The use of D- and L,D- cloprostenol to reduce diastral period of estrus cycle in mares

E. Solodova*, and L. Lebedeva
The All-Russian Research Institute for Horse Breeding, Riazan region, Russia

Abstract. In order to determine the minimum effective drug doses, the levels of decrease in progesterone concentration followed the injections of D and L, D - cloprostenol (the dosage 25 - 250 μg) were compared. The effect of treatment on the reduction of estrus duration and the time before ovulation were studied. It has been shown that the level of progesterone 24 hours after the first injection (at any dose except 50 μg of L, D-cloprostenol) decreased by 76-81%. Complete luteolysis was achieved (0.24-0.45 ng/ml) at any kind of treatment in 72 hours after injection. A significant difference in the average duration of estrus (when using large doses) and the period from injection to ovulation (when using small and large doses) was found between groups of mares that were injected with a follicle diameter of 22-28 or 29-37 mm in both the first and in the second half of diestrus. The study showed that the use of D-cloprostenol once in reduced doses (25 - 50 μg) or D, L-cloprostenol twice 50 μl in the presence of follicles more than 29 mm in diameter will decrease the risk of reducing the duration of estrus.

1 Introduction

In modern equine reproduction practice, cloprostenol (a synthetic analogue of prostaglandin F2α) are the most used hormonal agents to regulate the reproductive cycle of mares. In the mare's body, natural PGF2α, synthesized in the endometrium, travels through the systemic circulation to the corpus luteum and causes luteolysis, which results in the finish of the diestral phase of the estrus cycle. Cloprostenol is used for artificially interruption of this phase [1]. The PGF2α drugs may include two isomers: D-cloprostenol (has luteolytic activity) and L-cloprostenol (does not have luteolytic activity, but interacts with corpus luteum receptors, reducing the likelihood of D-cloprostenol molecules interacting with them). The content of L-cloprostenol can reach 40-60%, which significantly reduces luteolytic activity. Cloprostenol exhibits luteolytic and uterotonic properties 50-100 times more actively compared to natural PGF2α [2]. Therefore, the use of cloprostenol drugs can cause both systemic side effects and reproductive function effects. Systemic side effects are associated with its influence on the central nervous system (impaired coordination, stupor, and ataxia) and the vascular system (contraction of smooth muscle in organs such as the stomach, intestines, and bladder). Mares may manifest increased sweating, tachycardia,

* Corresponding author: l.solodowa2012@yandex.ru
diarrhea, and symptoms of colic. The strength and duration of these side effects may vary and depend on the dose of drug [3]. Typically, they the situation returns to normal within one to five hours after administration of PGF2a analogues. Since natural PGF2a is a powerful chemical, in order to prevent its negative effects on the body, protective mechanisms are provided: PGF2a synthesized in the endometrium has impact only on the corpus luteum, and its secretion occurs in the form of short impulses. The amplitude of the impulses increases as luteolysis progresses [3-5].

Side effects of the influence of PGF2a analogues on the reproductive sphere are due to both its indirect effect on the hypothalamic-pituitary axis and its direct effect on the follicle wall [3,6-8]. In this regard, it was noticed a reduction in the duration of estrus (as a result of accelerated development of the follicle and premature ovulation), an increase in polyovulations level and a decrease in pregnancy rates [9]. The central role of prostaglandins in the ovulation process explains the reason for the ovulation of large diestrual follicles (>36mm) within 48 hours after administration of a large dose (250-625 μg) of the cloprostenol analogue PGF2a. In this case, ovulation occurs before the progesterone concentration will reach the basal level and the mare will manifest the heat [10].

It has been established that in hormonally stimulated cycles with shortened estrus (2-3 days), the pregnancy rate is reduced to 60.9% compared to natural and stimulated cycles with estrus length of 4-6 days (86.2%) [11]. A decrease in the duration of estrous uterine edema (less than 3 days) as a result of an increase in estrogen levels, negatively affects both the pregnancy rate of embryo donor mares and the survival rate of transferred embryos [12,13]. This is associated with insufficient level of estrogens during estrus of such mares, that causes an inadequacy of the uterine environment for sperm activity during insemination [11] and with a decrease in the number of stromal estrogen and progesterone receptors in the endometrium, necessary for the maintaining of normal pregnancy [12, 14]. In addition, numerous studies in various animal species have shown that steroid hormones are necessary for the maturation and fertilization of eggs, proliferation and differentiation of granulosa cells of the follicle, subsequent ovulation and corpus luteum formation [15,16]. The intrafollicular environment saturated with estrogen has an antiatretic effect and is associated with good follicular growth. Estrogens enhance the cytoplasmic maturation of oocytes through a direct non-genomic effect at the plasma membrane level, inducing calcium influx into the cell and a specific pattern of Ca2+ oscillations [16]. The use of preovulatory follicular fluid as a medium for the maturation of equine oocytes in IVF and ICSI cycles increases the rate of fertilization and cleavage [17,18].

