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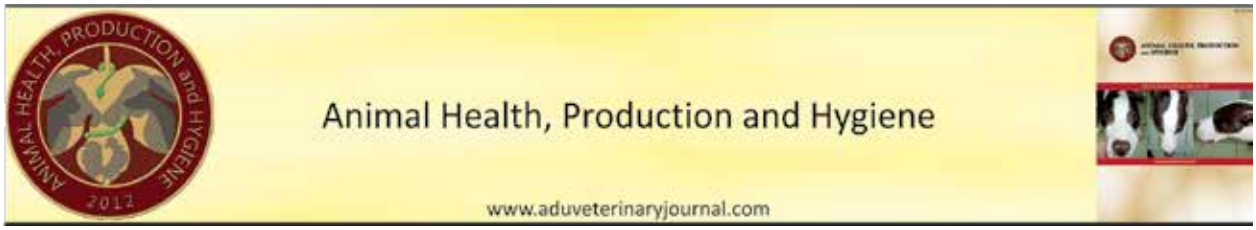

The Effectiveness of Progesterone-Releasing Intravaginal Device with Daily GnRH Injections on the Reproductive Parameters of Mares in Diestrus

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ABSTRACT

This study examines the effectiveness of PRID solely and combined with GnRH treatment on fertility parameters of mares. At day seven after ovulation, PRID (1.55 g P_4+10 mg E_2-benzoate) was inserted intravaginally for nine days in G1 (n=8) and G2 (n=7). In the same period, G1-mares received GnRH (2.5ml/mare, Receptal®) on a daily basis. At day 16 (day of PRID removal) $PGF_2\alpha$ (500µg/mare, Estrumate®) was administered to G1 and G2. Mares of G3 (n=8) served as controls. Follicle controls were started at day 16 in G1 and G2, and when the mares showed positive response to stallions in G3. After day 16 in G1 and G2, mares that did not show signs of oestrus and ovulation within eight days were considered to be non-responder (NR). Mating was performed every other day until ovulation occurred after determination of preovulatory follicle (POF). NR mares were found to be 12.5% (G1) and 14.3% (G2). The pregnancy rate was not statistically significant between the groups. Interval from PRID removal-ovulation was determined to be 6.4 days in G1 and 5.7 days in G2. Interval from POF-ovulation in G1 (1.2 day) was found to be significantly lower than in G2 (3.2 day) and G3 (3.6 day) ($P<0.05$). Diameter of POF (G1: 38.5 mm ; G2: 38.2 mm ; G3: 40.8 mm) and ovulatory follicle (G1: 41.5 mm ; G2: 42.7 mm ; G3: 43.0 mm) were not found significantly different among groups ($P>0.05$). The significance between P_4 concentrations of G1 and G2 was not found throughout PRID treatment ($P>0.05$), except second day of PRID insertion ($P<0.05$). It was concluded that application of PRID solely or combined with daily GnRH injection has no effect on fertility parameters in diestrus mares.

Keywords: Fertility, GnRH, Mare, PRID, Synchronization

Diöstrusteki Kısraklara Günlük GnRH Enjeksiyonuyla Birlikte Uygulanan Progesteron-Salgılayan İntravaginal Cihazın Reprodüktif Parametreler Üzerine Etkisi

