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Two hormonal protocols for timed artificial insemination in mares under different residence times of the progesterone intravaginal device

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Summary: The study aimed to evaluate two hormonal protocols for oestrus and ovulation synchronisation for timed artificial insemination (TAI) and their effect on the embryo recovery rate (ERR) in horses. Eleven crossbreed mares were allocated in three groups, taking 3 different oestrus cycles per mare, during two breeding seasons, totalling 17 cycles followed per group, with 11 cycles in the first season and 6 cycles in the second season. Conventional follicular follow-up in the control group (CG; n = 17) was performed by transrectal palpation and ultrasound (US) examination; the ovulation was induced with 500 μg histrelin (intramuscularly: IM) when the diameter of the follicles was ≥ 35 mm, the uterine oedema was grade 3 and cervical opening had begun. Artificial insemination with fresh semen (250×10^6 sperm cells) was performed 24 h after ovulation inductor administration, and embryos were collected eight days after ovulation (D8). The experimental groups had a progesterone-releasing intravaginal device (PRID) inserted for 9 (PRID9) or 11 days (PRID11). Day zero (D0) was defined as the day of TAI for both groups. The PRID was inserted at the beginning of the protocol (D-14 for PRID9 or D-16 for PRID11) plus US examination of the ovaries; the PRID was removed on D-5, 75 μg prostaglandin were administered (PGF2α; IM) and US was performed; 500 μg (IM) of histrelin (ovulation inductor) was administered on D-1; after 24 h (D0), TAI was performed with fresh semen (250×10^6 sperm cells) plus US; nine days after TAI (D9), transcervical embryo collection was performed. After the embryo collection, a dose of prostaglandin F2α was administered, with a delay of seven days before the start for the next group in order to desynchronise the mare's oestrus cycles between the protocols, so as not to interfere in the synchronisation results from one group to another. The order of submission of mares to protocols was randomly defined by sortition in each group. The groups were worked simultaneously: PRID11, PRID9 and CG in the first season and PRID11, CG and PRID9 in the second, during three consecutive months within the reproductive season. Treatment in both experimental groups (PRID9 and PRID11) was performed at any stage of the oestrous cycle. The administration of the protocols was applied to cyclical mares regardless of the US findings at the beginning of the study, and ovulation induction was performed regardless of the size of the follicle or uterine oedema. The US examinations of ovulations were only performed to verify whether they were early or late in relation to the protocol. Responsiveness (presence of preovulatory follicle diameter ≥ 30 mm on the day of ovulation induction D-1) for CG, PRID9 and PRID11 was 100 %, 58.8 % and 41.1 %, respectively ($P > 0.05$). The ERRs were 52.94 %, 29.41 % and 17.64 % for the CG, PRID9 and PRID11, respectively. There was a statistical difference only between the CG and PRID11 ($P < 0.05$). It was concluded that the hormonal protocol using PRID for nine days combined with histrelin administration four days after the PRID's removal showed promising results related to the ERR in mares, while reducing conventional follicular monitoring, the number of gynaecological examinations, animal management and visits to the properties. Because no statistical difference was detected between the PRID9 protocol and the CG, it could be used in large equine herds, reducing animal management and the number of visits by professionals. Effects such as early ovulations, the small size of preovulatory follicles at TAI, the interval between P4 removal and ovulation induction, and low ERR in treatment groups might be improved in future studies, aiming at the possibility using the method for commercial purposes under the acceptance of TAI protocols by veterinarians and breeders. Further studies about TAI in horses are recommended.

Keywords: embryo recovery rate, equine breeding, histrelin, progesterone-releasing intravaginal device, synchronisation of ovulation, timed artificial insemination.

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Introduction

Conventional artificial insemination (AI) in horses has some limiting factors, especially the need for follicular monitoring every 24–48 h. Timed artificial insemination (TAI) has gradually become significant, as it has already been established in cattle, replacing conventional AI (*Ferreira* et al. 2012, *Avanzi* et al. 2015), in order to reduce animal management, eliminate oestrus observations, increase the number of inseminated animals and concentrate parturitions (*Ferreira* et al. 2012).

