medium for the growth of bacteria [4]. McKinnon et al. noted a significant difference in pregnancy rate during foal heat between mares with the fluid in the uterus (33 %) and without it (84 %) [5].

Also, mares fertilized at the first heat after parturition have an increased level of early embryonic death [13], compared with this in subsequent cycles. In particular, mares with an ovulation occurred before the 10th day may have not time for recovery after foaling by the time of embryo entrance to the uterus (6.5 days after ovulation), as a result of which the embryo can die under adverse conditions. Dystocia and complications after foaling make an additional contribution to this problem. There have been special methods to delay the first ovulation in mares by progestin treatment or to reduce the luteal phase of the second cycle by injection of prostaglandin F2a in order to give the mare additional time to restore the uterus after parturition [5, 14].

The cause of early embryonic death may be the poorly clearance of the uterus, or bacterium may be additionally introduced during obstetric care, postpartum manipulations, swab procedure or mating / insemination [7, 9, 15]. Therefore, some authors recommend prophylactic flashing of the uterus with antibiotic solution in all mares after parturition [4]. In addition, the presence of fluid in the uterus (inflammatory sign) is associated with the production of prostaglandin F2 α , which can cause regression of the corpus luteum during a diestrus period [16]. The methods of the uterus cleaning (washing with antibiotics) after mating at foal heat were developed, which increased the pregnancy rate [17].

Due to the improvement of maintenance, veterinary services, usage of artificial insemination and, especially, introducing of the ultrasound diagnostics to the horse breeding practice, the pregnancy rate of mares mating at postpartum heat has become much higher [1]. In several studies, the authors did not find a decrease in reproduction level (conception rate in the first cycle, per season and per year, percentages of embryonic losses and a live foal birth) when covering mares at foal heat, compared to subsequent cycles, including when the placenta was retained and it was manually separated [18, 19]. So, when determining the efficiency of a mating at the first postpartum and subsequent cycles T.L. Blanchard et al. received comparable data (72 and 75 %, respectively). However, applying the method of logistic regression to take into account various factors influencing this indicator (year, stallion quality in a mating, mare's age, mating method, postpartum treatment of mares which had fluid in the uterus), the authors received other figures: 66 % in the natural coverage of mares and 83 % after artificial insemination [6]. This is the evidence of the benefit of more careful selection and more advanced methods of working with mares during postpartum heat.

Most specialists recognize the fact of lower mating rates and an increase in embryonic losses in aged mares (over 15–17 years old) [20, 2]. Anomalies in the structure of the oviducts epithelium, which do not ensure the normal development of the embryo in the early stages of cleavage, as well as the lower quality of oocytes in old mares, are considered to be the reasons for the decrease in fertility [21–23]. It is also noted that the clearance process of the genital tract after parturition in old multiparous mares can be delayed due to deep location of the uterus and slowed outflow of fluid from it [2, 7].

The problem of mares' conception rate directly depends on the type of semen used. At the foal heat, the usage of fresh sperm is recommended. It is allowed to inseminate the mares with chilled

semen but in this case fertility is reduced. J. Pycok reported about 60 % pregnancy rate (per 1 cycle) in mares with natural mating at postpartum heat and 73 % during subsequent cycles. When mares were inseminated with chilled semen, the results were 50 and 65 %, respectively [in 24].

In the case of cryopreserved sperm, its fertilizing ability after thawing is significantly reduced, mainly due to damage and degradation of acrosome, which leads to their enzyme deficiency [25]. Therefore, specialists advise not to work with expensive frozen sperm at foal heat and to inseminate mares not earlier the 30th day after parturition, that is, at the second cycle [2, 20, 26]. The same strategy is considered more preferable in embryo transplantation [1].

In general, the factors that determine the success in mating/insemination of mares at foal heat are following: normal parturition without dystocia, injuries, complications and placental retention, quick cleaning and restoration of the endometrium and reduction of uterus size, normal onset of foal heat with ovulation occurred not earlier the 10th day after parturition. The general opinion of specialists is that each mare should be carefully examined for deviations and delay in the process of postpartum repair of the uterus (especially regarding the fluid accumulation), taking into account the mare age and method of her mating / insemination. Only when all factors are taken into account, mating at the postpartum heat will be effective and economically justified.

In recent years, in Russian stud farms the skill level of specialists in horse reproductions has increased, the diagnostic base has improved; ultrasound scanner has been put into practice, which is reflected in growing reproduction rates in domestic horse breeding. The expensive frozen semen of valuable stallions of domestic and foreign breeding has become more widely used. In this regard, the responsibility for the result of insemination, partly at foal heat, is increased. The selection of mares for mating on farms becomes more thorough, and the effectiveness of artificial insemination with frozen sperm reaches 65-78 % in one cycle [27]. The purpose of this research was to analyze the current fertility level of mares in Russian stud farms at the foal heat and three subsequent cycles as a result of natural mating (NM) and artificial insemination with frozen semen (AIfs).