ÖZET

Sunulan çalışmada kısraklarda tek başına ve günlük GnRH uygulamasıyla kombine kullanılan PRID'in fertilite parametreleri üzerindeki etkisi araştırıldı. G1 (n=8) ve G2 (n=7)'de ovulasyondan yedi gün sonra PRID (1.55 g P_4+10 mg E_2-benzoat) intravaginal olarak uygulandı ve dokuz gün süreyle tutuldu. Aynı zamanda G1'deki kısraklara GnRH (2.5ml/kısrak, Receptal®) günlük uygulandı. G1 ve G2'ye PRID'in çıkartıldığı 16. günde $PGF_2\alpha$ (500µg/kısrak, Estrumate®) yapıldı. G3 (n=8) kontrol grubu olarak kullanıldı. Follikül kontrollerine G1 ve G2'de 16.günde, G3'te ise kısraklar aygıra pozitif cevap verdiğinde başlanıldı. G1 ve G2'de sekiz gün içinde östrus ve ovulasyon göstermeyen kısraklar tedaviye cevap vermedi (NR) olarak kabul edildi. Aşımlar preovulatorik follikül (POF) belirlendikten sonra ovulasyon şekilleninceye kadar gün aşırı olarak yaptırıldı. NR %12.5 (G1) ve %14.3 (G2) olarak belirlendi. Gebelik oranları arasında fark bulunmadı. PRID kaldırılması-ovulasyon aralığı G1'de 6.4 gün G2'de 5.7 gün olarak bulundu. POF-ovulasyon aralığı G1'de 1.2 günle G2 (3.2 gün) ve G3 (3.6 gün)'den düşük bulundu ($P<0.05$). POF çapı (G1: 38.5 mm; G2: 38.2 mm; G3: 40.8 mm) ve ovulator follikül çapı (G1: 41.5 mm; G2: 42.7 mm; G3: 43.0 mm) arasında fark bulunmadı ($P>0.05$). İkinci gün dışında ($P<0.05$) PRID uygulama süresi boyunca P_4 düzeyleri arasında fark belirlenmedi ($P>0.05$) Sonuç olarak PRID'in tek başına veya günlük GnRH uygulamasıyla kombine olarak kullanılmasının diöstrus dönemindeki kısraklarda fertilite parametrelerini etkilemediği sonucuna varıldı.

Anahtar Kelimeler: Fertilite, GnRH, Kısrak, PRID, Senkronizasyon

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Introduction

Modern and commercial horse breeders require their broad mares to birth live foals, preferably in January every year. However, in equine reproduction, estrus synchronization is performed for two main purposes; one is the embryo transfer programs, while the second is to synchronize a group of breeding mares because of the limited availability of a stallion.

The pharmacologic agents like prostaglandins, progestins, human chorionic gonadotropin (hCG) or deslorelin acetate and estradiol-17β can be used for such purposes. However, when a combination of these methods and pharmacologic agents are used, the highest degree of reliable and successful results are achieved.

Prostaglandin $F_2\alpha$ is extensively used to regulate the estrous cycle in mares, either solely or in combination with progestogens. Deslorelin acetate and hCG are preferred to induce ovulation (Pinto and Meyes, 2007).

Progestins inhibit LH secretion from the anterior pituitary (Evans et al., 1982; Bradecamp, 2007) and can be used as exogenously as peros or injectabl and intravaginal devices in mares. Application of either Altrenogest (0.044 mg/kg orally q24h), an exogenous form of progesterone (P_4) or using P_4 in oil (150 mg/day intramuscularly) suppresses follicular development. Studies showed that the usage of both forms of progestin daily for 10 days is effective for estrous synchronization in mares (Bradecamp, 2007; Sequires, 2008). However, they have some disadvantages, because Altrenogest is expensive, and P_4 in oil causes muscle soreness at the injection site (Nie, 2007). Moreover, daily administration can prove to be impractical for both reasons (Storer et al., 2009).

Progesterone-releasing intravaginal devices have been used extensively in cattle rearing. Different types of such devices are available besides natural P_4: PRID (progesterone releasing intravaginal device; 1.55 g of P_4 with 10 mg estradiol benzoat), CIDR (controlled internal drug release; 1.38 g of P_4), CIDR-B (1.9 g of P_4) and Cue-Mare (1.72 g of P_4).

The present study investigates the effectiveness of the use of PRID alone and its use combined with daily GnRH treatment on the fertility parameters in mares, which include an active corpus luteum.

Materials and Methods

Animals

In all, 23 mares (KWPN; Koninklijk Warmblood Paard Nederland, Netherlands Warmblood n=21, Cheval de Selle Français, n=1; Half-Blood English, n=1) between 8 and 21 years of age and mean weight of 550±200 kg were included in the study, from spring to summer (April-June). Each mare was generally and reproductively healthy. They were kept in large paddocks and fed on hay, grass, cereal grain and mineral supplements in the same stud farm, located at 40°12' N latitude. Mating was performed by natural service with stallions of known fertility within the same farm.

