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Estrus and ovulation synchronization in mares for timed artificial insemination using fresh or frozen semen followed by timed-embryo transfer

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Summary: Two protocols were evaluated for prostaglandin F2α-based ovulation induction during timed artificial insemination (TAI) and timed embryo transfer in mares: (1) deslorelin acetate (DESL), and (2) DESL and human chorionic gonadotrophin (DESL + hCG). Donor embryo recovery rate (ERR), recipient pregnancy rate (PR), and ovulation time (OT) were assessed in donors and recipients, allocated into 2 groups: DESL (n = 79) and DESL + hCG (n = 89). The DESL group was treated according: day zero (D0) ultrasonography examination (US)+PGF2α; D7/D8 US+DESL; D9/D10 US+TAI using frozen semen or TAI on D7/D8 with fresh semen; embryo recovery and embryo transfer were performed on D19 and in D23 the pregnancy diagnosis. The DESL+hCG group (n = 89) was treated according to the same protocol, except that on D7, DESL+hCG (IV) were administered. A difference ($P < 0.0001$) was observed in the time of ovulation after induction with DESL or DESL+hCG groups. The ERR of donors was 71.42% (DESL) and 64.28% (DESL + hCG) in mares inseminated with fresh semen, and 66.66% (DESL) and 73.68% (DESL+hCG) inseminated with frozen semen. The PR in mares that received embryos from fresh semen was 70% (DESL) and 55.55% (DESL+hCG) and that received embryos from frozen semen was 59.09% (DESL) and 60.71% (DESL+hCG) ($P > 0.05$). In conclusion, no difference in the ERR in the donor mares, or PR in the recipients was observed between the administered protocols. Compared to the DESL-only protocol, the combined DES+hCG protocol showed significant reduction in time between the administration of inductors and ovulation. No difference was observed in the ERR and PR with respect to use of fresh or frozen semen.

Keywords: deslorelin, hCG, mares, synchronization of ovulation, timed artificial insemination, timed embryo transfer

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Introduction

During the breeding season of mares, determining the correct time for artificial insemination (AI) is essential to maximize pregnancy rates (*McCue* et al. 2007). The pregnancy rate in mares may be impaired due to the long physiological period of estrus (5 to 7 days), making it difficult to predict the exact moment of ovulation (*Ginther* 1993). Precise prediction of the time of ovulation aids in increasing pregnancy rates, enabling semen to be deposited in the reproductive tract of the mare, closer to the time of ovulation (*Woods* et al. 1990, *Polo* et al. 2016, *Oliveira* et al. 2020). The best technique for the optimization of equine reproduction is AI with fresh, refrigerated, or frozen semen (*Polasek* et al. 2017). Furthermore, semen quality is one of the main determinants for successful outcomes (*Magistrini* et al. 1996, *Stradaioli* et al. 2004).

According to the average survival rate of sperm in the uterus of a mare, AI with fresh semen is recommended every 48 hours until ovulation occurs (*Pickett* et al. 1987). However, frozen horse semen shows reduced viability (6 to 12 hours), due to a series of cellular changes that occur during the cooling and freezing processes, resulting in lower embryo recovery rates (ERRs) and pregnancy rates (PRs) (*Loomis* and *Squires* 2005).

The use of ovulation inductors contributes significantly to the improvement of reproductive efficiency, promoting a reduction in the estrus cycle and synchronization of the time of ovulation and insemination. AI performed within 48 hours of ovulation induction results in reduced costs with regards to the transport of refrigerated semen and optimizes the use of frozen semen (*McCue* et al. 2007). Ovulation-inducing agents such as DESL or hCG stimulate the final follicular maturation and promote ovulation within 48 hours (*Barbacini* et al. 2000, *McCue* et al. 2007).

