Oestrous synchronization with progesterone impregnated device and prostaglandin F$_{2\alpha}$ both combined with human chorionic gonadotropin in transitional mares

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SUMMARY

The objective of this trial was to determine the efficacy of the progesterone removal inserted device (PRID) and PGF$_{2\alpha}$ analogue treatments combined with hCG administration on fertility in transitional mares. A total of forty-five thoroughbred mares were used in this experiment. Animals were divided into three groups. Progesterone-impregnated vaginal devices (PRID) were inserted into anterior vagina of the mares of the first group (n = 15). The devices were replaced on day 7 and removed on day 14. D-cloprostenol (0.075 mg) was intramuscularly injected twice at a 14-day interval in the second group (n = 15). Two milliliters of serum physiologic were intramuscularly injected two times at a 14-day interval in the third group (Control, n = 15). After injections and removal of progesterone-impregnated vaginal devices (PRID), the oestrous cycle of each mare was followed through teasing, palpation per rectum and transrectal ultrasonography. After the detection of oestrus, hCG (3000 IU) was intravenously injected to the mares of the first and second groups on the 4th day of œstrus. Pregnant mares were determined with ultrasonography on day 20 after the last mating. There was a statistical significant difference (P < 0.05) between groups I and III and also II and III, for the œstrus, ovulation, pregnancy rates, ovulation time, œstrus duration and mating number required per conception, respectively.

In conclusion, both progesterone impregnated device combined with human chorionic gonadotropin and PGF$_{2\alpha}$ combined with human chorionic gonadotropin applications were effective to induce œstrus and ovulation in transitional mares.

KEY-WORDS : PRID - PGF$_{2\alpha}$ - hCG - fertility - mare.

RÉSUMÉ

Synchronisation de l’œstrus avec un traitement associant la progestérone ou un analogue des prostaglandines F$_{2\alpha}$ à hCG chez la jument au cours de la phase de transition entre anoestrus et saison sexuelle. Par M. B. ATAMAN, A. GÜNAY, Ü. GÜNAY, A. BARAN et M. UZMAN.

L’objectif de cette recherche était de déterminer l’efficacité du traitement associant la progestérone délivrée par un dispositif vaginal (PRID) ou un analogue de synthèse des PGF$_{2\alpha}$ et l’hCG sur la fertilité. Un total de 45 juments, pur sang, a été utilisé dans cette expérience. Les animaux, au cours de la phase de transition entre anoestrus et saison sexuelle, ont été répartis en trois groupes. Les dispositifs vaginaux imprégnés de progestérones (PRID) ont été insérés dans le vagin des juments du premier groupe (n = 15). Les dispositifs ont été remplacés le 7ème jour et retirés le 14ème jour. Le D-cloprostenol (0.075 mg) a été injecté 2 fois à 14 jours d’intervalle par voie intramusculaire au deuxième groupe (n = 15). Deux millilitres de sérum physiologique ont été injectés par voie intramusculaire au troisième groupe (contrôle, n = 15). Après les injections et le retrait du dispositif vaginale, la cyclicité des juments a été examinée par passage à la barre, exploration et échographie transrectales. Après la détection de l’œstrus, l’hCG (3000 unités internationales) a été injecté par voie intraveineuse aux juments des premier et deuxième groupes, le 4ème jour de l’œstrus. Les populations folliculaires des ovaires a été examinée toutes les 12 heures par examen échographique jusqu’au moment de l’ovulation. Les juments étaient accouplées naturellement chaque jour. Le diagnostic de gestation a été réalisé par échographie le 20ème jour après le dernier accouplement. Une différence statistiquement significative a été mise en évidence (P < 0.05) sur les taux d’œstrus, d’ovulation et de gestation, la durée moyenne de l’œstrus et le temps d’ovulation, et le nombre d’accouplements requis par gestation des groupes I et II et III, respectivement.

En conclusion, les applications de progestérone délivrée sous la forme de dispositifs vaginaux imprégnés de progestérones sont efficaces pour induire l’œstrus et l’ovulation chez les juments en phase de transition entre anoestrus et saison sexuelle.

MOTS-CLÉS : PRID - PGF$_{2\alpha}$ - hCG - fertilité - juments.

Introduction

Mares are seasonally polyestrous breeders [3]. Transition from winter anoestrus to normal cyclicity is characterized by erratic œstrus behaviour, follicular growth, long and/or irregular œstrus period and œstrus periods not accompanied by ovulation [15]. Hormonal imbalance occurs during transitional period with high FSH and low LH secretions. Pituitary stores of LH and hypothalamic stores of GnRH are extremely depressed during winter. Concentration of GnRH in hypothalamus is readily replenished after the winter solstice, whereas pituitary stores of LH increase more slowly. This hormonal
environment results in follicular development without ovulation[14]. Most of the mares have 10 to 20 days of estrus during transitional period. Thus, the goal of any hormonal treatment during the transitional period is to hasten initial ovulation of the breeding season and to suppress the long erratic estrus period [15].