Study Design

All the mares were examined for the estrous cycle stage prior to the treatment period using transrectal palpation, ultrasonography (Agroscan L®, 5 MHz, ECM, Angouléme, France) and vaginoscopy. The day of ovulation was considered day 0. Mares were randomly assigned to the three groups. In group 1 (G1; n=8) PRID (1.55 g P_4 with 10 mg estradiol-benzoate, Ceva-DIF) plus GnRH (10 µg Buserelin acetate/mare, intramuscularly, Receptal®, Intervet) group. On the seventh day post ovulation, PRID was inserted intravaginally for nine days, and GnRH was injected on a daily basis. In group 2 (G2; n=7) or the PRID group, PRID alone was applied as in G1. On day 16, $PGF_2\alpha$ (500 µg cloprostenol/mare, intramuscularly, Estrumate®, Intervet) was injected in G1 and G2 immediately after the removal of the PRID. In group 3 (G3; n=8), the control group, no applications were done.

Examinations after PRID Removal

Mares were examined vaginally to determine vaginitis immediately after the PRID removal and no treatment was given. All the mares were thoroughly inspected daily for signs of estrus by exposing them to a stallion post PRID removal. Ovarian structures (follicles) and uterine examinations were done by transrectal palpation and ultrasonography. Controls were initiated on the day of PRID removal in G1 and G2, and when the mares responded positively to the stallions in G3. Follicle control was performed every 24 h initially, until the preovulatory follicle (POF; ≥ 35 mm) was identified and then the control was conducted every 12 h until ovulation occurred. The follicle diameters were recorded. After determining POF, no ovulations were induced and mating was performed every other day until ovulation occurred. In this study, no routine treatment, such as intrauterine infusions or antibiotics was given, post mating.

Pregnancy diagnosis was performed by transrectal ultrasonography at three different periods: on days 14, 21 and 45, post ovulation. If the embryonic vesicle was observed on day 14 post ovulation, the mare was declared to be pregnant.

Blood Samples and Progesterone Analysis

Blood samples were drawn daily to determine the serum P_4 concentrations obtained from the jugular vein using a vacutainer vial before every examination and application. Blood sample collections were begun on the day of the PRID insertion in the G1 and G2 groups and when the mares in the G3 group responded positively to the stallions. It ended when ovulation occurred in all the groups. Blood samples were centrifuged immediately after collection, for over 10 minutes at 3000 rpm; the serum was decanted and stored at -20°C until assay was done.

Progesterone concentrations were measured by radioimmunoassay (Immunotech®, France) described earlier by Alaçam et al., 2009. The results of the intra- and inter-assay coefficient tests were 5.8% and 9.0%, respectively.

Reproductive Parameters

The following parameters were evaluated in the study.

Rate of non-responder mare (NR; %): After the removal of PRID, the mares that revealed no estrus, no follicle development or no ovulation within eight days were considered as non-responders. They were given one dose of $PGF_2\alpha$ on day eight post PRID removal. After this injection, they showed estrus, ovulated and pregnancy occurred. Therefore, the fertility parameters of these mares were not evaluated during statistical analysis.

Table 1. Time of estrus, ovulation and pregnancy after PRID removal (day)
Tablo 1. PRID çıkarıldıktan sonra östrus, ovulasyon ve gebelik zamanı (gün)

Parameters (day)	G1 (n=7)	G2 (n=6)	G3 (n=8)	P value
PRID removal-estrus	3.3 ± 1.8	3.5 ± 1.0		ns.
*PRID removal-ovulation	6.4 ± 2.3	5.7 ± 1.5		ns.
*Estrus-ovulation	3.1 ± 1.3	2.2 ± 0.9	3.4 ± 1.1	ns.
*Preovulatory follicle-ovulation	1.2 ± 1.3[a]	3.2 ± 1.3[b]	3.6 ± 1.9[b]	P<0.05
Preovulatory follicle-pregnancy	1.6 ± 1.1	1.3 ± 0.5	1.8 ± 1.5	ns.
PRID insertion-pregnancy	15.0 ± 2.7	13.7 ± 1.4	13.6 ± 1.5	ns.

ns.: non -significant
*The first ovulation date in double ovulated mare was used during statistical analysis.
Values with different superscripts (a, b) in the same row are significantly different (P<0.05) .