DESL is a gonadotrophin releasing hormone (GnRH) agonist. The systemic administration of DESL during the estrus cycle

in mares promotes a prolonged pulsatile release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, inducing final follicular maturation and ovulation (*Meinert* et al. 1993, *Mumford* et al. 1995). hCG is a glycoprotein, with a biological activity similar to that of LH, binding to the ovarian LH receptors and acting in the final stages of maturation and ovulation of the dominant follicle (DF) (*Rossdale* and *Lambercht* 1998, *Barbacini* et al. 2000). To reduce costs and time spent by professionals, ovulation inductors are used alone or in combination.

The hypothesis of the present study was that the combination protocol will result in better synchronization of ovulation by reducing the ovulation time as well as the variation in ovulation time among the mares, as compared to the conventional protocol, (using a single hormone as inductor), commonly employed in embryo transfer programs.

The objective of the study was to test 2 hormonal protocols: timed AI (with frozen or fresh semen) and embryo transfer in horses using the ovulation inductors (1) deslorelin acetate (administered alone; DESL) or (2) a combination of deslorelin acetate and human chorionic gonadotrophin (DESL + hCG). The parameters of the study to be assessed were donor embryo recovery rate (ERR), recipient pregnancy rate (PR), and ovulation time (OT) in donor and recipient mares after administration of the inductors.

Material and Methods

Animals and location

A total of 168 estrous cycles from 110 mares were evaluated during the breeding season (November 2017 to March 2018; South Hemisphere). The mares (donors or recipients) were of different breeds (Brazilian Jump Horse, English Thoroughbred, Pure Arabian Blood, Quarter Horse, Mangalarga Marchador, and Criollo). The average age of the mares was 6.5 years (range 4–16 years) and the average weight was 525 kg (range 400–650 kg). The average of body condition score was 3.0 (1 = thin; 5 = fat) (*Henneke* et al. 1983). The study was conducted in farms located at latitude 25° 32′ 05″ S, longitude 49° 12′ 23″ W and latitude 22° 44′ 22″; longitude 45° 35′ 29″ W. The mares were kept in a semi-extensive grazing system with daily access to alfalfa hay, paddocks of Cynodon dactylon, and water ad libitum. In addition, pelleted feed composed of calcium, phosphorus, methionine, lysine, iron, selenium, zinc, vitamin A, vitamin D3, vitamin E, vitamin B1, vitamin B2, biotin, copper, iodine, manganese, cobalt, mineral matter (9%), protein (13%), and carbohydrates (SUPRA Rações, Rio Claro, São Paulo, Brazil) was provided.

Embryo donor mares

At D0 (start of the study), only mares with corpus luteum and follicles < 20 mm (n = 99 per estrus cycles) and free of clinical abnormalities of the reproductive tract, as determined via ultrasound examinations (Mindray 2200, 75L50esv endorectal transducer, Shenzhen, China), were selected as embryo donors. Donor mares with or without embryos were reused during the next estrus cycle and the hormonal protocol reapplied in order to obtain a new embryo.

The mares were assigned to groups based on the ovulation inductor employed (DESL alone or DESL + hCG in combination) and type of semen used (fresh or frozen). The mares were monitored by 2 experienced professionals who performed ultrasonographic evaluations to determine the diameter of the preovulatory follicle (POF, in mm), ovulation time (OT; hours) after the administration of the ovulation inductor and ERR. Ovulation was induced when the POF measured ≥ 35 mm (diameter) and grade 3 uterine edema was confirmed (*Gastal* et al. 2006).

Groups of embryo donor mares

- Group DESL, fresh semen (n = 14) received 1 mg of deslorelin acetate (IM) to induce ovulation and were inseminated with fresh semen.
- Group DESL + hCG, fresh semen (n = 14) received 1 mg of deslorelin acetate and 1,000 IU of human chorionic gonadotropin (IV) and were inseminated with fresh semen.
- Group DESL, frozen semen (n = 33) received 1 mg of deslorelin acetate (IM) and were inseminated with frozen semen.
- Group DESL + hCG, frozen semen (n = 38) received 1 mg deslorelin acetate + 1,000 IU of human chorionic gonadotropin (IV) and were inseminated with frozen semen (Figure 1).