The AI can be performed when preovulatory follicles become larger than 35 mm in diameter and the mare shows cervical opening and uterine oedema (*Davies Morel* et al. 2010).

Once the day and time of the TAI have been established, follicular monitoring can be reduced by administering hormonal protocols. Few reports are presented about equine TAI in the literature.

Some studies mention ovulation synchronisation and TAI for frozen semen, but little is reported about hormonal protocols (*Almeida* et al. 2001, *Avanzi* et al. 2015).

The use of hormonal protocols for this purpose is still not widely practiced. Conventional daily monitoring of follicular dynamics has remained efficient, despite the number of transrectal examinations required, involving professional visits and considerable additional costs (*McKinnon* et al. 2011, *Bortot* and *Zappa* 2013).

The TAI protocol should be based on the follicular wave and ovulation synchronisation providing a dominant follicle (*Bó* et al. 2016). The use of progesterone-releasing intravaginal devices (PRIDs), combined with estradiol benzoate, is part of most TAI protocols in cattle. This association promotes the temporary suppression and atresia of follicular growth, regardless of the period of the oestrus cycle, through negative feedback on the follicle-stimulating hormone. A new follicular wave emerges about 3.5 to 5 days after estradiol benzoate administration (*Moreno* et al. 2001, *Bó* et al. 2002). The PRIDs have been used in horses in the anoestrus phase (*Polo* et al. 2016) or the transitional phase at the beginning of the breeding season, aiming to synchronise the first ovulation, anticipating the beginning of the season (*Wilde* et al. 2002, *Handler* et al. 2007, *Hanlon* and *Firth* 2012).

Several drugs can be used for the induction of ovulation, which is fundamental for TAI success. Human chorionic gonadotropin (hCG) is effective in inducing ovulation in mares in 48 h (*Silva* et al. 2016). Moreover, gonadotropin-releasing hormone agonists (deslorelin and histrelin) are effective at increasing the release of luteinizing hormone, inducing ovulation in 36 to 48 h; histrelin has been highlighted as the most powerful current synthetic analogue (*Lindholm* et al. 2011, *Voge* et al. 2012). The administration of 0.5 or 0.25 mg of histrelin can induce ovulation within 48 h in mares in the breeding season, reaching 96 % efficiency (*Kiser* et al. 2013).

The aim of this study was to evaluate the efficiency of two different hormonal protocols for oestrus and ovulation synchronisation with a view to TAI in mares, using PRID for nine or eleven days combined with histrelin, and to compare embryo recovery rates (ERRs) between groups.

Materials and methods

Location and animals

The study was carried out at the Gralha Azul Experimental Farm of the Pontifical Catholic University of Paraná, located at 25° 25' 40" S, 49° 16' 23" W, during the 2018–2019 and 2019–2020 southern hemisphere reproductive seasons (September to February). Eleven crossbred mares (Crioulo, Arabian, Quarter Horse and Mangalarga) with no commercial purpose and a crossbred stallion (Lusitano and Breton) were evaluated. Their ages ranged from 6 to 14 years, average weight was 425 ± 75 kg, and their body condition score (BCS) was between 3 and 4 (1 = thin to 5 = obese; *Speirs* 1997). They were managed and fed in fields with *Avena sativa*, *Lolium multiflorum*, *Medicago sativa* and 2 kg of concentrate/animal/day (ProEquine Agraria®; 12 % protein, 12 % fibre, 14 % mineral matter, 2.5 % ether extract; Guarapuava, Paraná State, Brazil), and water and mineral salt ad libitum.

Animal inclusion criteria were the presence of corpora lutea (CL), shown by transrectal ultrasound (US) evaluation (*Ginther* and *Pierson* 1989) at the beginning of each reproductive season and at the beginning of the study to prove cyclicity (*Ginther* et al. 2005). The exclusion criteria were reproductive incompatibility of the anatomy of the cervix or mares being in the spring transitional period in September, without CL, dominant follicle or uterine oedema (*Cerqueira* et al. 2019).