2. Methods and materials

The data of mating and foaling results in mares of trotter (Orlov Trotter, Russian Trotter, Standardbred), Arabic and Trakehner horse breeds in 6 Russian stud farms (the Moscow stud (Moscow region)), the Tersky stud (Stavropol region), Ryazan stud (Ryazan region), Lokotskoy stud (Bryansk region), Vorontsov stud (Yaroslavl region) and A. A. Kazakov' stud farm (Ryazan region) during 2000-2016 years were calculated. In general the information about fertility of 716 breeding mares in 1093 estrus cycles after NM (534 mares, 850 cycles) or AIfs (182 mares, 243 cycles) was analyzed. The conception rate at the foal heat and during each of three subsequent cycles, as well as the pregnancy rate at the end of the season and per 1 cycle were counted. The calculation of the embryonic lost level in each cycle was carried out for 478 mares in 742 estrus cycles, for which there was relevant information.

The stallions of proven fertility with tested sperm quality (volume, concentration, activity, longevity) were used in natural mating. Frozen stallion' semen from private horse owners and from the bioresource collection of Cryobank of Genetic Resources of the All-Russian Research Institute for Horse Breeding was used for artificial insemination. The quality of frozen sperm was checked after thawing. The degree of follicle maturity was determined by ultrasound control. Mares were covered when a mature follicle was present in the ovary with an interval of 24–48 hours until ovulation was established. Insemination of mares with frozen semen was carried out in the range from (–12) to (+6) hours relative to ovulation.

The normal foal heat was considered the estrus, manifested within 25 days after parturition. The number of mating cycles, the number of intentionally missed cycles (without mating), and also the cycles with delayed estrus (>25 days) and lactational anestrus (more than 1–3 months) were counted. According to the results of mating/insemination, the percentage of pregnancies and embryonic loses from the number of fertilized mares were determined. The mares, which not became pregnant, were

covered/inseminated repeatedly, so they were taken into account in the next cycle. The number of early embryonic losses in each cycle and at the end of the year was calculated.

The data obtained were statistically analyzed by Student-Fischer t-test.

3. Results and discussion

The results of NM and AIfs of mares is presented in Table 1. A significantly ($p < 0.001$) lower percentage of pregnant mares was revealed at the foal heat (42.95 %) compared with the second (57.85 %), the third (67.96 %) and the fourth (81.48 %) cycles. The same trend was noticed from the first to the third cycle among mares inseminated with frozen sperm (51.79 % – 55.56 %, – 63.26 %, respectively), but the difference was not significant. At the fourth cycle, the mare of this group has a sharp decline in the pregnancy rate (from 63.26 to 25.0 %). This may be explained by the fact that the mares not became pregnant in the previous cycles ($n=12$) most likely were problematic and subfertile, but valuable for breeding programs, so specialists tried to inseminate them with frozen sperm till the end of breeding season.

The total pregnancy rate per 1 cycle for artificial inseminated mares was 54.71 %, which is 2.6 % higher than the result of natural mating (52.12 %). However, at the end of the year, the significantly increased ($p < 0.001$) pregnancy level was founded in naturally covered mares compared with those inseminated by frozen semen (78.65 % versus 66.5 %, respectively).

Table 1. Pregnancy rate of foaling mares per cycles during breeding season

The kind of mating	Mares		Estrus cycles after foaling				Per 1 cycle	At the end of the breeding season
			1	2	3	4		
Natural mating	covered	n	440	280	103	27	850	534
		n	189	162	70	22	443	420
	pregnant		42.95	57.85	67.96	81.48	52.12	78.65±1.78 ^a
		%	±2.36 ^e	±2.95 ^g	±4.60 ^f	±7.48 ^c	±1.71	
Artificial insemination with frozen semen	inseminated	n	56	126	49	12	243	182
		n	29	70	31	3	133	121
	pregnant		51.79	55.56	63.26	25.0	54.73	66.48±3.50 ^b
		%	±6.68	±4.43	±6.87 ^h	±12.5 ^d	±3.19	

^{a,b} $p < 0.001$, ^{c,d} $p < 0.001$, ^{e,g} $p < 0.001$, ^f $p < 0.001$, ^h $p < 0.01$

Table 2. The rates of pregnancy and pregnancy lost in foaling mares during breeding season