Donor mares inseminated with fresh semen

At D0, the reproductive tract of the mares was evaluated by ultrasound examination. Mares with corpus luteum and a follicle < 20 mm in diameter received 5 mg of prostaglandin F2α (Lutalyse, ZoetisTM, Guarulhos, SP) (IM). At D7-D8, females that presented with uterine edema (*Samper* et al. 2002) and ovaries with follicles ≥ 35 mm were induced for ovulation by administration of 1.0 mg deslorelin (Sincrorrelin, Ouro Fino, Cravinhos, São Paulo, Brazil) (IM) or 1,000 IU hCG IV (Vetecor, Hertape Calier/CEVA - Animal Health, Paulinea, São Paulo, Brazil) (IV) according to the experimental group and were inseminated with fresh semen (*Pietrani* et al. 2019).

The semen was collected by an artificial vagina (Botupharma®, Botucatu, Brazil) at 40 °C with the aid of a mannequin or a mare in estrus. The semen quality was evaluated by an optical microscope with a hot plate. A semen droplet was placed between the lamina and coverslip, previously warmed to 37 °C. The total and progressive motility of the sperms was determined based on a percentage scale (0–100%) (*CBRA*, 2013). Fresh semen was diluted in a commercial diluent medium based on skimmed milk (Botu-Semen® Botupharma®, Botucatu, São Paulo, Brazil) at a ratio of 1 part semen to 1 part diluent (1:1). The semen was directly placed in the uterus by a rigid pipette and manually guided via transcervical ultrasonography. The sperm dose for the use of fresh semen was calculated as 800 to 1000×10^6 spermatozoa with progres-

sive movements, after determining the sperm concentration using a Neubauer camera. At D9/D10 (after initiation of the protocol) and 36 hours after the injection of the ovulation inductors, ovarian follicular dynamics were evaluated every 2 hours to monitor the time of ovulation.

Donor mares inseminated with frozen semen

Mares that were artificially inseminated with frozen semen were monitored with ultrasound examinations pre and post ovulation (D9/D10). Ovulation was confirmed when a dominant follicle observed in the last ultrasonographic examination, was no longer detected.

The frozen semen was deposited in the uterine horn, ipsilateral to the ovary, with the preovulatory follicle. Inseminating doses (before and after ovulation) were 800×10^6 sperm (0.5 mL semen); thawing of the semen straws was performed in a water bath at 46 °C for 20 sec, followed by analysis of motility and vigor. At D19, embryo collection and transfer to the recipient was conducted.

Criteria for the use of frozen semen was as follows: ≥ 30% progressive sperm motility; fresh semen ≥ 60% sperm motility. It was mandatory that both types of semen (fresh/frozen) should have a spermatic vigor between 2 and 3 (CBRA 2013). Diagrammatic representation of protocols used for donors, based on the types of semen used (Figure 1).

Embryo recovery in donor mares

Embryo recovery was performed by a catheter (Minitube, Porto Alegre, RS, Brazil), via the transcervical route (*Roser* et al. 2019), following which, it was placed in the uterine body. The catheter balloon was inflated with 40 mL of air and immediately drawn to obtain contact of the balloon with the first cervical ring. After fixation of the catheter in the uterine body, 1000 mL of Ringer's Lactate solution (heated to 37 °C) (Fresenius Kabi - Brazil Ltda, Barueri, SP, Brazil) was infused intrauterinely. Thereafter, the solution was removed from the uterus by gravity. This procedure was repeated 3 times, totaling 3000 mL of uterine lavage (*Camargo* et al. 2020). All the fluid collected from the uterus was passed through a filter (75 µm diameter) to trap the embryo. Around 30 mL of the medium was allowed to remain in the filter. After obtaining the uterine lavage, embryos were sought and evaluated based on the developmental stage and quality. The recovered embryos are required to be in the initial blastocyst, blastocyst, or expanded blastocyst stage. Only excellent and good-quality embryos were transferred to the recipients. The criteria for determining the quality of the embryos was in accordance with the International Manual Embryo Transfer Society Manual (*Manual of the International Embryo Transfer Society*, 1998). After evaluation, the embryos suspended in the BotuEmbryo Holding embryo maintenance medium (Botupharma®, Botucatu, São Paulo, Brazil), were washed and freshly transferred to the recipients. The embryos were deposited directly into the uterine body using an artificial insemination pipette (PROVAR; Provar Produtos Veterinários, São Paulo).