The interval from PGF2a-induced luteolysis to ovulation is highly variable, occurring within a range of 2 to 15 days. The large difference in interval length mainly depends on the diameter of the largest follicle present in the ovary at the time of PGF2a application. The interval decreases with increasing of follicle size [19].

So, it is very important to predict the time of onset of the estrous phase of the cycle, to decrease the likelihood of shortening its duration and to determine the minimum effective doses of drugs that cause luteolysis, both when applying the embryo transfer method, planning the aspiration of follicular fluid and oocytes from preovulatory follicles, and in routine work on reproduction.

Currently, the Russian pharmaceutical industry produces drugs D-cloprostenol "Estrophantin" (LLC "NPK "Askont+", Serpukhov) and L, D-cloprostenol "Magestrophan" (CJSC "Mosagrogen", Moscow). The manufacturer's recommendations about doses of both drugs (250 μg, 1 ml) do not reflect the difference of its potency. The difference in the effectiveness of the drugs is confirmed by studies conducted on farms in the Moscow and Nizhny Novgorod regions on black-and-white and Holstein dairy cows. The results showed that the percentage of estrus synchronization after the use of estrophanth in was 17.14% higher than after the use of estrophan (L, D-cloprostenol). On average, cows treated with
estrophanthin had lower progesterone levels in 24 hours than cows treated with estrophan [2]. In order to determine the minimum effective doses of D and L, D - cloprostenol, the aim of the work was to compare the drop in progesterone concentration when using domestic drugs Magestrophan (L, D - cloprostenol) and Estrofantin (D-cloprostenol). The reduction of the estrous phase, as a criterion for the advisability of using drugs, depending on the diameter of the follicle and the time of injection (the first or second half of diestrus) was monitored.

2 Materials and methods

The studies were carried out on mares of horse breeding farm Lag-Service Agro LLC (Ryazan region, Russia) and mares of the experimental stable of The All-Russian Research Institute for Horse Breeding. The weight of mares was 400-600 kg. The tested doses of estrophanthin and magestrofan were administered intramuscularly: 1 ml (250 µg) once, 0.6 ml (150 µg), 0.2 ml (50 µg) and 0.2 ml (100 µg) twice. At a dose of 0.1 ml (25 µg), only estrophantine was tested once. Blood was taken on days 5-7 of the estrus cycle before injection of the drug, 24 hours and 72 hours after injection, and with a double injection: before each injection and in 48 hours after the second injection (that is: in 96 hours after the first injection). In the group of mares that received a single dose of magestrophan at a dose of 0.2 ml (to monitor the restorative ability of the corpus luteum function), blood was taken additionally in 120 hours after the injection. Blood was collected from the mares from the jugular vein in the morning, before feeding, left for 30 minutes and centrifuged at 3000 rpm for 15 minutes, and then the serum was frozen. The concentration of progesterone was determined by chemiluminescence immunoassay in the laboratory of Health Center LLC, Ryazan.

The duration of the period from injection to ovulation and the length of estrus when using small doses (0.1-0.4 ml) and usually practiced doses of 0.6-1 ml were analyzed for both drugs in the first half (from 5 to 9 day) and in the second half of diestrus (from the 10th day of the cycle) with small, medium and large follicles with diameter of ≤21 mm, 22-28 mm, 29-37 mm (I, II, III groups of mares, respectively). The analysis of low doses of drugs included: single injections of 0.1 ml (in mares weighing 350 - 400 kg) and 0.2-0.3 ml of estrophantine (400 - 600 kg); double injections of 0.2 ml of both Estrophanthin and Magestrophan (350-600 kg). In the analysis for high doses (0.6-1.0 ml) both drugs were taken into account.

