Anim. Reprod., v.1.n.1, p.91-95, Oct./Dec. 2004

Transfer of equine embryos into anovulatory recipients supplemented with short or long acting progesterone

A.N. Rocha Filho[1,3], M.A. Pessôa[2], M.M. Gioso[1], M.A. Alvarenga[1]

[1]Departamento de Reprodução Animal e Radiologia Veterinária, FMVZ, UNESP, Botucatu, SP 18.618-000, Brazil.
[2]MP Reprodução Eqüina, Bauru, SP, Brazil.

Abstract

Pregnancy and early embryonic death rates in cycling and noncycling progesterone-treated recipient mares were examined in a retrospective study. During three breeding seasons, 264 embryos were transferred into either cycling (control, n=152) or noncycling (n=112) mares in a commercial embryo transfer program in Brazil. The anovulatory mares were treated with either 200 mg/day (Treatment 1, n=54) or 400 mg/every other day (Treatment 2, n=13) short acting progesterone, or 1500 mg long acting progesterone every 7 (Treatment 3, n=30) or 6 (Treatment 4, n=15) days. Embryos were transferred non-surgically into cycling recipients 4 to 8 days after ovulation and 5 to 8 days after the beggining of progesterone supplementation into anovulatory mares. Pregnancies were checked on days 12, 25 and 50 by transrectal ultrasonography. Data were analyzed by Chi-square. Pregnancy rates on days 12 (75.0, 75.9, 76.9, 76.6 and 73.3%) and 50 (61.8, 61.1, 61.5, 53.3 and 60.0%) as well as embryonic death rates (17.5, 19.5, 20.0, 30.4 and 18.2%) were similar (P>0.05) for control mares and treatments 1, 2, 3 and 4, respectively, and between control and all anovulatory mares combined. Concerning pregnancy and embryonic death rates in anovulatory recipients, similar (P>0.05) results were obtained after embryo transfers into recipients receiving short or long acting progesterone for 5 to 8 days before transfer, as well as after transfers performed during spring or fall and during winter. Additionally, daily administration of short acting injectable progesterone provided similar (P>0.05) results as administration of a high dose (400 mg) on alternate days. In conclusion, anovulatory mares treated with short or long acting progesterone for 5 to 8 days before transfer can be successfully used as embryo recipients during the period of the year in which few cycling recipients are available.

Keywords: progesterone, mares, anovulatory, embryo transfer, recipients.

Introduction

Utilization of anovulatory mares treated with progestagens as embryo recipients is extremely desirable in commercial embryo transfer programs, especially at the beginning of the breeding season when a limited number of cycling mares is available. Additionally, hormone-treated recipients do not need to be synchronized with donors or teased, requiring less palpation, and are reliably in the optimal stage to receive an embryo (Hinrichs and Kenney, 1987). The use of progestins to prepare ovariectomized mares for receiving embryos have been described. Hinrichs et al. (1985) administered 300 mg progesterone (P4) daily for 5 days before transfer and 3 of 7 mares became pregnant. In another study, ovariectomized mares were assigned to one of the following treatments before receiving an embryo: 1) 22 mg altrenogest for 5 days, po; 2) 66 mg altrenogest for 6 days, po; or 3) 300 mg injectable P4 for 5 days. Intact embryo recipients were synchronized and served as control. Pregnancy rates were 1/6, 2/6, 2/5 and 13/19, respectively (Hinrichs *et al.*, 1986).

On the other hand, McKinnon *et al.* (1988) obtained similar pregnancy rates after transferring embryos into intact mares or into ovariectomized mares administered 300 mg P4. Additionally, transfers into ovariectomized mares receiving 22 mg altrenogest orally resulted in exactly the same pregnancy rate as

[3] Corresponding author: arochavet@hotmail.com
Received: May 26, 2004
Accepted: July 12, 2004

transfers into mares receiving 300 mg injectable P4 (70%, 14/20).

Recently, Carnevale *et al.* (2000) compared cycling recipients (n=590) and transitional recipients given altrenogest for 5 to 7 days before transfer (0.044 mg/kg, po; n=18) and no significant differences (P>0.05) were found in pregnancy and embryonic death rates (67.8 vs. 55.6% and 14.5 vs. 30.0%, respectively). Progestin-treated mares have also been successfully used as oocyte recipients in oocyte transfer and GIFT programs (Carnevale *et al.*,1999; Hinrichs *et al.*, 1999, 2000).

Different progestin preparations are commercially available for administration to mares. McKinnon *et al.* (2000) have compared the ability of different progestagens to maintain pregnancy in mares after induced luteolysis of the primary corpus luteum on day 18 of pregnancy. Medroxyprogesterone, hydroxyprogesterone hexanoate, altrenogest, norgestomet and megestrol acetate were used. Mares given altrenogest remained pregnant whereas all mares given any of the other preparations lost their pregnancies 2 to 8 days after prostaglandin administration. Recently, a long acting progesterone preparation (P4 LA) was given to cycling mares after prostaglandin administration. Injection of 1500 mg every 7 days effectively maintained normal luteal phase levels of P4 in mares with no endogenous source (Bringel *et al.,* 2003).