Embryo recipient mares

Embryo recipient mares, aged 5 to 15 years, weighed between 380 and 500 kg and had well developed mammary glands (*Squires* et al. 1999). Recipient mares were treated as per the same protocol for ovulation induction as that of the donors, except for a difference of 2 days for D0, so that they did not ovulate at the same time as the donors. Therefore, the donor mares would lead by 2 days in terms of corpus luteum formation on the day of embryo transfer. The recipients underwent to ultrasound examinations to measure the POF diameters, as well as to check the OT after the administration of the inductor (s). The mares were monitored every 2 hours, after 36 hours following administration of the inductors. The PR with embryos originating from frozen or fresh semen was noted. Pregnancy was confirmed in the recipients 4 days after embryo transfer (= D23).

Figure 2 shows the diagrammatic representation of the protocols used for the recipients.

Statistical analysis

The average POF diameter and OT were compared in donor and recipient females, based on the ovulation inductor

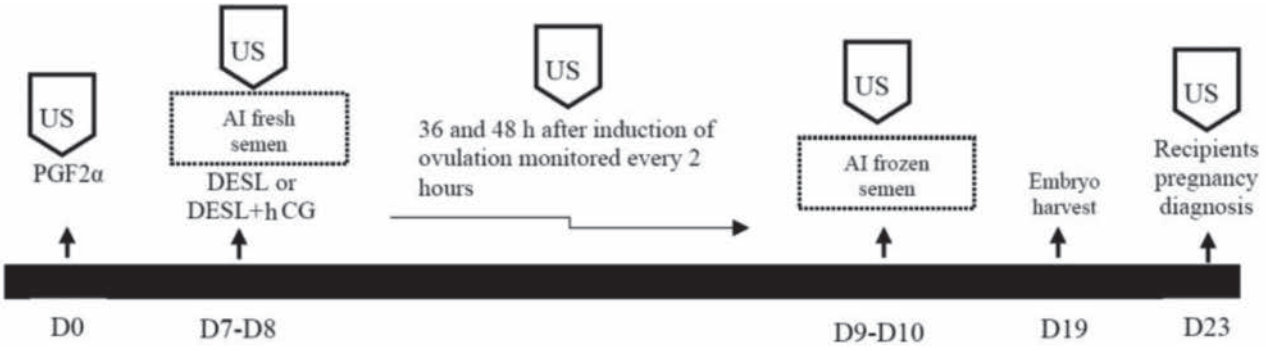

Fig. 1 Protocol diagrarn applied to embryo donors submitted to DESL (n = 14) or DESL + hCG (n = 14) and inseminated with fresh semen; application of DESL (n = 33) or DESL + hCG (n = 38) and inseminated with frozen semen. US (Ultrasound examinations); $PGF_{2\alpha}$ (Lutalyse, Zoetis TM, Guanilhos, Brazil; DESL (Sincrorrelin, Ouro Fino, Cravinhos, Brazil); HCG (Vetecor, Hertape Calier, CEVA-Atüinal Health, Paulinea, Brazil)

(DESL or DESL + hCG) used and the type of semen (fresh or frozen) by ANOVA, using the Tukey test (significance at $P < 0.05$). The chi-square test was used to verify the difference in ERR in donors and PR in recipients, based on the type of semen and ovulation inductor used. The logistic regression of the ERR was performed considering the following variables: POF diameter, OT elapsed after induction of ovulation, age, and weight. The location of the stud farm was evaluated by the chi-square goodness of fit test. Pearson's correlation was used to determine the correlation between POF and OT. Data were tested for normal distribution. The statistical models were adjusted using the Statistical Analysis System (SAS, version 9.1 for Windows; SAS Inst, Cary, NC, USA).