Experimental design

The protocol diagrams for the control group (CG) and the experimental groups PRID9 and PRID11 are represented in Figure 1 A, B and C.

The experiment was carried out over two consecutive breeding seasons – but not all mares were used both years – on three groups: a CG, a group with PRID inserted for 9 days (PRID9) and another group with PRID inserted for 11 days (PRID11). Each group consisted of 17 observations (oestrus cycles), using 1 cycle per animal per group, and three different oestrus cycles per mare in a crossover design, computing 11 cycles in the first season and 6 cycles in the second season. Day zero (D0) was defined as the day of AI for all groups.

The embryo transfer in mares was performed in the CG as usual. Conventional follicular follow-up for the CG (n = 17) was performed by rectal palpation and US examinations of the ovaries at different times according to the mare's oestrus cycle and follicle size, every 24 or 48 h (*Pimentel* et al. 2014). A dose of prostaglandin (D-Cloprostenol 75 µg, intramuscular: IM; Croniben, Biogenesis Bagó) was given to induce luteolysis when CL were detected, in order to start a new cycle. When follicles larger than 35 mm, uterine oedema grade 3 and cervical opening were detected by rectal palpation and US, ovulation was induced with histrelin (500 µg IM, Strelin, Botupharma, Botucatu, São Paulo); this was on D-1. After 24 h of ovulation induction (on D0), US examination and an AI with fresh semen (250×10^6 sperm cells) were performed. Ovulation was confirmed after 24 h of AI (D1). Embryo collection (EC) was performed eight days after ovulation (D9) (Figure 1A).

The group with PRID for nine days (PRID9) (*n* = 17) received an intravaginal device with controlled-release progesterone (1 g P4, Biogénesis Bagó) and US on D-14; the PRID was removed after nine days (D-5) and PGF2α (D-Cloprostenol 75 µg IM; Croniben, Biogenesis Bagó) was administered plus US. Ovulation was induced with histrelin (500 µg IM; Strelin, Botupharma, Botucatu, São Paulo) plus US after four more days (D-1). The TAI was performed with fresh semen (250×10^6 sperm cells) after 24 h (D0) plus US, and EC was achieved nine days later (D9) (Figure 1B).

The PRID11 group was submitted to the same treatment as PRID9, except the PRID remained for 11 days (Figure 1C).

Treatment in both experimental groups (PRID9 and PRID11) was performed at any stage of the oestrous cycle. The administration of the protocols was applied to cyclical mares regardless of the US findings at the beginning of the study, according to the inclusion or exclusion criteria mentioned previously. Ovulation induction was performed regardless of the size of the follicle or uterine oedema. The US examinations of ovulations were only performed to verify whether they were early or late in relation to the protocol.

A dose of hCG (1250IU IM, Vetecor, Hertape Calier, São Paulo, Brazil) was administered in both experimental groups to mares with follicles larger than 33mm, uterine oedema and cervical opening on the first day of the protocol (D-14 for PRID9/D-16 for PRID11), in order to induce ovulation and start a new oestrus cycle in the presence of the P4 device.

Before the PRID was inserted, it was sprayed with oxytetracycline plus hydrocortisone (Terracam Spray®, Agener União, São Paulo, Brazil) and the extractor cord was removed in order to avoid external contact and minimise vaginitis. The manipulator wore palpation gloves to insert the devices into the vagina.

The US (SonoScapeA5v, rectilinear transducer L561v 3 to 8 MHz, China) examinations were performed every 48h in addition to the mares' management days in order to evaluate the follicular growth during the protocols. The follicle sizes were based on the means of the follicular heights and diameter (*Ginther* and *Pierson* 1989).

