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THE EFFECT OF HORMONAL TREATMENTS ON THE PREGNANCY RATES OF MARES

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To determine the effect of hormonal drugs on the pregnancy rates of mares, authors analyzed this indicator in natural (n=303) and stimulated cycles (n=170). They used cloprostenol preparations to shorten the diestral period, and human chorionic gonadotropin [hCG] to induce ovulation. Natural cycles are divided into groups, depending on the duration – normal and short (19–24 and 12–18 days, respectively). Stimulated cycles for the following groups: (1) short (8–18 days); (2) normal (19–24 days) with the use of cloprostenol and hCG; (3) normal – with the use of hCG only. Short, both natural and stimulated cycles, are divided into groups – with normal (4–6 days) and short estrus (2–3 days). The analysis of natural and stimulated cycles showed that the level of pregnancy rates in stimulated cycles (73.5%–80.8%) corresponds to this indicator in normal natural cycles (79.6%). In natural short cycles, the pregnancy rate significantly decreases (26.3% ± 7.1%). When analyzing short cycles, it turned out that, both in natural and stimulated cycles, the pregnancy rate depends on the length of the estrus and decreases in cycles with a short estrus. In general, the difference in pregnancy rates was 41.0% ($p \leq 0.001$). In short stimulated cycles, with an estrus length of 4–6 days, the pregnancy rate is higher (91.5% ± 4.06%) than in cycles with an estrus of 2–3 days (51.7% ± 9.3%). Thus, the shortening of the estrous period in stimulated cycles has a significant effect on the pregnancy rate.

Keywords: *cloprostenol; human chorionic gonadotropin; sexual cycle; estrus; diestrus; ovulation; pregnancy rates*

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ВЛИЯНИЕ ГОРМОНАЛЬНЫХ ОБРАБОТОК НА ЗАЖЕРЕБЛЯЕМОСТЬ КОБЫЛ

Е.В. Солодова, А.В. Калинова

С целью определения влияния гормональных препаратов на зажеребляемость кобыл проведён анализ этого показателя в естественных (n=303) и

стимулированных циклах (n=170). Для сокращения диэстрального периода применялись препараты клопростенола, для индукции овуляции – хорионического гонадотропина человека (ХГЧ). Естественные циклы разделены на группы в зависимости от продолжительности: нормальные и короткие (19-24 и 12-18 дней соответственно). Стимулированные циклы на следующие группы: 1) короткие (8-18 дней) и 2) нормальные (19-24 дня) с применением клопростенола и ХГЧ; 3) нормальные – с применением только ХГЧ. Короткие, как естественные, так и стимулированные циклы разделены на группы: с нормальным (4-6 дней) и коротким эструсом (2-3 дня). Анализ естественных и стимулированных циклов в целом показал, что уровень зажеребляемости в стимулированных циклах (73,5-80,8%) соответствует этому показателю в нормальных по длине естественных циклах (79,6%). Установлено, что в естественных коротких циклах зажеребляемость значительно снижается (26,3±7,1%). При анализе коротких циклов выяснилось, что, как в естественных, так и в стимулированных циклах, зажеребляемость зависит от длины эструса и снижается в циклах с коротким эструсом. В целом разница показателей зажеребляемости составила 41,0 % (p ≤0,001). В коротких стимулированных циклах, с длиной эструса 4-6 дней, зажеребляемость выше (91,5±4,06%), чем в циклах с эструсом 2-3 дня (51,7±9,3%). Таким образом, сокращение эстрального периода в стимулированных циклах оказывает значительное влияние на зажеребляемость.

Ключевые слова: *клопростенол; хорионический гонадотропин человеческий; половой цикл; эструс; диэструс; овуляция; зажеребляемость*

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Introduction

The application of hormonal drugs has become widely used in the practice of horses reproduction. Currently, the most used drugs are prostaglandin PGF2a analogs - dinoprost and cloprostenol [4; 8; 21]. The most used ovulation-inducing drugs are human chorionic gonadotropin [hCG] and gonadotropin releasing hormone [GnRH] agonists – deslorelin, buserelin, and others.

A distinctive feature of the sexual cycle of mares from the cycle of other types of farm animals is a long hunt (estrus or follicular phase). Its length averages 7.3 days, with fluctuations from 2 to 13 days [16]. The use of hCG and GnRH agonists makes it possible to predict ovulation time within 36–48 hours after injection [1; 3; 17]. The hCG and GnRH preparations are often used (1)

when inseminating mares with frozen seed to increase economic efficiency (to save sperm and labor costs of the inseminator technique); (2) if single insemination with fresh semen is necessary in a mare, prone to post-breeding endometritis; (3) rational use and reduction of the load on the stallion of the producer [2; 14] Cloprostenol and dinoprost are used to return mares to estrus before the 15th–16th day of the cycle and interrupt prolonged diestrus. Both groups of drugs help in synchronizing donor and recipient mares when using the fresh and chilled embryo transplant method [5; 18; 21].