In the present study, data of 264 embryo transfers performed from late fall to early spring in a commercial program in Brazil, during 2001, 2002 and 2003, were examined. The aim of this study was to compare pregnancy and embryonic death rates among cycling recipients and anovulatory recipients supplemented with short or long acting P4.

Materials and Methods

Animals and experimental groups

From late fall to early spring (April to October) in South America, Quarter Horse and Paint Horse donor mares were monitored by transrectal palpation/ultrasonography every other day. After detection of a 35-mm-folicle, mares were inseminated every 48h until ovulation. Fresh or cooled transported semen from different stallions were used for artificial insemination.

Light-horse mares (n=264) between 5 and 15 year of age and weighing 350 to 600 Kg were used as embryo recipients. The mares were kept in outdoor paddocks and were maintained on grass hay with free access to water and trace-mineralized salt. Mares had no reproductive abnormalities, as indicated by transrectal palpation and ultrasonography. The cycling recipients (n=152) had their ovarian activity monitored the same way as donors and served as control. The anovulatory mares (n=112) were examined at regular intervals and characterized by absence of a corpus luteum and presence of follicles <20 mm in diameter in subsequent examinations.

By the time a donor mare was in estrus, unless there was a synchronized cycling recipient available, an anovulatory recipient was hormonally treated to receive an embryo. After two days of estradiol cypionate administration (10 mg/day, i.m.; ECP; Pharmacia and Upjohn Co., Kalamazoo, Michigan, USA), anovulatory recipients were supplemented with either short or long acting P4 for 5 to 8 days before embryo transfer. Mares receiving short acting P4 (P4; Northside Pharmacy, Lexington, USA) were intramuscularly treated with either 200 mg/day (n=54) or 400 mg every other day (n=13). Recipients given long acting P4 (P4 LA 150; B.E.T. Laboratories, Lexington, USA) received 1500 mg i.m. every 7 (n=30) or 6 (n=15) days. Recipients that became pregnant after transfer were supplemented until day 100 of pregnancy.

Embryo recovery and handling

Embryos were recovered 7 to 9 days after ovulation of the donor mare by nonsurgical uterine lavage using 3 L of warmed lactated Ringer´s solution, as described (Alvarenga *et al.*, 1993). Upon identification, the embryo was washed at least 10 times and held in filtered DPBS plus 0.4% BSA for less than 1 hour.

Embryo transfer

Embryos (n=264) were transferred nonsurgically by a single technician into cycling and noncycling recipients. Briefly, the embryo was placed into a sterile insemination pipette attached to a 3-mL-syringe, between columns of air and medium to minimize movement within the pipette and to assure expulsion of the embryo. Then, the pipette was covered with a sterile plastic sheath.

Before transfer, recipients had their tails wrapped, fecal contents removed and the external genitalia cleaned. The pipette was handled by the technician, using a plastic glove, and gently guided through the cervix of the recipient. The embryo was deposited within the body of the uterus.

Pregnancy diagnosis

Recipients were examined by transrectal ultrasonography on days 12, 25 and 50 of pregnancy. Mares not showing an embryonic vesicle on day 12 were reexamined on day 15. If a vesicle was found, the recipient was regarded as pregnant on day 12.

Statistical analysis

Pregnancy and embryonic death rates were compared using Chi-square analysis. Significance was indicated by a probability of P<0.05.

Results

Pregnancy rates on days 12 and 50 and embryonic death rates were similar (P>0.05) for cyclinand P4-treated recipients, irrespective of the type of progesterone supplementation given (Tab.1). There ere no significant differences (P>0.05) in these end points when data of all P4-treated groups were combined and compared to cycling recipients (Tab.2). Embryonic death rates in the intervals studied (days 12 to 25 and 25 to 50 of pregnancy) for cycling and anovulatory recipients were similar (P>0.05) within each group and between groups (Tab.3). Embryo transfers into anovulatory recipients from 5 to 8 days after the start of P4 supplementation resulted in similar (P>0.05) pregnancy and embryonic death rates (Tab.4). Embryo transfers into anovulatory recipients during spring or fall yielded similar (P>0.05) pregnancy and embryonic death rates compared to those performed in the winter (Tab.5).

Table 1. Pregnancy rates on days 12 and 50 and embryonic death (ED) rates after transfer of equine embryos into cycling and noncycling recipients receiving different progesterone treatments.