A dose of prostaglandin F2α was administered after EC, with a delay of seven days before the start for the next group in order to desynchronise the mare's oestrus cycles between the protocols, so as not to interfere in the synchronisation results from one group to another. The ERR (%) and the responsiveness to treatment (presence of a preovulatory follicle at the time of ovulation induction; *Davies Morel* et al. 2010) were evaluated to observe the efficiency of each protocol. Early ovulations were considered to be those that occurred before ovulation induction. The animals within each group were weighed and evaluated for BCS on the first day of the protocol and on the day of TAI to evaluate whether these factors influenced the result.

The order of submission of mares to protocols was randomly defined by sortition of each group. The groups were worked simultaneously: PRID11, PRID9 and CG in the first season and PRID11, CG and PRID9 in the second, during three consecutive months within the reproductive season.

Semen collection and manipulation

A mare in oestrus was used for stallion stimulation for semen collection. After exposure and cleaning the penis with water and compresses, the stallion was led to a dummy, where, after

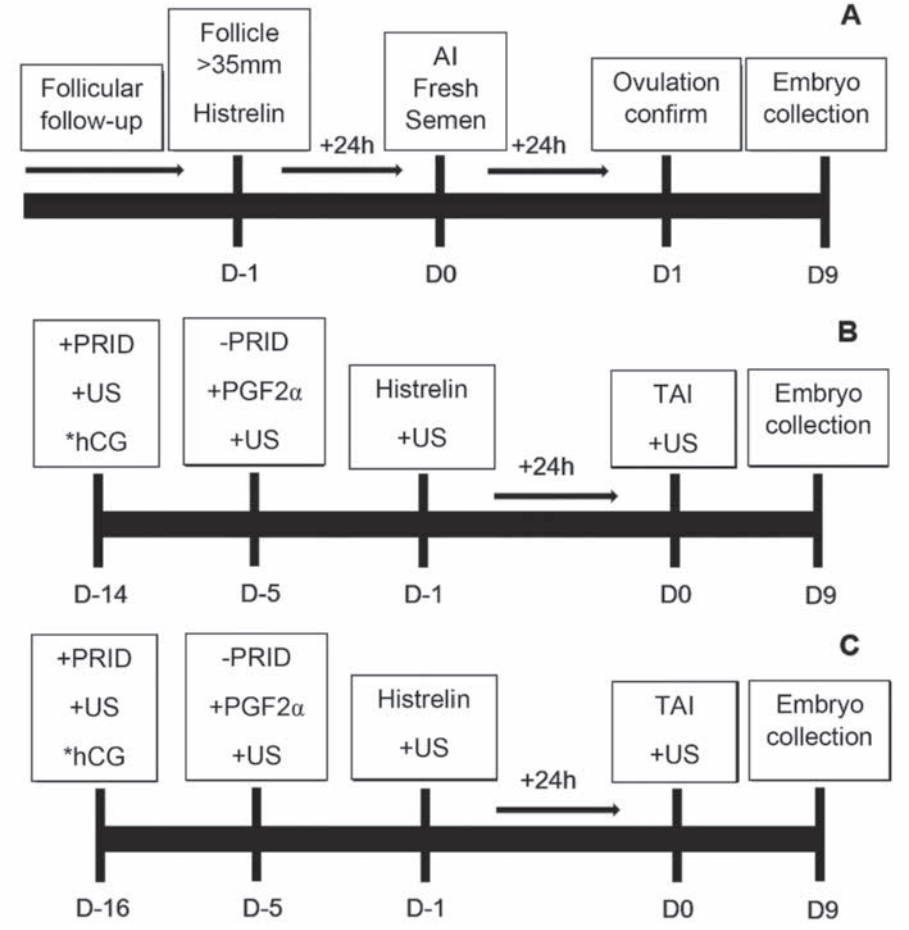

Fig. 1 A, B, C Protocol diagrams for timed artificial insemination in mares to control group and the treated groups PRID9 and PRID11, respectively. AI = artificial insemination; PRID = progesterone-releasing intravaginal device (1g P4, Biogénesis Bagó); US = ultrasound; hCG = human chorionic gonadotropin (1250IU M, Vetecor, Hertape Calier, São Paulo, Brazil); PGF2α = prostaglandin F2α (D-Cloprostenol 75μg IM, Croniben, Biogenesis Bagó); Histrelin = 500 μg IM, Strelin, Botupharma, Botucatu, São Paulo; TAI = timed artificial insemination. | *Protokoll-Diagramme für die terminierte künstliche Besamung von Stuten der Kontrollgruppe (KG) und jeweils der Behandlungsgruppen PG9 und PG11. AI = künstliche Besamung; PRID = intravaginales Progesteron Pessar (1g P4, Biogénesis Bagó); US = Ultraschall; hCG: humane Choriongonadotropin (1250 IE IM, Vetecor, Hertape Calier, São Paulo, Brazil); PGF2α = Prostaglandin F2α (D-Cloprostenol 75μg IM, Croniben, Biogenesis Bagó); Histrelin = 500 μg IM, Strelin, Botupharma, Botucatu, São Paulo; TAI = terminierte künstliche Besamung.*

mounting, its penis was diverted laterally to the artificial vagina, model Botucatu® (Botupharma, Botucatu, São Paulo), and the semen was collected. The ejaculate was filtered in a nylon filter to separate the gel fraction and then analysed for motility and vigour by optical microscope. The sperm concentration was counted in a Neubauer chamber and the semen was diluted in the proportion of one part of semen to one part of skimmed milk diluent, centrifuged at 2200 rpm (600 × g) for 10 min and resuspended with BotuSemen® at a total dose of 250 million sperm cells (2 mL) with progressive motility (*Xavier* et al. 2009). It was deposited in the uterine horn ipsilateral to the preovulatory follicle by flexible pipette for deep intrauterine AI because of the low dose of semen (Minitube®, Minitube do Brasil, Porto Alegre, Rio Grande do Sul, Brazil).