The interval from luteolysis induced by PGF2a analogs to ovulation is very variable and occurs with a range of 2 to 12 days [20; 21]. The large difference in the length of the interval is mainly due to the presence of follicles of different sizes in the ovaries of mares during the diestrus period [13]. This interval has a negative correlation with the diameter of the largest follicle, present in the ovary at the time of injection. If a relatively large follicle (≥35 mm) is present in the ovary, the onset of estrus and ovulation will depend on the follicular status (growth phase or follicle atresia). Accordingly, mares with follicles approaching the diameter of the preovulatory ones can quickly come into estrus and ovulate 2 to 5 days after injection. If the follicle is atretic, it will continue to regress slowly. When ovulating within 48 hours after injection, mares often do not show any behavioral signs of estrus. The reaction of the corpus luteum to PGF2a also depends on its degree of maturity [6; 7; 19]. Thus, up to 5 days after ovulation, one injection is not enough for complete luteolysis. However, when using PGF2a in the second half of the diestrus or in the case of a prolonged diestral phase, regression of the corpus luteum occurs faster. An inversely proportional correlation is observed between the dose of the drug and the interval before ovulation [10; 15].

However, when using hormonal drugs, there are side effects that negatively affect pregnancy rate: (1) rapid onset of ovulation as a result of accelerated follicle development; (2) an increase in the level of polyovulation; (3) the formation of hemorrhagically anovulatory follicles [9; 11]. There is a decrease in pregnancy rates with a reduction in the time interval from the injection of cloprostenol to ovulation [22].

From the above, it follows that drugs analogs of PGF2a and hCG drugs significantly reduce both the diestrus and estrous periods. Authors assume that the reduction of these periods may have a negative impact on the pregnancy rate of mares.

Scientific novelty

The pregnancy rate analysis in hormone-stimulated cycles depending on the duration of the estrous period of the cycle of mares was conducted for the first time.

Materials and methods

This research aims to evaluate the effect of hormonal treatments on the level of pregnancy rate and its dependence on the length of the cycle and duration of estrus.

The authors conducted a retrospective analysis of data for several breeding seasons (2014–2021) on the cycles of mares, private horse owners (Stud Farm "Lokotskoy" CJSC; "Lag-Service Agro" LLC), and mares that were inseminated on an experimental farm of the Research Institute of Horse Breeding. The conditions of keeping and feeding mares in all farms corresponded to zootechnical standards.

The authors divided all cycles into natural (n=303) and stimulated (n=170) – without and with the use of hormonal drugs, respectively. Natural cycles had two groups:

- Normal (19–24 days);
- Short (12–18 days).

Cycles, with the use of hormonal drugs, belonged to one of the following groups:

- Short (8–18 days, estrus 2–6 days);
- Normal (19–24 days, estrus 4–6 days) (in groups 1 and 2, cloprostenol or cloprostenol and hCG were used);
- Normal (19–24 days, estrus 4–6 days, with the use of hCG only).

The second group included cycles in which cloprostenol not only shortened the diestrus phase, but also interrupted prolonged diestrus. Short natural and stimulated cycles are divided into groups with normal (4–6 days) and short estrus (2–3 days).

The set of ultrasound characteristics, characterizing the uterus and ovaries in a given period, determined the beginning and end of the estrous period of the cycle: (1) the beginning – by the appearance of endometrial edema, in the presence of one or two follicles at least 30–32 mm in diameter and the simultaneous absence of a pronounced corpus luteum (more than 1.5 cm in diameter); (2) the end - by ovulation. Additional signs of the beginning of the estrous phase, revealed by external and rectal examination, were moistening of the vaginal mucosa and the appearance of characteristics inherent in the cervix and horns of the uterus in estrus. During the estrous period, ultrasound and rectal examination were performed every 12–24 hours.

Cloprostenol preparations were used in the work - magestrophan ("Mosagrogen" CJSC, Russia) or estrophantine ("Ascont+" LLC, Russia). When processing the results, cycles were taken into account, in which 150–200 mcg

(0.6–0.8 ml) of the drug was used intramuscularly, both for interrupting diestrus (from day 5 to day 14 of the cycle) and interrupting prolonged diestrus (after day 17 of the cycle). Preparations of chorionic gonadotropin - hCG ("Moscow Endocrine Plant," Russia) or Chorulon ("Intervet International B.V.," the Netherlands) were used in the presence of an estrous follicle ≥ 35 mm in diameter and maximum endometrial edema at a dose of 1500–2500 IU. Ultrasound examinations of the reproductive organs of mares were carried out on Mindrey DP-50 and Exago devices.