Oestrus can be regulated through the use of oral progestin [10, 17], injectable progesterone plus oestradiol benzoate [15, 18], progestin implants [1] and intravaginal sponges [5] at the various times during breeding season and transitional period. Varying effects of oral [15], injectable [19] and implant [1] progestins have been found in transitional mares with regard to oestrous and ovulatory response. In general, oral progestin [Altrenogest] is only efficacious during late transitional period [15]. On the other hand, injectable and/or oral implant of Norgestomet is not useful [1, 19].

The objective of this trial was to determine the efficacy treatments associating of the progesterone removal inserted device [PRID] containing 1.55 g progesterone and 10 mg oestradiol 17-benzoate and/or a PGF2α analogue combined with hCG to induce estrus in transitional mares.

Materials and methods

ANIMALS

A total of forty-five nonlactating thoroughbred mares, aging 8-12 years and 6 stallions aged 6-10 years with a known fertility and health were used in this experiment. This study was carried out in between February 1 and March 15 which is accepted period of time for transitional period for horses in Turkey.

TREATMENT OF MARES

Animals were divided into three groups. Prior to assignment, mares met the following criteria : 1) largest follicle < 35 mm in diameter and 2) no evidence of a corpus luteum based on ultrasonography of the ovaries for at least 22 days prior to assignment. Vaginal devices (PRID 2mm, Sanofi Veterinaria, Alges-Portugal) containing 1.55 g progesterone and 15 mg Oestradiol Benzoate were inserted into anterior vagina of the mares of the first group (n = 15). The devices were replaced on day 7 and removed on day 14. D-cloprostenol (0.075 mg), an analogue of prostaglandin F2α (Dalmazin, Vetas, Istanbul-Turkiye) was intramuscularly injected two times at a 14-day interval to the mares of the third group (n = 15).

OESTRUS DETECTION AND MATING

After injections and removal of the vaginal devices, the oestrus cycle of each mare was followed through teasing, palpation per rectum and transrectal ultrasonography. The oestrus cycle of each mare of the third (control) group was followed for 22 days and those who had spontaneous estrus were detected. After the detection of estrus, 3000 IU hCG (Chorulon, Intervet, Istanbul, Turkiye) were injected intravenously to the mares of the first and second groups on the 4th (84 hours after the detection of oestrous) day of the estrus. Follicles were monitored every 12 hours with ultrasonography until ovulation occurred. Mares in estrus were mated naturally every 48 hours beginning from 84 hours after detection of estrus until the end of estrus. The time interval between the end of treatments (removal of the vaginal devices and the injection of the second PGF2α) and the onset of estrus is recorded as injection-œstrus interval.

The time interval between the end of treatments (removal of the vaginal devices and the injection of the second PGF2α) and ovulation detection is recorded as ovulation time.

Pregnant mares were determined with ultrasonography on day 20 after the last mating. Estrous, ovulation and pregnancy rates were determined based on the total mares in each group.

STATISTICAL ANALYSIS

Data was analysed with Chi-square analysis to compare the estrous, ovulation and pregnancy rates between groups and a student’s-t test was performed to compare average injection-œstrus interval (hr), ovulation time (hr), estrus duration (hr) and mating number required per conception between groups.

Results

Estrous, ovulation, pregnancy rates, injection-œstrus interval (hr), ovulation time (hr), estrus duration (hr) and mating number required per conception (mean ± SEM) in groups I, II and III were summarised in Table I.

There was a statistically significant difference between the groups I and III (P < 0.01) and II and III (P < 0.05) for the estrous and ovulation rates.

The differences of pregnancy rates between the groups I and II were not significant (P > 0.05). On the other hand, there was a statistically significant difference (P < 0.05) between groups I and III, and also II and III for the pregnancy rates.

The differences of injection-œstrus interval (hr) between the groups I and II were not significant (P > 0.05). There was a statistically significant difference (P < 0.01) between the groups I and III and also II and III for the mean ovulation time.

There was a statistically significant difference (P < 0.01) between the groups I and III and also II and III for the mean estrus duration and mating number required per conception.
Discussion and conclusions

In our study, vaginal devices applications were highly found to be effective in controlling estrous which is similar to the reports of PALMER [11] who cited that sponges impregnated in the vagina resulted in a high degree of synchronization of estrous and ovulation. DINGER et al [4] reported that higher synchronization rate could be obtained with the use of vaginal devices (PRID) containing 2 mg progesterone compared to vaginal devices (PRID) containing 1 mg progesterone in two consecutive seven-day periods. We have also observed higher synchronization rates with the use of vaginal devices (PRID) containing 1.55 g progesterone in two consecutive seven-day periods.

Estrous and ovulation rates were higher following in PRID + hCG treated group than nontreated group. Our results are in agreement with the findings of SQUIRES et al [17] who showed that a great number of treated mares exhibited estrous, and ovulated, compared to untreated controls when mares were treated with progesterone for 2 weeks during mid to late transition phase. Vaginal devices usually did not induce inflammation with adherence to vaginal mucosa. Inflammation was observed only in two mares and one became pregnant in our study. It was reported that although applications of injectable progesterone results in sufficient synchronization of estrus and ovulation, it is time consuming because mares must be injected on a daily basis [2], but this disadvantage could be overcome by the use of vaginal devices (PRID).