Embryo collection

The mare's perineum was washed with soap and water, and the base of the tail covered with cotton bandage before EC. A sterile silicone catheter was manually introduced through the cervix to flush the uterus, using up to 4 L of lactated Ringer's solution (JP Pharmaceutical Industry, São Paulo, Brazil), heated to 37 °C. The fluid was recovered by gravity flow in an embryo filter and placed under a stereoscopic microscope model Leica Zoom 2000 (increase 4×, Germany) for the embryo search. The recovered embryos were frozen by vitrification (*Diaz* et al. 2016).

Statistical analysis

The data were analysed by Statgraphics Centurion XVI Software, Virginia (USA, 2013). The differences in the ERR between groups, and the differences in treatment responsiveness rates and early ovulations were calculated using Fisher's exact test. This test was used as an alternative to the Chi^2 test because 2 × 2 tables and expected values below 5 were analysed. Fisher's exact test is based on calculating the probability distribution of the table frequencies in a situation with fixed margins because the probability of a given frequency distribution is a function of unknown value parameters (*Fisher* 1934).

The differences between the mean follicular diameters of the experimental groups at D-5 and D-1 were tested by Student's t test, assuming non-homogeneous variances (*Meyer* 1983).

Binary logistic regression was used to test the influences of age, group, BCS and weight on the ERR. Generalized linear models are defined by a probability distribution for the response variable Y belonging to the exponential family, a set of explanatory variables that can be numeric or categorical and a link function. The response variable of the model has Bernoulli (or Binomial) distribution and the link is the logistic function in binary logistic regression, which is a case of a generalized linear model (*McCullagh* and *Nelder* 1989).

All variables were considered significant if $P < 0.05$.

Results

The mean weights (kg) for the CG, PRID9 and PRID11 were 417.29, 433.73 and 434.55, respectively, and the mean BCS (1–5) were 3.29, 3.30 and 3.29, respectively. Neither parameter showed any significance between groups. The mares' mean age was 10.35 years. Binary logistic regression tests were performed to evaluate the influence of these variables on the ERR. The *P* values for binary logistic regression ranged from 0.106 to 0.469.

Table 1 represents the follicular dynamics of the groups on the days of management, the percentage of responsiveness of the mares at each treatment and the ERRs.

