

Ovulation induction for embryo transfer: hCG versus GnRH analogue

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EXPANDED ABSTRACT

INTRODUCTION

During an individual mare's physiological breeding season, she may show variation in the length of oestrus, the interval from onset of oestrus to ovulation and the size of the follicle(s) at ovulation. Modern breeding technologies, such as embryo transfer and artificial insemination (AI) with fresh, chilled and frozen semen, require precise prediction of the time of ovulation. The use of ovulation inducing agents can improve breeding efficiency by reducing the duration of oestrus, increasing the number of ovulations occurring within 48 hours after injection of the drug, decreasing the number of inseminations or matings per oestrus and synchronizing ovulation and insemination. There is a wide range of products on the market to hasten ovulation in cycling mares.

MATERIALS AND METHODS

Data on embryo recovery rates were collected during 3 physiological breeding seasons (March – September, 2003 – 2005) from 63 Thoroughbred and 7 Pony experimental mares aged 2 – 20 ($\bar{x} = 8.9$) years. The mares were treated once during a total of 443 oestrous cycles with one of 4 ovulation inducing agents when they exhibited a dominant ovarian follicle of >35 mm diameter in association with adequate endometrial oedema. Semen was collected by artificial vagina from 2 identical twin Pony stallions of known high fertility. The mares were inseminated once with 500×10^6 progressively motile spermatozoa extended in a skim milk-glucose extender. The ovulation inducing agent; i) crude equine pituitary extract (CPE, INRA, Nouzilly, France, 20mg i.v.); ii) human Chorionic Gonadotrophin (hCG; Chorulon, Intervet, Milton Keynes, Buckinghamshire, 3000 i.u. i.v.); iii) gonadotrophin-releasing hormone (GnRH) analogue, deslorelin acetate (Ovuplant, Peptech Animal Health, Christchurch, New Zealand, 2.1.mg s.c. in vulval lips) and, iv) GnRH analogue, BioRelease Deslorelin (BET Pharm, Lexington, Kentucky, 0.75 or 1.5.mg, i.m.) was administered coincidentally with the insemination. Ovulation was diagnosed by daily ultrasonographic examinations combined with daily bloodsampling for serum progesterone measurements. On day 7 after ovulation the uterus of each mare was flushed twice with 1 – 2l of a commercial flushing medium (Emcare; ICP Ltd, Auckland, New Zealand) and recovered embryos were evaluated microscopically and graded on the basis of their morphology.

RESULTS

Grouping all 4 ovulation inducing drugs together, ovulation occurred within 24h after treatment in 17.5% of cases, between 24 and 48h in 80.4% and >48h in 2.1% of cases. Embryo recovery rates ranged from 15 embryos from 40 attempts (37.5%) in mares treated with CPE, to 26 embryos from 49 flushes (53%) in mares treated with Ovuplant, to 44 embryos from 80 flushes (55%) in mares treated with Chorulon, to 63 embryos from 92 flushes (68.5%) in mares treated with 0.75 mg BioRelease Deslorelin, to 126 embryos from 182 flushes (69%) in mares treated with 1.5 mg BioRelease Deslorelin.

	flushes	embryos	% embryo recovery
CPE	40	15	37.5
Ovuplant	49	26	53
hCG	80	44	55
0.75 mg BioRelease Deslorelin	92	63	68.5
1.5 mg BioRelease Deslorelin	182	126	69.2

Tab. 1: Total embryo recovery

For experimental purposes, when being used as embryo donors or recipients, totals of 47 mares were treated with 1.5 mg of BioRelease Deslorelin in 2004/2005 in 1 – 7 consecutive oestrous cycles for a total of 249 cycles, and 55 mares were treated with 0.75 mg BioRelease Deslorelin in 2005 during 1 - 7 consecutive cycles for a total of 139 cycles.