Since the performance of insemination of mares affected by many factors, treatments were excluded: (1) cycles with signs of endometritis detected on ultrasound; (2) cycles, usually, of old mares with reduced fertility; (3) cycles that used fresh or frozen-melted seed of poor quality (less than 500 x 10^6 of motile sperm dose). The authors of this research carried out work on rectal and ultrasound examination of mares, their insemination or mating.

The authors processed the data according to the generally accepted method for calculating statistical characteristics, assessing the reliability of differences between groups and using the Student-Fisher t-test.

Results

Comparison of pregnancy rate in natural and stimulated cycles of different lengths revealed reduced pregnancy rate in natural short cycles (Table 1).

Table 1.

Pregnancy rate in natural and stimulated cycles of different duration

Cycle characteristics		Number of cycles,		Pregnant,	
		n	heads	M±m, %	
Normal, 19–24 days	Natural				
		265	211	79.6±2.5[a]	
Short, 12–18 days		38	10	26.3±7.1[d]	
Short cycles with cloprostenol and hCG* 8–18 days, estrus 2–6 days	Stimulated				
		76	58	76.3±4.8	
Normal cycles with cloprostenol and hCG* 19–24 days, estrus 4–6 days		26	21	80.8±7.7	
Normal cycles with hCG		68	50	73.5±5.3	

*Note**: – hCG injections in 5 cycles in each group, $p^{ad} \leq 0{,}001$.

The level of pregnancy rate in stimulated cycles, both with normal duration and in shortened ones, is quite high (73.5%–80.8%). It has no significant difference with the pregnancy rate index of mares in normal natural cycles (79.6 ± 2.5).

Analysis of the pregnancy rate of mares, depending on the length of the estrus, showed that it is lower in cycles with a short estrus (2–3 days), both in stimulated and natural cycles (by 39.8% and 33.0%, respectively) (Table 2).

Table 2.

Fertility in short natural and stimulated cycles in groups with short and normal estrus

Estrus length 2–3 days	Inseminated, n	Cycle characteristics		
		Stimulated	Natural	Total
		29	29	58
	Pregnant, heads (M±m, %)	15 (51.7±9.3)	13 (44.8±9.2)	28 (48.3±6.5)[c]
4–6 days	Inseminated, n	47	9	56
	Pregnant, heads (M±m, %)	43 (91.5±4.06)	7 (77.8±13.8)	50 (89.3±4.1)[b]

*Note**: $p^{c\,b} \leq 0{,}001$.

Discussion

This research showed that the use of hormonal drugs in general (both hCG and cloprostenol) does not reduce the level of pregnancy rate. However, the analysis of pregnancy rate in cycles with short and normal estrus duration explains the statement that mares with a short interval from injection of cloprostenol to ovulation (4–7 days) have a reduced level of pregnancy rate, compared to mares who ovulated 8–10 and more than 11 days after injection [9]. Evidently, the short interval from injection to ovulation includes a short estrous period. The results of the researches are consistent with the positive correlation found between the number of estrus days, during which the uterus of mares has a pronounced estrus-like echo texture, and pregnancy rate, both in natural and stimulated cycles [16].

It is logical to assume that in hormone-stimulated cycles with short estrus, reduced pregnancy rate is associated with accelerated development and ovulation of follicles. In this case, the necessary changes characteristic of normal hunting do not have time to occur in the uterus. Inappropriate physico-chemical and organoleptic properties of mucus prevent the advancement of sperm. Insufficient cervical relaxation leads to post-breeding endometritis.

A decrease in the survival rate of transplanted embryos in recipient mares, with a previous short hunt (2–3 days) [12], also speaks about the inconsistency of the conditions of the uterine environment for the successful development of the embryo.

Reduced pregnancy rate (26.3% ± 7.1%, $p \leq 0.001$) in natural short cycles may also be associated with the presence of latent endometritis in such mares. The release of uterine prostaglandin in it, which causes lysis of the corpus luteum, occurs earlier than 14 days [4].

The research results allow recommending, to achieve a higher pregnancy rate, to administer PGF2a preparations to mares only in the absence of large follicles in the ovaries (≥ 35 mm) at the time of injection. If it is necessary to interrupt diestrus in the presence of a large follicle, do not use hCG or GnRH analogues to induce ovulation.

Conclusion. In hormone-stimulated cycles, the pregnancy rate significantly depends on the length of the estrus. Thus, with an estrus length of 4–6 days, it is high, and in stimulated cycles with a shortened estrus of up to 2–3 days, it decreases. This conclusion can help practitioners in making decisions about the appropriateness of using hormonal drugs in each specific case, contributing to improving the efficiency of working with mares.

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