First service pregnancy rate (FSPR; %): The rate of pregnant mares after the first estrous cycle (First breeding cycle).

Overall pregnancy rate (OPR; %): The total pregnancy rate after the first and second services. *Twinning rate (TwPR; %)*: Twin pregnancies were ascertained when a double embryonic vesicle was seen on day 14 post ovulation. In such cases, the crushing method was used to provide singleton pregnancy.

Pregnancy index (PI; number): The number of natural mating encounters required for pregnancy to occur.

Early embryonic death rate (EED; %): The rate of spontaneously terminated pregnancy before 45 days post ovulation was termed EED in the study.

Cross-cover rate (CCR; %): The rate of mares that were mated more than once in the same estrous cycle.

Rate of foaling mare (FM; %): The rate of mares that completed the normal gestation period.

Statistical analysis

Data were statistically analyzed using SPSS® 16.0 (SPSS Inc, Chicago, Illinois, USA). Descriptive statistics were used to determine the mean and standard deviation values. Non-parametric tests (Kruskal-Wallis H test) were used for inter- and intra-group analyses. Chi-square test was used to compare rates (%). Results were considered significant at P<0.05.

Results

After PRID removal, all the mares revealed mild vaginitis characterized by mucopurulent vaginal discharge with mild odor.

The rate of non-responder mares in the G1 and G2 was found to be quite similar. One of the eight mares in G1 and one of the seven mares in G2 were found to be non-responders (P>0.05). Thus, the rate of mares that ovulated (responder mare) in both groups was found to be 87.5% and 85.7%, respectively.

Interval from PRID removal to estrus was determined as 3.3 and 3.5 days in G1 and G2, respectively. Interval from PRID removal to ovulation was found to be less in G2 (5.7 days) than G1 (6.4 days), however, no significant difference was found between the groups (P>0.05). In all groups, interval from estrus to ovulation was found to be less in G2 than in G1 or G3. On the other hand, no significant difference was found between the values (P>0.05). Interval from preovulatory follicle to ovulation was determined to be significantly lower in G1 (1.2 day) than other groups (P<0.05; Table 1).

When compared with follicular size, the diameters of the preovulatory follicle and ovulatory follicle in G1 were found to be the smallest, at 38.5 mm and 41.5 mm, respectively. During the same period, the same parameters were determined to be the largest in the control group, at 40.77 mm and 43.0 mm, respectively. Despite these numerical differences, no significant difference was observed among the groups (P>0.05; Table 2).

Although the FSPR was found to be lower in G1 (71.4%) than in G2 and G3 (100%), no significance was determined (P>0.05). However, EED occurred between day 14 and 21 of pregnancy in one mare in the control group (1/8; 12.5%). This mare and the other non-pregnant mares in G1 were re-mated during the subsequent estrus. Therefore, the OPR achieved 100% for all the groups in the study (Table 3).

No triple or more ovulations occurred in this study. Double ovulations, however, occurred only during the first service period. The results were found to be close to each other in G1 and G3 at 28.6% and 25.0%, respectively (P>0.05). However, when all of the double ovulated mares in G1 had twin

Table 2. Diameters of preovulatory follicle and ovulatory follicle (mm) for all groups
Tablo 2. Tüm grupların preovulator ve ovulator follikül büyüklükleri (mm)

Diameters of follicle (mm)	G1* (n=7)	G2 (n=6)	G3* (n=8)	P Value
Preovulatory	38.5 ± 3.5	38.2 ± 2.5	40.8 ± 4.3	ns.
Ovulatory	41.5 ± 4.4	42.7 ± 5.7	43.0 ± 7.7	ns.

ns.: non -significant
* Both of the follicles size in double ovulations was evaluated during statistical analysis.