Results

Donors

Of the 168 stratified cycles evaluated, 99 cycles belonged to the embryo donor mares. No difference was observed in the POF diameter and ERR on comparing the ovulation induction protocols or types of semen (fresh or frozen) used. Differences were noted in the OT after administration of the inductor between the groups inseminated with fresh semen ($P = 0.0001$) and those inseminated with frozen semen ($P = 0.0001$) (Table 1). Table 2 presents the ERR based on the ovulation inductor used (DESL vs. DESL + hCG). It was found that the DESL + hCG treatment led to a shorter OT (39.65 h) and less time variation (1.49 h) between the groups ($P = 0.0001$). The ERR in the DESL + hCG groups was 71.15 %, while that in the DESL groups was 68.08 %. No difference was noted in the POF diameters between the groups or in the ERR, when insemination was performed with fresh or frozen semen.

Recipients

A total of 168 estrous cycles were evaluated, amongst which, 69 cycles belonged to embryo recipient mares (Table 3). No difference was observed in the PR after embryo transfer between the groups (DESL vs. DESL + hCG, or insemination with fresh/frozen semen). Further, no difference was noted in the POF diameter between the groups inseminated with fresh or frozen semen. Table 3 indicates that the OT was lower when the DESL + hCG protocol was followed as compared to that when the DESL protocol was followed, after AI with fresh or frozen semen ($P = 0.0001$). Table 4 shows the POF diameter, OT, and PR in the embryo recipient mare groups after administration of DESL or DESL + hCG, disregarding the type of semen (fresh/frozen) used.

The logistic regression test was based on the ERR data and the following variables: POF diameter ($P = 0.8993$), OT ($P = 0.4468$), age ($P = 0.268$), weight ($P = 0.6318$), and the location effect of the animals (latitude 25° 32′ 05″ S, longitude 49° 12′ 23″ W and latitude 22° 44′ 22″ S; longitude 45° 35′ 29″ W) ($P = 0.6375$). No significant differences were observed. The Pearson correlation test did not reveal any correlation between the POF diameters and OT after ovulation induction.

Discussion

One of the objectives of the present study was to determine the number of hours in which ovulation would occur after the detection of the POFs (diameter ≥ 35 mm, by US) following administration of the ovulation inductors. Additionally, the study aimed to determine ERR (donor mares) and PR (embryo recipients) after AI with fresh or frozen semen.

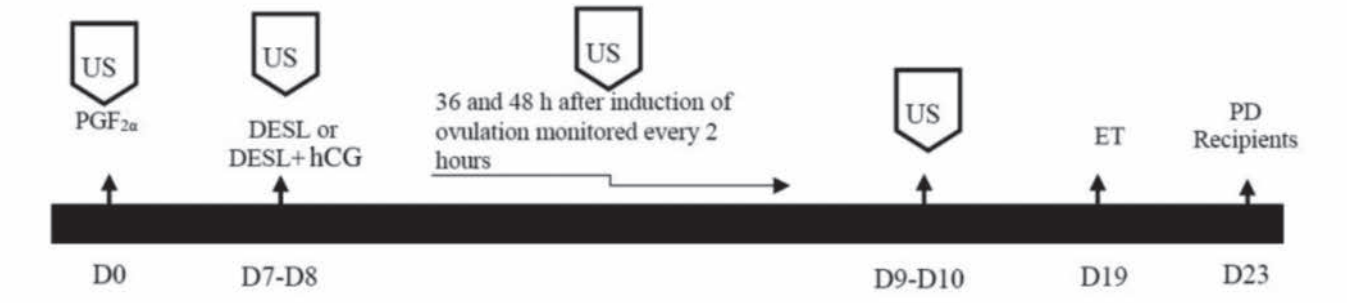

Fig. 2 Diagram of the protocol applied to embryo recipient mares, submitted to DESL (n = 10) or DESL + hCG (n = 09) and transferred with embryos from fresh semen; or to the application of DESL (n = 22) or DESL + h CG (n = 28) and embryos transfer with embryos from frozen semen.