Ovulation and pregnancy rates were 60.0 % and 53.3 % and 20.0 % and 13.3 % in groups II and III, respectively. These findings are similar with the reports of TAYLOR et al [18] who reported that ovulation rates were higher in treated group than untreated (control) group. In addition to this, JÖCHLE et al [8] cited that PGF$_{2\alpha}$ analogue treatments of the transitional mares with low progesterone level induced immediate secretion of FSH and LH, although GnRH was elevated consistently only after FSH and LH peak level. PGF$_{2\alpha}$ appears directly to stimulate gonadotropin secretion. In our study, prostaglandin F$_{2\alpha}$ treatments applied to the mares with no corpus luteum may have caused the rise of FSH and LH secretion.

After the second PGF$_{2\alpha}$ injection, there were no signs of estrous behaviour and follicular development in some of the mares. This was may be due to no response to PGF$_{2\alpha}$ application.

Injection-estrus interval were 72.0 and 80.0 hours in groups I and II, respectively, which is consistent with the results of SEMACAN et al [13] and SQUIRES et al [17]. Most of the mares showed estrus 3.4 days after the treatment with progesterone and 3.0 ± 1.41 days after the second PGF$_{2\alpha}$ treatment.

Ovulation time and estrous duration were 125.0 and 141.2 ; 129.0 and 157.5 ; and 168.7 and 195.0 hours in groups I, II and III, respectively, which are consistent with the results of other workers [12, 13, 16]. Most of the mares ovulated 6 to 9 days after the second PGF$_{2\alpha}$ treatment [12, 13] in contrast to 5.4 ± 1.5 days of progesterone treatment [10]. However, in our study, hCG injection also shortened the estrous duration as compared to the control animals. Similar results were reported by SQUIRES et al [16]. In our study, longer estrous duration in the control mares was apparently due to the growth and maintenance of large follicles for extended periods of time without ovulation.

Estrous, ovulation and pregnancy rates were highly low in the control group. This was may be due to high FSH and low LH secretions with the resultant effect of irregular follicular development without ovulation in the transitional period.

The numbers of mating required per conception were 1.3, 1.7 and 2.3 in groups I, II and III, respectively, which are similar to 1.9 reported by HENDERSON et al [6] and

<table>
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<tr>
<th>Groups</th>
<th>Estrous rate (%)</th>
<th>Ovulation rate (%)</th>
<th>Pregnancy rate (%)</th>
<th>Injection-estrus time (hr)</th>
<th>Ovulation time (hr)</th>
<th>Estrous duration (hr)</th>
<th>Mating number/per conception</th>
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<tr>
<td>I*</td>
<td>80.0$^a$</td>
<td>73.3$^a$</td>
<td>66.7$^a$</td>
<td>72.0 ± 3.10$^d$</td>
<td>125.0 ± 3.61$^d$</td>
<td>141.2 ± 6.21$^f$</td>
<td>1.3 ± 0.21$^f$</td>
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<tr>
<td>II**</td>
<td>66.7$^{ab}$</td>
<td>60.0$^{b}$</td>
<td>53.3$^a$</td>
<td>80.0 ± 4.00$^d$</td>
<td>129.0 ± 3.71$^d$</td>
<td>157.5 ± 7.63$^f$</td>
<td>1.7 ± 0.21$^f$</td>
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<tr>
<td>III***</td>
<td>26.7$^c$</td>
<td>20.0$^c$</td>
<td>13.3$^b$</td>
<td>-</td>
<td>168.7 ± 4.02$^c$</td>
<td>195.0 ± 4.02$^g$</td>
<td>2.3 ± 0.21$^g$</td>
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Different superscripts in column differ significantly
a, b : p < 0.05 ; a, c : p < 0.01 ; ab, c : p < 0.05 ; d, e : p < 0.01, f, g : p < 0.01, and a, ab : p > 0.05

** : PRID : Vaginal devices containing 1.55 g progesterone and 15 mg Oestradiol Benzoate were inserted into anterior vagina of the mares of the first group. The devices were replaced on day 7 and removed on day 14.

*** : Control : Two-milliliters of saline were intramuscularly injected two times at a 14-day interval per animal of the third group.

| TABLE I. — Estrous, ovulation, pregnancy rates average injection-estrus interval (hr), ovulation time (hr), and estrous duration (hr) (mean ± SEM) in groups I, II and III. |
1.7 MEYERS et al [9]. On the other hand, HOLTAN et al [7] cited that the numbers of insemination per conception were 3.9 for controls and 2.5 for hCG treated group. Our results showed that hCG application minimizes the over use of a stallion to shorten the duration of estrous.

As a conclusion, both progesterone impregnated device and PGF$_{2\alpha}$ combined with human chorionic gonadotropin applications were effective to induce estrous and ovulation in transitional mares. Administration of hCG is an effective method to minimize the over use of a stallion during the long estrous period and ovulation induction can alleviate the increased use of valuable stallions for more mares.

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References