Table 3. Fertility parameters obtained in the study
Tablo 3. Çalışmada elde edilen fertilite parametreleri

Parameters (%)	G1 (n=8)	G2 (n=7)	G3 (n=8)	P Value
Rate of non-responder mares	12.5	14.3	-	ns.
First service pregnancy rate	71.4	100	87.5	ns.
Early embriyonic death	0.0	0.0	12.5	ns.
Overall pregnancy rate	100	100	100	ns.
Double ovulation rate	28.6	0.0	25.0	ns.
Twins pregnancy rate	28.6	0.0	12.5	ns.
Foaling rate	100	83.33	100	ns.
Cross-cover mate	57.1	50.0	62.5	ns.
Pregnancy index (number)	2.2	1.5	1.62	ns.

ns.: non -significant (P>0.05).
*Early embryon ic death between days 14 and 21 of pregnancy was determinate in one mare.

pregnancies, only one of the two double ovulated mares in G3 (12.5%) showed twin pregnancy (P>0.05; Table 3).

Although, the rate of cross cover mating was between 50.0% and 62.5%, no significance was noted between the groups (P>0.05). In the study, the foaling rate was found to be very high, ranging between 83.3% and 100%. No significance, however, was found between the groups (P>0.05; Table 3).

Measurement of P_4 concentration in blood serum demonstrated that P_4 levels were less in G1 than G2. On the other hand, a significant difference was only found on the second day after the PRID insertion (P<0.05). There was no significant difference found on the other days (P>0.05; Figure 1).

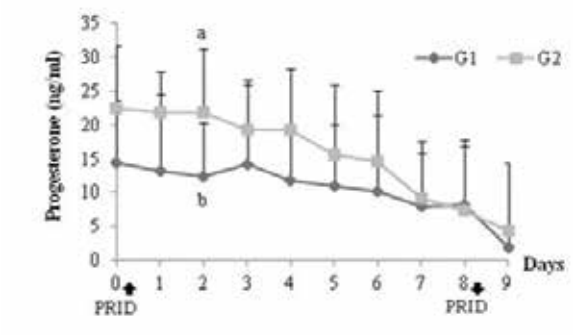

Figure 1. Progesterone concentrations (ng/ml) during treatment of PRID. Values with different superscripts (a, b) on the same day are significantly different (P<0.05).

Şekil 1. PRID uygulaması sırasındaki progesterone konsantrasyonları (ng/ml). Aynı günde farklı harflerle (a,b) belirtilen değerler önemli düzeyde farklıdır (P<0.05).

The P_4 concentration was found to be the lowest in G2 on days when the preovulatory follicle and ovulatory follicle were determined at 0.4 ng/ml, respectively. These outputs were considered significantly lower than the control group (P<0.05; Table 4).

Discussion

Intravaginal devices including P_4 have been used for many years in cattle rearing and their effect on fertility is well known in cattle. However, only a limited number of publications related to the use of these devices are available on mares. Different intravaginal devices have been used in mares during various stages; subestrus or acycle (Arbeiter et al., 1994; Beceriklisoy et al., 2006) or transitional period (Ataman et al., 2000). Nevertheless, their effect on the fertility parameters of seasonal mares, has not yet been fully determined.

The use of intravaginal devices has been reported as safe in cattle and mares. However, all intravaginal devices cause some degree of vaginitis in mares (Arbeiter et al., 1994; Newcombe, 2007; Sequires, 2008). In this study, all the mares showed mild vaginitis characterized by mucopurulent vaginal discharge with mild odor and hyperemia after PRID removal. Despite the no administration of local antibiotics or disinfectant for vaginitis treatment, it cleared up spontaneously within two to three days.