Table 1 Types of semen, preovulatory follicle diameter, ovulation time after deslorelin (DESL) or deslorelin + human chorionic gonadotropin (DESL + hCG) and embryo recovery rate (ERR) in embryos donors mares.

Groups	Semen type (n = 99 cycles)	Ø Preovulatory follicle (x ± s; mm;)	Ovulation time after inductor (x ± s; hours)	Embryo recovery rate % (n)
DESL	Fresh (n = 14)	39.42 ± 1.78	45.14 ± 3.30a	71.42 (10/14)
DESL+hCG	Fresh (n = 14)	39.42 ± 3.87	39.7 ± 2.12b	64.28 (9/14)
DESL	Frozen (n = 33)	39.39 ± 1.98	43.75 ± 3.10a	66.66 (22/33)
DESL+hCG	Frozen (n = 38)	39.23 ± 2.13	39.63 ± 1.21b	73.68 (28/38)

Different letters in the same column indicate significance at the $P < 0.05$ level.

Results of the two protocols did not show significant difference in the POF diameter in donors and recipients. Nonetheless, the POF diameter (mm) of donors and recipients was within the expectations (≥35 mm) of the proposed methodology. Ovulation occurred in 100% of donor and recipient mares within the average time interval of 39.67 hours after administration of DESL + hCG and 44.44 hours after administration of DESL (Tables 1 and 4). *Hemberg* et al. (2006), in a similar study, also obtained 100% ovulation in mares, however, they reported a longer interval time between ovulations (ranging from 36 to 48 hours). After administration of the inductors, the animals were monitored by ultrasonography at 2 hour intervals, until ovulation was confirmed. This monitoring enabled the accurate detection of the ovulation time (in hours) in order to establish a mean and, importantly, a relatively low standard deviation (as observed primarily in the DESL + hCG group).

Ultrasound examinations of POF, performed every 2 hours in the present study, resulted in more precise determination of the OT than that reported by *Meinert* et al. (1993), *Meyers* et al. (1994) and *Mumford* et al. (1995). These authors reported ovulation rates of 83% to 93%, within 48 hours after the administration of DESL or hCG. Indeed, a greater time lapse between induction and ovulation, might impact the reproductive outcome following AI. Moreover, the synchronization of ovulation is an important factor while using frozen semen in AI due to the low viability exhibited by equine sperms once the semen has been thawed. The results of the present study are consistent and will be of use to professionals, as the ovulation time after induction is greatly reduced and can be precisely predicted. Therefore, our protocol offers advantage over previously reported ones, where a longer time lapse between the administration of the inductor and ovulation was noted. Indeed, the identification of the precise moment of ovulation allows for the optimization of semen usage (mainly frozen semen) in AI, considering the low sperm viability (4 to 6 hours only) after it has been thawed (*Ginther* et al. 1972, *Woods* et al. 1990, *Hemberg* et al. 2006).