Table 1 shows that 10 of the 17 cycles evaluated in PRID9 group, were responsive to treatment (58.8 %), presenting preovulatory follicles on the day before TAI, and seven ovulations occurred up to 48 h after induction with histrelin (70 %), with five embryos recovered. The CL were observed on the day of AI (early ovulation) in two oestrus cycles (11.7 %). One ovulation was observed four days after induction, with a positive result for the EC performed eight days after ovulation, therefore, this result was discarded as it was performed outside D9 of the protocol and was not computed as ERR relative to the TAI.

Table 1 Diameters (Ø) of the largest follicles on the days of management of the mares, preovulatory follicle diameter (POF), protocol responsiveness rates, and embryo recovery rates. | *Durchmesser (Ø) der größten Follikel am Untersuchungstag der Stuten, Präovulatorischer Follikeldurchmesser (POF), Protokoll-Reaktionsfähigkeitsrate, Embryogewinnungsrate.*

Group	Diameter of the > follicle during the protocol (x±s) mm			Ø POF (x±s) mm	Protocol Responsiveness Rate n (%)	Embryo Recovery Rate n (%)
	D-16	D-14	D-5	D-1		
CG (n=17)	-	-	-	36.5±3.3[a]	17/17 (100.0)[a]	9/17 (52.94)[a]
PRID9 (n=17)	-	22.9±11.4	25.3±10.6[a]	30.7±8.9[b]	10/17 (58.8)[b]	5/17 (29.41)[ab]
PRID11 (n=17)	23.1±6.6	-	22.9±10.9[a]	26.7±9.6[b]	7/17 (41.1)[b]	3/17 (17.64)[b]
P	-	-	0.54	CG × PRID9: 0.02 CG × PRID1: 0.001 PRID9 × PRID1: 0.29	CG × PRID9: 0.003 CG × PRID1: 0.0001 PRID9 × PRID1: 0.24	CG × PRID9: 0.14 CG × PRID1: 0.03 PRID9 × PRID1: 0.34

Different letters in the same column indicate statistical significance ($P < 0.05$).

After the administration of hCG to PRID9 on D-14, four of the six mares induced ovulated within 48 h (66.66 %); one mare showed regression and subsequent growth of the follicle induced, with an embryo recovered; and one mare was not responsive to induction, and the follicle continued to grow until its ovulation prior to TAI, which resulted in no embryo.

Seven of the 17 cycles were responsive to treatment (41.1 %) in the PRID11 group but only four ovulated within 48 h after induction with histrelin (57.14 %), with three embryos recovered. On the day before insemination, there was a predominance of small follicles and CL were observed in four cycles (23.5 %; indicating early ovulation). One of the two mares induced with hCG on D-16 showed ovulation in 48 h and the other after 6 days, but neither resulted in embryo recovery.

The CG had daily follicular follow-up assisted by transrectal US, resulting in an average of 6.4 palpations per mare (ranging from 2 to 18 controls), until the emergence of a preovulatory follicle whose mean diameter was 36.5 mm at the time of ovulation induction (D-1), which differed significantly from both experimental groups ($P < 0.05$). In this group, 100 % of the ovulations occurred within 48 h after histrelin administration and nine embryos were recovered.

The CG and PRID9 did not differ significantly in the ERRs obtained ($P > 0.05$). The same applied to the comparison between the PRID9 and PRID11 group, therefore, the result of the PRID9 group showed statistical similarity to both the CG and the PRID11 group. However, both experimental groups showed differences ($P < 0.05$) in responsiveness to the protocol compared to the CG.

The PRID11 group showed a lower efficacy in the ERR, with only three embryos recovered from the 17 cycles evaluated. The lowest mean follicular diameter at the time of ovulation induction (D-1) was observed in this group (Table 1). Some mares presented follicular regression and atresia, while others were not inhibited by P4 when the follicular diameter was around 25 mm at the beginning of the protocol, thus, their follicular growth and early ovulation persisted.

All of the early ovulations occurred soon after the removal of the PRID and before the day of ovulation induction (D-1), with no significant difference between the experimental groups ($P = 0.65$).