Group	Transfers	Pregnant 12d (%)	Pregnant 50d (%)	ED (%)
Cycling	152	114/152 (75.0)	94/152 (61.8)	20/114 (17.5)
P4 200 mg/day	54	41/54 (75.9)	33/54 (61.1)	8/41 (19.5)
P4 400 mg/2 days	13	10/13 (76.9)	08/13 (61.5)	2/10 (20.0)
P4 LA 10 ml/7 days	30	23/30 (76.6)	16/30 (53.3)	7/23 (30.4)
P4 LA 10 ml/6 days	15	11/15 (73.3)	09/15 (60.0)	2/11 (18.2)
Total	264	199/264 (75.4)	160/264 (60.6)	39/199 (19.6)

Results within the same column are not significantly different (P>0.05).

Table 2. Pregnancy rates on days 12 and 50 and embryonic death (ED) rates in cycling and anovulatory progesterone-treated recipient mares after embryo transfer.

Group	Transfers	Pregnant 12d (%)	Pregnant 50d (%)	ED(%)
Cycling	152	114/152 (75.0)	94/152 (61.8)	20/114 (17.5)
P4-treated	112	85/112 (75.9)	66/112 (58.9)	19/85 (22.3)
Total	264	199/264 (75.4)	160/264 (60.6)	39/199 (19.6)

Results within the same column are not significantly different (P>0.05).

Table 3. Embryonic death (ED) rates between days 12 to 25 and 25 to 50 of pregnancy in cycling and anovulatory progesterone-treated recipient mares after embryo transfer.

Group	ED 12-25d (%)	ED 25-50d (%)
Cycling	11/114 (9.6)	09/114 (7.9)
P4-treated	07/85 (8.2)	12/85 (14.1)
Total	18/199 (9.0)	21/199 (10.6)

Results within the same column and within the same row are not significantly different (P>0.05).

Table 4. Pregnancy rates on days 12 and 50 and embryonic death (ED) rates after transfer of equine embryos into anovulatory recipients receiving progesterone for 5 to 8 days before transfer.

Days of P4 treatment	Transfers	Pregnant 12d (%)	Pregnant 50d (%)	ED (%)
5	34	26/34 (76.5)	21/34 (61.7)	5/26 (19.2)
6	33	25/33 (75.7)	21/33 (63.6)	4/25 (16.0)
7	28	19/28 (67.8)	13/28 (46.4)	6/19 (31.5)
8	17	15/17 (88.2)	11/17 (64.7)	4/15 (26.6)
Total	112	85/112 (75.9)	66/112 (58.9)	19/85 (22.3)

Results within the same column are not significantly different (P>0.05)

Table 5. Pregnancy rates on days 12 and 50 and embryonic death (ED) rates in anovulatory progesterone-treated recipient mares after embryo transfer during spring or fall and winter months.

Season	Transfers	Pregnant 12d (%)	Pregnant 50d (%)	ED (%)
Spring/Fall	50	40/50 (80.0)	33/50 (66.0)	7/40 (17.5)
Winter	62	45/62 (72.6)	33/62 (53.2)	12/45 (26.7)
Total	112	85/112 (75.9)	66/112 (58.9)	19/85 (22.3)

Results within the same column are not significantly different (P>0.05).

Discussion

All progesterone treatments evaluated in this study showed to be effective for preparation of anovulatory mares as embryo recipients, as far as the figures yielded by the different treatments were comparable to the control ones. Pregnancy rates after non-surgical embryo transfer usually range between 50 and 75% (Squires *et al*., 1999). The comparison between data combined for all P4-treated mares and cycling mares reinforce the suitability of anovulatory hormone-treated mares as embryo recipients, as previously demonstrated (McKinnon *et* al., 1988; Carnevale *et al.,* 2000).

The same picture was observed when embryonic death rates in the different groups were compared. However, in spite of no significant differences, the percentage of embryonic loss in mares receiving P4 LA every 7 days appeared to be higher. The disparity in the number of animals among groups and the reduced number observed in some of them possibly prevented the occurrence of any significant effect of group. Bringel *et al,*. (2003) reported that circulating progesterone concentrations were compatible to luteal phase levels after injection of 1500 mg P4 LA every 7 days in mares with no endogenous progesterone, suggesting that it would be a reliable regime for anovulatory embryo recipients. However, further studies are necessary to conclude if administration in 6-day intervals is a more suitable protocol. When the combined data of all P4-treated mares were compared to controls no differences on embryo loss were found. The figures of the present study for cycling and noncycling embryo recipients combined are in agreement with the 5 to 24% range in embryo loss rates in fertile mares reported by Ball (1988).

Carnevale *et al.* (2000) compared embryonic death at different intervals after embryo transfer. The percentage tended to be higher (P=0.06) between days 17 and 25 of pregnancy. We have compared embryo loss at two intervals (12 to 25 and 25 to 50 days of pregnancy) in cycling and P4-treated recipients and no significant (P>0.05) differences were found within or between groups.