Ultrasound examinations of mares were carried out using Mindrey DP50 and Exago ultrasound scanners. Mares were examined daily during estrus preceding the shortened estrus cycle, on the day of injection (5–7 days after ovulation) and then before each blood draw, noting the presence and size of follicles in the ovaries. The average diameter of follicle was calculated as half sum of the maximum and minimum distance in horizontally and vertically projections. In diestrus, mares were checked depending on the size of the leading follicle and the degree of uterine edema. During the estrous phase, monitoring was carried out daily until ovulation was confirmed.

The data were analyzed by Microsoft Excel 2010 and Statistica 12 program.

3 Results and discussion

Analysis of blood serum progesterone levels in mares (Table 1) showed that the initial level of progesterone (5.79-9.54 ng/ml) was reduced by 76-81% in 24 hours after the first injection when using both drugs in any doses (except for Magestrophan in a dose 50 µg, with a single dose) and reached a level of ≤2 ng/ml (1.25-1.95 ng/ml).
Table 1. The average concentration of progesterone in the blood serum of mares before and after of Magestrophan (M) or Estrophanthin (E) injection.

<table>
<thead>
<tr>
<th>Groups of mares magestroph an/estrophanine (dose)</th>
<th>n</th>
<th>Progesterone injection, (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>before injection</td>
</tr>
<tr>
<td>M E</td>
<td></td>
<td>M E M E M E M E M</td>
</tr>
<tr>
<td>I/Ia (250 μg, 1 ml)</td>
<td>3/3</td>
<td>5,79 8,06 1,32 1,95 0,29 0,42</td>
</tr>
<tr>
<td>II/IIa (150, μg, 0,6 ml)</td>
<td>3/3</td>
<td>5,87 8,50 1,25 1,77 0,24 0,45</td>
</tr>
<tr>
<td>III/IIIa (50 μg, 0,2 ml twice)</td>
<td>3/3</td>
<td>9,54 7,65 1,81 1,49 0,53 0,47</td>
</tr>
<tr>
<td>IV/IVA (50 μg, 0,2 ml)</td>
<td>3/6</td>
<td>7,06 8,09 2,3 1,73 0,43 0,33</td>
</tr>
<tr>
<td>V (25 μg, 0,1 ml)</td>
<td>3/3</td>
<td>7,26 1,55 0,32</td>
</tr>
</tbody>
</table>

72 hours later both after a single injection or after the second injection (groups III and IIIa), the level of progesterone decreased to 0.24-0.45 ng/ml and was 4-6% of the initial level. Thus, complete luteolysis (plasma progesterone concentration <1.0 ng/ml) was achieved by this time.

The least significant decrease in 24 hours is observed with a single injection of 50 μg of Magestrophan (group IV) - by 67.4%. Only in 5 days (120 hours) after injection, the level of progesterone in this group decreased to 4.5% of the initial level. Therefore, we did not test Magestrophan in a lower dose (< 50 μg). The drop in progesterone concentration when using 25 μg of Estrophanthin corresponds to the values obtained when using higher doses of the drug, which proves the greater effectiveness of the luteolytic effect of D-cloprostenol on the mare’s corpus luteum.

It was shown by C.H.G. Irvine et.al., that twice-administered reduced by 10-20 times (0.5 mg) doses of dinoprost (a natural analogue of PGF2a, standard single dose of 5-10 mg) were effective to mares, although in a single application, does not have any luteolytic effect [3].

Thus, the study of the relative decreasing in progesterone levels showed that the use of various doses of both Magestrophan (from 250 μg to 50 μg (twice)) and Estrophanthin (250 - 25 μg) in the first half of diestrus led to the same drop in progesterone levels and comparable values in 48-72 hours after injection. Since the administration of exogenous PGF2a in diestrus not only affects the corpus luteum, but also leads to rapid growth and maintenance of elevated LH levels in the blood for 72 hours [4], it can be assumed that the duration of follicular development before ovulation will be influenced by the dose of cloprostenol. The effectiveness of cloprostenol also depends on the degree of maturity of the corpus luteum: a decrease in progesterone concentration, associated with the onset of natural regression of the corpus luteum, occurs from the 11th day of the cycle. Therefore, we analyzed the duration of estrus cycles in mares using reduced and recommended doses of cloprostenol depending on the diameter of the largest follicle at the moment of injection and the diestrus period.

Data analysis (Table 2) showed a significant difference of the average (3.2-4.2 days) duration at the period from injection to ovulation between groups II and III when using both small and large doses in both periods (p<0,001). It was found the significant difference (2.3 days) in the length of estrus between groups II and III when using recommended (large) doses of cloprostenol (150-250 μg, 0.6-1 ml) in both the first and second half of diestrus (p<0,001).
The duration of the period before ovulation and the length of estrus depending on the diameter of the follicle, the dose and time of injection of cloprostenol.