Interestingly, the OT observed in our study, after administration of the ovulation inducers, differs from that reported by *Farquhar* et al. (2000). They observed that 84.3% of mares had ovulated up to 48 hours after treatment with deslorelin, impacting the reproductive efficiency when frozen semen was used in AI. Reports on the use of DESL + hCG in mares, such as those employed in our study, are scarce. The present study evaluated 2 ovulation induction protocols in order to determine which one of the two would be able to predict the ovulation time more precisely. Our study assessed the protocols not only on the basis of the shorter time taken for ovulation, but also based on better synchronization of ovulation. Therefore, the findings of this study will be of utility for the development of TAI in mares. The combined use of DESL + hCG (as ovulation inductors) is an innovative approach, exhibiting significant reduction in time between induction and ovulation, compared to the conventional protocol, using DESL alone ($P < 0.05$). Moreover, the DESL + hCG protocol introduced in our study, exhibited increased ovulation synchronization, with low standard deviation (1.4 hours) (Table 3); a substantial reduction of up to 5 hours in OT was observed in the DESL + hCG groups (AI with fresh or frozen semen). Indeed, synchronization of ovulation in the donor will greatly impact reproductive outcome, mainly in the view of low sperm viability of frozen semen.

Serum LH levels in mares, induced to ovulate with DESL (alone), were shown to be higher than those of mares induced only with hCG (*Boakari* et al. 2017). hCG has a molecular structure similar to that of luteinizing hormone (LH) and promotes the increase of LH levels, inducing ovulation. In addition, DESL promotes stimulation of the pituitary for LH secretion and FSH production, stimulates final follicle maturation, and promotes ovulation (*Wilson* et al. 1990, *Ishida* et al. 1999, *Gigli* et al. 2006).

In the present study, donor mares (ovulation inducer: DESL) inseminated with fresh or frozen semen had an ERR of 71.42% and 66.66% respectively (Table 1). This ERR is higher than that reported by *Raz* et al. (2009) (36%), who used deslorelin as the ovulation inductor and performed AI with fresh semen. This discrepancy in the results can be attributed to the more frequent (2 hourly) ultrasonographic monitoring of ovulation that was conducted in our study, which resulted in a higher ERR. In fact, this practice enables the prediction of OT with greater accuracy and allows the synchronization of AI. Therefore, it is suggested that the dominant follicle of the mares be closely observed with the help of ultrasonography at short intervals for the improvement of reproductive outcomes.

Table 2 Preovulatory follicle diameter, ovulation time after injection of inductor and embryo recovery rate after application of deslorelin (DESL) or deslorelin + human chorionic gonadotrophin (DESL + hCG) in embryo donor mares.

Groups	Ø Preovulatory follicle (x ± s; mm;)	Ovulation time after inductor (x ± s; hours)	Embryo recovery rate % (n)
DESL (n = 47)	39.40 ± 1.90	44.17 ± 3.19[a]	68.08 (32/47)
DESL+hCG (n = 52)	39.28 ± 2.67	39.65 ± 1.49[b]	71.15 (37/52)

Different letters in the same column indicate significance at a level of $P < 0.05$.

Table 3 Embryos derived from fresh or frozen semen, preovulatory follicle diameter (Ø), ovulation time after inductor application and pregnancy rate in embryo recipient mares treated with deslorelin (DESL) or deslorelin + human chorionic gonadotrophin (DESL + hCG).

Groups	Semen type (n = 69)	Ø Preovulatory follicle (x ± s; mm;)	Ovulation time after inductor (x ± s; hours)	Pregnancy rate after embryo transfer % (n)
DESL	Fresh: 10	39.9 ± 1.57	44.6 ± 2.83[a]	70.00 (7/10)
DESL+hCG	Fresh: 09	39.77 ± 2.23	39.44 ± 1.13[b]	55.55 (5/09)
DESL	Frozen: 22	38.31 ± 2.19	44.81 ± 2.44[a]	59.09 (13/22)
DESL+HCG	Frozen: 28	39.17 ± 2.22	39.60 ± 1.44[b]	60.71 (17/28)

Different letters in the same column indicate significance at a level of $P < 0.05$.