Previous studies on the administration of progestagens to maintain pregnancy in mares have not focused on the use of long acting injectable P4 preparations. The use of P4 LA is extremely advantageous due to the greater labor and animal stress associated with the daily injections required by the short acting forms. We have also demonstrated that P4 in oil can be administered every other day without altering pregnancy or embryonic death rates in noncycling embryo recipients.

According to the results of the present study, anovulatory mares administered exogenous progesterone might be used as embryo recipients during their transitional and deep anestrus periods, as far as no significant (P>0.05) differences in pregnancy and embryonic death rates were observed after transfers performed in the spring or fall and winter months.

Equine embryos have been transferred into cycling mares within a large range of days after ovulation, depending on the availability of recipients and on the personal preferences of technicians. Data of several breeding seasons in commercial embryo transfer programs in Brazil have been examined and no significant (P>0.05) differences were found in pregnancy rates for recipients that had received embryos from 3 to 8 days after ovulation (Fleury *et al.,* 1989; Jacob *et al.,* 2002). The results of the present study show that hormone-treated recipients allow almost the same flexibility, as far as pregnancy and embryonic death rates were similar (P>0.05) for recipients given P4 for 5 to 8 days before transfer, what is quite interesting due to its practical implications in commercial programs.

In conclusion, anovulatory mares in deep anestrus or transitional phase given short or long acting progesterone for 5 to 8 days before transfer can be successfully used as embryo recipients during the period of the year in which few cycling recipients are available.

References

Alvarenga MA, Landim e Alvarenga FC, Meira C. 1993. Modifications in the technique used to recover equine embryos. *Equine Vet J Suppl,* 15:111-112.

Ball BA. 1988. Embryonic loss in mares: incidence, possible causes and diagnostic considerations. *Vet Clin North Am Equine Pract*, 4:263-290.

Bringel BA, Jacob JCF, Zimmerman M, Alvarenga MA, Douglas RH. 2003. Biorelease progesterone LA 150 and its application to overcome effects of premature luteolysis on progesterone levels in mares. *Rev Bras Reprod Anim*, 27:498-500.

Carnevale EM, Alvarenga MA, Squires EL, Choi YH. 1999. Use of noncycling mares as recipients for oocyte transfer and GIFT. *In*: Annual Conference Society for Theriogenology, 1999, Nashville, TN, USA. *Proceedings*. Nashville, TN, USA. p.44. (abstract).

Carnevale EM, Ramirez RJ, Squires EL, Alvarenga, MA, Vanderwall DK, McCue PM. 2000. Factors affecting pregnancy rates and early embryonic death after equine embryo transfer. *Theriogenology*, 54:965-979.

Fleury JJ, Costa Neto JBF, Alvarenga MA. 1989. Results from an embryo transfer programme with Mangalarga mares in Brazil. *Equine Vet J Suppl*, 8:73-74.

Hinrichs K, Kenney RM. 1987. Effect of timing of progesterone administration on pregnancy rate and embryo transfer in ovariectomized mares. *J Reprod Fertil Suppl*, 35:439-443.

Hinrichs K, Provost PJ, Torello EM. 1999. Birth of a foal after oocyte transfer to a nonovulating, hormone-treated recipient mare. *Theriogenology*, 51:1251-58.

Hinrichs K, Provost PJ, Torello EM. 2000. Treatments resulting in pregnancy in nonovulating, hormone-treated oocyte recipient mares. *Theriogenology*, 54:1285-93.

Hinrichs K, Sertich PL, Kenney RM 1986. Use of altrenogest to prepare ovariectomized mares as embryo transfer recipients. *Theriogenology*, 26:455-460.

Hinrichs K, Sertich PL, Cummings MR, Kenney RM. 1985. Pregnancy in ovariectomized mares achieved by embryo transfer: a preliminary study. *Equine Vet J Suppl*, 3:74-75.

Jacob JCF, Domingues IB, Gastal EL, Gastal MO, Silva AG, Mello CM, Gasparetto F. 2002. The impact of degree of synchrony between donors and recipients in a commercial equine embryo transfer program. *Theriogenology*, 57:545. (abstract).

McKinnon AO, Squires EL, Carnevale EM, Hermenet MJ. 1988. Ovariectomized steroid-treated mares as embryo transfer recipients and as a model to study the role of progestins in pregnancy maintenance. *Theriogenology*, 29:1055-1063.

McKinnon AO, Lescun TB, Walker JH, Vasey JR, Allen WR. 2000. The inability of some synthetic progestagens to maintain pregnancy in the mare. *Equine Vet J*, 32:83-85.

Squires EL, McCue PM, Vanderwall D. 1999. The current status of equine embryo transfer. *Theriogenology*, 51:91-104.