The kind of mating	Mares		Estrus cycles after foaling				Per 1 cycle	At the end of the breeding season
			1	2	3	4		
NM*	covered	n	302	180	73	15	507	348
		n	135	109	57	14	315	304
	pregnant	%	44.7±2.86	60.56±3.64	78.08±4.84	93.33±6.46 ^g	55.26±2.08	87.36±1.78 ^a
		n	17	7	1	0	25	25
	pregnancy lost	%	12.60±2.86	6.42±2.35	1.75±1.74	0	7.94±1.52	8.22±1.57
		n	29	97	38	8	172	130
AIfs**	inseminated	n	29	97	38	8	172	130
		n	13	53	24	2	92	80
	pregnant	%	44.83±9.24	54.64±5.05	63.16±7.82	25.0±15.31 ^h	53.49±3.80	61.54±3.51 ^b
		n	0	5	0	0	5	5
	pregnancy lost	%	0	9.43±4.01	0	0	5.43±1.27	6.25±2.71
		%	0	9.43±4.01	0	0	5.43±1.27	6.25±2.71

^{a,b} $p < 0.001$, ^{g,h} $p < 0.001$

*NM – natural mating,

**AIfs – artificial insemination with frozen semen

The level of embryonic losses in successive cycles (Table 2) was counted for mares with relevant information ($n=478$). However, a difference was found in the dynamics of the pregnancy lost percentage from the first to the fourth cycle. Thus, in the group of natural mating, the highest level of embryonic losses was noted in the first cycle (foal heat) – 12.6 %, and in the artificial insemination

group – in the second cycle (9.43 %), when the number of inseminated mares were increased (from 29 to 97 mares).

No significant differences were found per cycle between NM and AIfs in pregnancy rate (55.26 % and 53.49 %) and embryonic losses (7.94 % and 5.43 %), respectively. At the same time, a highly significant difference ($p < 0.001$) in the pregnancy rate remained at the end of the year (87.36 % and 61.54 %, respectively) in favor of the group of NM. The final level of pregnancy lost in both groups was close (8.22 % and 6.25 %).

The results obtained indicate a more thorough selection of mares after foaling for AIfs. We analyzed the number of mares actually covered or inseminated at foal heat in the programs of NM and AIfs (Table 3).

Table 3. The strategy of mare selection for mating at the foal heat in different breeding programs

The kind of mating	Mares, n	Mating mares		Not mating mares (foal heat consciously missed)		Mares with prolonged period to the 1 st heat	
		n	%	n	%	n	%
NM*	534	425	82.3 % $\pm$ 1.65 ^a	87	16.3 % $\pm$ 1.60 ^c	22	4.1 % $\pm$ 0.86
AIfs**	179	54	30.2 % $\pm$ 3.43 ^b	99	55.3 % $\pm$ 3.72 ^d	26	14.5 % $\pm$ 2.63

^{a,b} $p < 0.001$, ^{c,d} $p < 0.001$

NM – natural mating

AIfs – artificial insemination with frozen semen

In NM program 82.3 % mares (425/534) were covered in the first cycle (foal heat). At the same time, only 54 from 179 mares (30.2 %) of AIfs group were selected for insemination at foal heat, and in 55.3 % of these mares the first cycle was consciously missed, probably because of the conclusion that their uterus were not ready to accept new pregnancy. The number of mares with a prolonged (more than 30 days) period from the parturition to the first estrus was 10.4 % more in the group of AIfs (14.5 %) than in NM (4.1 %). These data are comparable with T.L. Blanchard et al. (2004) on the incidence of postpartum anestrus in mares in Southeast Texas [6].

4. Conclusion

Thus, it can be concluded that nowadays in Russian stud farms, where qualified specialists work and artificial insemination are used, the differentiated approach to selection of mares for natural mating and insemination with frozen semen is practiced. Apparently this situation is the result of more widely usage of expensive cryopreserved semen in Russia and the accumulated negative experience in working with mares at foal heat.

Stallions covered the most part of mares (over 80 %) in the natural mating program in the first cycle with the lowest level of pregnancy (44.7 %) and the highest level of embryonic loss (12.6 %). In subsequent cycles, the success of mating significantly ($p < 0.001$) increased (up to 80–90 % in the fourth cycle), and the percentage of embryonic death decreased to 0 %.

More thorough monitoring of the uterus after parturition in the group of mares selected for insemination with frozen sperm led to the preferential missing of postpartum heat in approximately 70 % of mares. Obviously, only gynecologically normal mares were fertilized with frozen semen, therefore, the efficiency of insemination in the first cycle increased almost to the level of natural mating (43–52 %). In this regard, our observations coincide with the opinion of colleagues [13, 22, 26] that the efficiency of mating and artificial insemination with frozen sperm at foaling heat is acceptable and can provide normal pregnancy rate, when a qualified approach is used to evaluate the functional state of the genital tract of mares after parturition.

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