Luteinizing hormone is the main factor responsible for follicular growth and maturation as well as ovulation, after selection of the dominant follicle. In most mammals, P_4 exerts an inhibitory effect on LH release and follicular growth (Gastal et al., 1999). Nevertheless, in mares, the endogenous P_4 concentrations present during the mid-luteal stage is insufficient to suppress

Table 4. Progesterone concentrations (ng/ml) during the preovulatory follicle and ovulatory follicle stages
Tablo 4. Preovulator ve ovulator follikül dönemindeki progesteron konsantrasyonları (ng/ml)

Follicles	Progesterone (ng/ml)			P Value
	G1 (n=7)	G2 (n=6)	G3 (n=8)	
Preovulatory stages	$0.7 \pm 0.4^{a,b}$	0.4 ± 0.2^{a}	0.7 ± 0.3^{b}	P< 0.05
Ovulatory stages	$0.5 \pm 0.6^{a,b}$	0.4 ± 0.5^{a}	1.1 ± 1.2^{b}	P< 0.05

the gonadotropin secretion, follicular development and ovulation (Bradecamp, 2007). This event leads to the diestrous ovulations, unique to mares and it typically occurs during first eight days of diestrus. Furthermore, the exogenous P_4 support in mares does not always prevent ovulation. Approximately, 15 to 25% of the cyclic mares with the inserted CIDR-B tended to ovulate during the application period (Lübbecke et al., 1994; Klug and Jöhle, 2001). Due to the $PGF_2\alpha$-resistant young CL (<5 days) that occurred from diestrus ovulations, the $PGF_2\alpha$ may not induce to luteolisis (Pinto and Meyes, 2007). Therefore, the most important reason for non-responder mares in our study is the diestrous ovulation. Others could be related some yet-to-be identified factors. As 7% of the mares with mature CL do not respond to the single luteolytic level (0.5 mg; Irvine et al., 2002) or higher dose (15 mg; Kenney et al., 1975) of $PGF_2\alpha$.

In some studies, CIDR-B had been used and synchronized estrus and ovulations occurred between 1.3 to 4 days and 2.5 to 6.15 days, respectively after device removal (Lubbecke, 1992; Sequires, 1993; Horn, 1997; Ataman et al., 2000; Kumar et al., 2011). In this study we found similar PRID removal-estrus day, but longer PRID removal-ovulation (5.7 and 6.4 days) day in contrast to previous studies.

The follicle size during insertion and removal of the intravaginal devices was reported to be one of the main factors affecting the time of estrus and ovulation in mares (Handler et al., 2007). The follicle needs a couple of days to develop and ovulate after the P_4 source was removed (CL or exogenous P_4 supports). Normally, the follicle diameter grows up to 2-3 mm in a day (Aurich, 2011). In this study, there was no difference in the time of estrus and ovulation after PRID removal. Besides, the diameters of the preovulatory and ovulatory follicles in the PRID-applied group were almost similar. After analyzing this data, we suggested that the follicle diameters during the insertion and removal of the device were similar in the PRID-applied groups, although the follicle diameters were not measured. This similarity between the follicle sizes in all the groups may have caused an insignificant interval between PRID removal-estrus and –ovulation.

The PRID application was not changed to diameters of preovulatory and ovulatory follicles. There were no difference between previously studies (Bergfelt and Adams, 2007) and control group of this study. Interestingly, the interval between determining the preovulatory follicle and ovulation was found to significantly shorten in the PRID plus GnRH group (1.2 days) than in the PRID alone and control groups (3.2 and 3.6, respectively). These findings suggest that the daily GnRH injection accelerated the follicle development post identifying the preovulatory follicle without changing the maximum diameter of the ovulatory follicle. Histological maturation is well known by the characteristic expansion of the granulosa cell layer and accumulation of the extracellular matrix (Irvine-Rodgers and Rodgers, 2006; Palma et al., 2012). According to McNeill-Weist et al., (1988) P_4 increased the FSH response to GnRH. The GnRH injections could have accelerated the histological maturation of the preovulatory follicle by activating some local ovarian mechanisms such as IGF and VEGF. The IGF proteins play a critical role in follicle development (Donadeu and Watson, 2007). This protein cooperates with the gonadotropins in follicular growth, steroidogenesis and increase in the genes associated with preovulatory maturation (Adashi et al., 1988). Besides, the IGF-1 stimulates VEGF production from the equine follicle cells (Ginther et al., 2005) and VEGF provides tropic support for the ovulatory follicles to develop by increasing the vascularisation (Acosta and Miyamoto, 2004).