Embryo donor mares (ovulation inductors: DESL + hCG), inseminated with fresh or frozen semen had an ERR of 64.28% and 73.68% respectively (Table 1). *Loomis* and *Squires* (2005) reported an ERR of 48.1% in mares inseminated with frozen semen (pre and post ovulation AI). They reported that ultrasound monitoring of ovulation and insemination 4 to 6 hours after ovulation, significantly increased ERR. In the present study, the ERR using frozen semen (73.68%) was superior to that reported by *Hemberg* et al. (2006) (45.4%), *Reger* et al. (2003) (71.4%) and *Sieme* et al. (2003) (50%), employing HCG in one group and deslorelin and HCG combined, in another. The improvement in ERR may be attributed to the 2 AIs performed (pre and post ovulation AI), as well as to the frequent ultrasound monitoring to confirm ovulation. Moreover, these practices enabled the optimum use of frozen semen, as sperm viability was greater for up to 4 hours post ovulation (*Sieme* et al. 2003). It should be noted that in the present study, the ERR was higher than that reported by *Sieme* et al. (2003) possibly because of the combination of the 2 inductors used and the frequent ultrasonographic monitoring.

No significant difference ($P = 0.921$) was observed in the PR of the recipient mares belonging to the various groups (Table 3): 70.00%, 55.55%, 59.00%, and 60.71% for the DESL (fresh semen), DESL + hCG (fresh semen), DESL (frozen semen), and DESL + hCG (frozen semen) groups, respectively. *Fleury* et al. (2001) reported a PR of 72% by transferring embryos derived from fresh semen, which is close to that obtained in the present study (70.00%). Lower ERR and PR was expected in the groups utilizing frozen semen, because of the damage inflicted on the sperm cells by the freeze-thawing process. However, the 2-hourly ultrasonographic monitoring of ovulation, performed in the present study, allowed AI to be carried out close to the OT, increasing the success rates. This finding was consistent with the reports of *Samper* (2001), *Reger* et al. (2003), *Sieme* et al. (2003), *Loomis* and *Squires* (2005) and *Hemberg* et al. (2006).

In the present study, it was found that both ERR and PR from frozen semen were close to those obtained using fresh semen ($P > 0.05$). PR of the recipient mares induced by DESL (62.50%) was slightly higher than that of the recipient mares induced by DESL + hCG (59.45%). However, the difference was not significant between the groups ($P = 0.79$) (Table 4). The variables that may influence PR in recipients include the transfer method, synchronization of ovulation between donors and recipients, the quality of the embryo and intrauterine environment, and the management of the recipients (*Squires* et al. 1999), among others. Therefore, the hypothesis of the present study may be partially supported by the results obtained (such as ovulation in significantly less time) in the DESL + hCG protocol. This provided greater precision and less time variation (standard deviation of 1.49 hours) (Table 2) that had a positive influence on the PR. This knowledge can aid field professionals in the breeding season to obtain better pregnancy outcomes in mares. Additionally, this information could guide future research directed at timed AI in equines.

Table 4 Preovulatory follicle diameter (Ø), ovulation time and pregnancy rate after administration of deslorelin (DESL) or deslorelin + human chorionic gonadotrophin (DESL + hCG) in embryo recipient mares.

Groups	Ø Preovulatory follicle (x ± s; mm;)	Ovulation time after inductor (x ± s; hours)	Pregnancy rate % (n)
DESL (n = 32)	38.81 ± 2.12	44.75 ± 2.52[a]	62.50 (20/32)
DESL+hCG (n = 37)	39.32 ± 2.21	39.56 ± 1.36[b]	59.45 (22/37)

Different letters in the same column indicate significance at a level of $P < 0.05$.

Conclusions

There was no difference in the embryo recovery rate (donors) and pregnancy rate (recipients) between the two protocols used; the combination of DESL + hCG induced ovulation in significantly less time than DESL alone. In embryo recovery programs and for establishment of equine pregnancy, fresh or frozen semen may be used for AI. The results support the practice of frequent ultrasound examinations by professionals, optimizing the use of frozen semen, and for reduction of animal stress.

Animal welfare statement

The procedures described here were approved by the PUCPR Animal Use Ethics Committee under number 01197.

Conflict of interest statement

none

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