DISCUSSION

Ovulation inducing agents are necessary for modern breeding programmes. Unfortunately, the drug most often used for this purposes, human Chorionic Gonadotrophin (hCG; Chorulon), can stimulate the formation of anti-hCG antibodies after repeated injections (Duchamp *et al.* 1987). The half-life of such anti-hCG antibodies can range between 30 days to several months after 2 - 4 injections of the hormone (Roser *et al.* 1979) and their crossreaction with endogenous equine LH can reduce the efficiency of treatment and significantly delay or even completely suppress the occurrence of ovulation, especially in older mares. McCue (2004) recommended that hCG should be used once or twice only during a breeding season and that an alternative gonadotrophin, like CPE, should be used instead; it induced ovulation in 78% of mares that had been previously immunized against hCG. Johnson *et al.* (2002) investigated the effects of multiple s.c. implants of deslorelin acetate (Ovuplant) in cycling mares and noted that such treatment caused a prolongation of the interovulatory interval and suppression of follicular size. A single implant caused a consistent suppression of serum LH and FSH concentrations for 10 – 14 days, indicating a degree of „down-regulation“ of pituitary responsiveness to endogenous GnRH, occasionally leading to a complete suppression of ovarian activity for some weeks.

The data collected in the present study show clearly that the lower dose of BioRelease Deslorelin (0.75 mg) is adequate to consistently induce ovulation in Thoroughbred-size mares (500 – 580 kg). Fleury *et al.* (2004) reduced the dose of BioRelease Deslorelin even further to 0.5 mg and noted that mares treated with this

amount, when they exhibited follicle diameters of only 30-35 mm took about 5 h longer to ovulate than mares given same dose of drug when the dominant follicle had reached ≥ 35 mm in diameter. It was surprising to observe a definite reduction in the diameter of the dominant follicle 24h after administration of BioRelease Deslorelin in the present study but this did not prevent normal ovulation within the 24 – 48h interval. Overall, in the mares treated with 0.75 mg BioRelease Deslorelin, ovulation occurred within 24h after treatment in 27.3% of cases, between 24 and 48h in 71.9% of cases and >48h in 0.75% of cases. And in the mares treated with 1.5 mg BioRelease Deslorelin, ovulation occurred within 24h after treatment in 18.9% in cases, between 24 and 48h in 79.1% and >48h in 2.0% of cases.

One day after flushing their uteri, the donor mares were injected with a prostaglandin analogue to induce luteolysis and hasten their return to oestrus. During the past two seasons, no adverse effects of maturation on follicular growth were noted and there was no prolongation of the interovulatory interval in mares treated in several consecutive oestrous cycles with the GnRH analogue, BioRelease Deslorelin. Furthermore, embryo recovery rates suggest that this drug is a possible alternative to hCG, CPE and Ovuplant for induction of ovulation in mares.

REFERENCES

- Duchamp G., Bour B., Combarous Y. and Palmer E. (1987) Alternative solutions to hCG induction of ovulation in the mare. *J. Reprod. Fert.; Suppl.* **35**, 221-228
- Roser Janet F., Kiefer Barbara L., Evans J.W., Neely D.P. and Pacheco C.A. (1979) The development of antibodies to human chorionic gonadotrophin following ist repeated injection in the cyclic mare. *J. Reprod. Fert.; Suppl.* **27**, 173 – 179

- McCue Patrick M., Hudson Jason J., Bruemmer Jason E. and Squires Edward L. (2004) Efficacy of hCG at inducing ovulation: a new look at an old issue. *Proc. Am. Assc. Equ. Pract.* Vol. 50, 510 - 513
- Johnson C.A., McMeen S.L., Thompson Jr. D.L. (2002) Effects of multiple GnRH analogue (deslorelin acetate) implants on cyclic mares. *Theriogenology* 58, 469 – 471
- Fleury P.D.C., Alonso M.A., Alvarenga M.A. and Douglas R.H. (2004) Intervals to ovulation after treatment with oestradiol cypionate (ECP) or BioRelease Deslorelin (BRT-DES). *Equine Embryo Transfer. Havermeyer Foundation Monograph Series No. 14*, 89 Eds: M. Alvarenga and J.F.Wade