The various results of the pregnancy rate obtained in studies with synchronized mares depend upon the season, synchronization method or method of ovulation induction. In this study, the FSPR were found to be higher than the results of the earlier researchers (75.9% Cuvergo-Arango and Clark, 2010; 53.9% Hanlon and Firth, 2012) who used CIDR-B at the beginning of the ovulatory season. In this study, the overall pregnancy rate in the PRID-applied groups (100% for each group) was also found to be higher than the findings of the previous studies; in cyclic mares 81% to 88.23% (Alt et al, 2004; Card and Green, 2004) and in mares at the end of breeding season 46.15% (Kumar et al., 2011). On the other hand, our results showed no significance between the first service and overall pregnancy rate of all the groups. Thus, we suggest that the PRID application either done alone or with daily GnRH injection does not affect the pregnancy rates.

The double ovulations, and particularly the twin pregnancies, are undesirable conditions in horse breeding (England, 2005). Their occurrence is affected by breed, season, reproductive stage and hormonal approaches (England, 2005; Aurich, 2011). Both the rates of double ovulations and twin pregnancies were found to concur with the literature.

The increased number of mating encounters could be the cause for the endometritis and busy stallions in horse breeding (LeBlanc and Causey, 2009; Cuervo-Arango and Clark, 2010). Therefore, the higher CCR rate and PI are undesirable. In this study, there was no significant difference between the CCR in all the groups, when compared with the results (3.4%) of Cuervo-Arango and Clark (2010). However, those studies had been conducted with mares in the vernal period and had hCG-induced ovulation. These could explain the insignificant difference we found in this study. It is evident that the ovulations had occurred within 48 to 72 hours after application of either the hCG or Deslorelin implant to induce ovulation (Beceriklisoy et al., 2006; Bradecamp, 2007).

The PRID-released P_4 is absorbed through the vaginal wall; it enters the bloodstream, increasing the plasma P_4 concentration. The P_4 concentration is higher than 10.5 nm/L for the first four days, while during the following days the released or absorbed amount eventually decreases (Newcombe, 2002). Therefore, the initial increase in plasma P_4 concentrations is expected during PRID or CDIR-B treatment. In parallel with this expectation, peak P_4 concentrations were reported two days post the PRID insertion (Handler et al., 2007) and four days after the CIDR-B insertion (Klug and Jöhle, 2001; Kumar et al., 2011). In these studies, the P_4 concentrations during the PRID or CIDR-B insertion were reported to be between 1.6 and 9.7 ng/ml. In contrast to these author findings, we observed a continuous decrease in the P_4 concentration during the PRID treatment. At the time of PRID insertion, the higher P_4 concentrations (14.32 ng/ml and 22.54 ng/ml) in this study could have lead to this situation. This suggestion is supported by Handler et al., (2007) who reported a negative correlation between the increased P_4 and P_4 concentrations at the time of PRID insertion.

Fertility parameters obtained from any synchronization protocol affects by many factors such as season, follicle size or protocol. Previously authors in this article were used intravaginal device in mares with vernal period, deep anestrus or problematic animals. In these stages, hormonal balance between hipotalamus-hipofiz-ovarian axes has not

yet establishment. Besides ovulations were induced by hCG or Ovuplant. In contrast, our study was performed in the middle of mating season when the hormonal balance have already establishment and ovulations were not induced.

In conclusion, we suggest that successful results could be obtained if this protocol is combined with the induction of ovulation. The results obtained from this study showed that the treatment with PRID or PRID plus GnRH in diestrous mares does not cause any complication and does not affect the fertility parameters. Therefore, instead of resorting to PRID treatment, a regular control of ovulation appears to be effective for improvement of fertility in diestrous mares.

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