A mild vaginal inflammatory reaction in 5 of the 17 cycles evaluated was observed clinically.

Discussion

The TAI has not been widely performed in this species because of the peculiarity of a mare's oestrus cycle, physiologically having a long oestrus (practically one-third of the duration of the oestrus cycle), and blind hormonal regulation of the cycle has not been performed successfully in many cases. In addition, mares can ovulate despite the presence of a corpus luteum or P4 treatment. This makes blind hormonal treatment (without US examination) even more difficult.

The present study is one of the first to develop and test an efficient hormonal protocol for TAI in mares, aiming to reduce the number of veterinary visits to properties for gynaecological interventions (with a consequent cost reduction) and promote animal welfare by decreased management in large equine herds.

Factors such as the age, weight and body condition of mares can influence the success of embryo recovery (*McCue* 2011, *Zúccari* et al. 2013, *Araújo e Oliveira* 2018). The *P* values for binary logistic regression indicated the homogeneity of the sample and that these variables had no influence on the results of the ERR ($P > 0.05$).

The PRID9 protocol stood out for its better suitability for TAI, since the ERRs of this group showed no statistical difference ($P > 0.05$) from that of the CG. This result should be considered promising because, hypothetically, the CG should result in better reproductive efficiency since it is the conventional method for insemination and embryo transfer in horses.

Some mares in the PRID11 group presented follicular regression and atresia, while others were not inhibited by P4 when the follicular diameter was around 25 mm at the beginning of the protocol, showing early ovulations. This may be related to the reduced total number of follicles per animal by the end of treatment, regardless of the number of follicles at the beginning of the protocol. The increase in the diameter of the largest follicle indicated that P4 for 11 days induced gradual ovarian cyclicity and reduced the number of follicles. This has also been reported by *Handler* et al. (2006) and *Polasek* et al. (2017) using PRID for 11 days with good results for oestrus synchronisation in anoestrus mares. In the latter study, the average diameter of the largest follicles on the day of ovulation induction was similar to the present study, and the results were satisfactory for acyclic mares, highlighting the importance of a low concentration of endogenous hormones in the success of this protocol. *Handler* et al. (2006) also reported that 23.3 % of the mares presented early ovulation during the permanence of the P4 device, corroborating the results of the present study (23.5 %).

A mean follicular diameter of 30.7 mm was verified at the time of ovulation induction in the PRID9 group. If there was a longer interval between device removal and ovulation induction, this mean could possibly be higher, as observed by *Polasek* et al. (2017), whose ovulation induction was performed six days after device removal with a 3 mm increase in follicular diameter. The duration and concentration of P4 was sufficient to prompt a new follicular wave without a lot of early ovulations (11.7 %). In the same way, a seven-day period between protocols could not be enough to allow mares to return to normal cycling, signalling a potential limitation impacting the study.

In addition, the ovulation that was observed in the PRID9 group after four days of induction, with positive embryo recovery but not coinciding with the EC day of the TAI protocol, indicated that the TAI may lead to a better pregnancy rate than ERR, since the success of EC depends directly on the day of ovulation.

Effects such as early ovulations, the small size of follicle at TAI, the interval between P_4 removal and ovulation induction,

and the low ERR in treatment groups might be improved in future studies, aiming at the possibility of using this method for commercial purposes under the acceptance of TAI protocols by veterinarians and breeders.

The percentage of embryo recovery observed in the CG (52.94%) is concordant with literature data, which show that it can vary from 40 to 80% (*Fleury* et al. 2001, *Squires* et al. 2003, *Vazquez* et al. 2010, *Gomes* et al. 2014). Some factors, such as the professional's experience and variations in environmental temperature, influence the ERR in mares (*Oliveira* et al. 2015, *Cuervo*-Arango et al. 2018). The average temperatures for the first and second year in the present study were 20.66 and 19.04 °C, respectively. According to *Oliveira* et al. (2015), the ERR is expected to be approximately 60% at average ambient temperatures lower than or equal to 24 °C.