<table>
<thead>
<tr>
<th>Dose, µg</th>
<th>No. group p</th>
<th>Injection time (diestrus period)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 5-10 of estrus cycle</td>
<td>day 11 of estrus cycle and later</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>diameter M/m, mm before ovulation</td>
<td>diameter M/m, mm estrus</td>
<td>duration, days (min-max)</td>
</tr>
<tr>
<td>25-50</td>
<td>I</td>
<td>15, 8±1.2</td>
<td>9,7±0.5 (8-15)</td>
<td>4,5±0.2 (3-6)</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>24,4±0.7</td>
<td>8,6±0.6 (6-11)</td>
<td>4,1±0.3 (3-6)</td>
</tr>
<tr>
<td>III</td>
<td>13</td>
<td>32,1±0.8</td>
<td>5,3±0.3 (3-7)</td>
<td>3,4±0.2 (2-5)</td>
</tr>
<tr>
<td>150-250</td>
<td>I</td>
<td>15, 7±0.9</td>
<td>10,0±0.3 (7-13)</td>
<td>3,4±0.2 (2-6)</td>
</tr>
<tr>
<td>II</td>
<td>18</td>
<td>24,3±0.4</td>
<td>7,6±0.4 (5-12)</td>
<td>4,3±0.3 (3-6)</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>36,0±1.4</td>
<td>3,4±0.5 (1-5)</td>
<td>2,0±0.3 (1-3)</td>
</tr>
</tbody>
</table>

*- groups according to the diameter of the largest diestral follicle: I≤21 mm, II -22-28 mm, III - 29-37 mm
p<1.2<0.01; p1.3<4.5; 4.5; 6.7; 8.9; 11.12; 13.14<0.001;
p2.5<12.16<0.01; p7.15<0.001;
p2.9<0.01;

Since a reduction in estrus to 3 days has a negative impact on pregnancy rates, injection of cloprostenol in such doses at the time of presence of a follicle of more than 29-30 mm is undesirable. The average length of estrus when using recommended doses of the drug in the presence of large follicles was 2 and 1.7 days, and when using small doses - 3.4 and 2.9 days in the first and second half of diestrus, respectively. Thus, the use of D-cloprostenol in doses of 25 - 50 µg or D,L-cloprostenol twice in doses of 50 µg in the presence of follicles more than 29 mm in diameter will decrease the risk of estrus reducing.

A slight tendency was observed towards a reduction in the duration of the period before ovulation and the length of heat between periods of diestrus. However, due to the small sample, significant differences (p<0.01) in the reduction of time to ovulation were found only in group III when using low doses of drugs. This trend is explained by the effect of cloprostenol on the corpus luteum, which is already beginning to undergo natural regression.

The ability of cloprostenol to accelerate the maturation of follicles and reduce the time to ovulation is confirmed by R. Newcombe et.al [20] analysis of the effect of different doses (from 8.75 to 625 µg) of cloprostenol on large follicles (28-31 and ≥36 mm). The shortest interval (2.4 days) was observed after injection of 625 µg of cloprostenol in mares with follicles of ≥36 mm, while the longest interval (4.9 days) was observed after injection of 8.75 µg for follicles of 28 - 31 mm in diameter. This research did not reveal a significant difference in the intervals to ovulation between doses of cloprostenol from 8.75 to 250 µg, which is confirmed by the results of our studies (no significant differences in the drop in progesterone concentration when using different doses).
4 Conclusions

Our research has shown that:
1. The effects of both injections of Magestrophan (from 250 μg to 50 μg (twice)) or Estrophanthin (from 250 to 25 μg, once) in the first half of diestrus of mares were similar. Two treatments resulted in the drop of blood serum progesterone concentrations with similar values in 48-72 hours after injection.
2. Use of manufacturer-recommended doses of both D-cloprostenol and L, D-cloprostenol in the presence of large (≥29 mm in diameter) follicles increased the likelihood of reducing period of follicle development and onset of ovulation. The use of D-cloprostenol once in reduced doses (25 - 50 μg) or D, L-cloprostenol twice 50 μg in the presence of follicles more than 29 mm in diameter will decrease the risk of reduced duration of estrus.

References

11. E. Solodova, J.Horse breeding and Equestrian sports, 1, 20-23 (2019).