Some studies have tested oestrus synchronisation in horses with PRID. *Newcombe* (2002) used PRID in mares for 10.9 days during the transition period and observed 89% of the ovulations 6.6 days after the device was removed, with 4.6% ovulations occurring before its removal. *Handler* et al. (2006) used progesterone implants (PRID®) in eight mares for 11 days at different seasons, with satisfactory results for oestrus synchronisation (73.4%). *Hanlon* et al. (2010) tested PRID in mares for 10 days during the transition period and noticed 100% ovulating about three days after device removal. Thus, there are discrepancies among authors related to the percentage of ovulations, with variations that can be attributed to the concentrations of endogenous hormones (anoestrus/transition/reproductive season), the status of follicles and the timing of the insertion of the PRID regarding the stage of cycle, geographic region, or variations in breed, age or climate.

Studies to anticipate the onset of ovarian cyclicity in Quarter Horse mares in anoestrus were developed by *Oliveira Filho* et al. (2012) using PRID for 10 days. Seventy per cent of the mares presented 35 mm follicles 3.8 days after PRID removal, with ovulations 48 h after induction with hCG and desloreline. These data corroborate those of the present study, despite the different methodologies, since ovulation induction in the TAI protocols administered was predecided four days after device removal, with more than 50% ovulations occurring up to 48 h after histrelin administration. The PRID resulted in a 75% ERR in *Oliveira Filho* et al. (2012) compared with 29.41% in the present study (PRID9); this ERR (*Oliveira Filho* et al. 2012) was probably more promising because the diameter of the preovulatory follicle was larger. In addition, the animal breed used (Quarter Horse) has been bred specifically for reproduction, a different geographical location was used, with different device timing permanence (10 days), ovulation inducers and seasonality, all of which could be influencing factors among the animals surveyed.

Researchers from our group recently evaluated 15 mares with PRID for ten days, followed by AI with fresh semen after four days of PRID removal, and obtained an ERR very close to the CG (80 and 82.35% respectively – personal communication – *Macan* et al. 2021). These higher percentages of ERR than those of the present study may be attributed to the use of a PRID with a higher concentration of progesterone (1.9 g), AI with 500 million viable spermatozoa and the use of dinoprost tromethamine instead of D-cloprostenol. In that study, the authors also realised a 65% cost reduction in veterinary visits by applying the protocol compared to the CG (personal communication – *Macan* et al. 2021).

According to *Polasek* et al. (2017), the use of PRID in mares can trigger transitory vaginitis, which is spontaneously controlled after device removal without interfering with pregnancy rates. The use of oxytetracycline with hydrocortisone (spray) on the device at the time of its insertion in the mare reduces the severity of the vaginitis, with no influence on the follicular diameter, ovulation period, uterine oedema or pregnancy. Thus, PRID can be used in equine TAI (*Martinez* et al. 2016). *Almeida* et al. (2001) demonstrated the efficacy of oestrus synchronisation in mares using a subcutaneous implant of norgestomet or oral administration of altrenogest for nine days. Neither progestogen showed any significant difference in the reproductive indices of the mares, signalling that these progestogens can be used for nine days for TAI in mares as alternative forms of P4 besides PRID.

The hypothesis of the present study can be confirmed, because PRID9 gave results close to the CG, which is considered standard in the assisted reproduction of horses.

Conclusion

The proposed protocol for TAI in mares combining PRID for nine days and histrelin four days after progesterone removal showed promising results related to the ERR, while reducing the conventional follicular monitoring and the number of gynaecological examinations, animal managements and visits to the properties. Although the ERRs of PRID groups were low, the PRID9 protocol showed better results than PRID11; no difference was detected between the PRID9 protocol and the CG. Further studies about TAI in horses are recommended.

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Conflict of interest statement

The authors state no conflicts of interest.

Animal welfare statement

The study was approved by the Ethics Committee on the Use of Animals (CEUA) of the Pontifical Catholic University of Paraná (PUCPR) under number 01263.

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