Vol.54, n. 3: pp. 517-521, May-June 2011
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

# Induction of Ovulation in Quarter Horse Mares through the Use of Deslorelin Acetate and Human Chorionic Gonadotrophin (hCG)

**Tatiana Figueiredo[1], Roman Paiva[2], Luiz Ernandes Kozicki[3*], Fernanda Kaercher[1], Romildo Romualdo Weiss[4], Ivo Walter dos Santos[5] and Priscilla Ricabone Muradas[6]**

*[1]Pontifícia Universidade Católica do Paraná; São José dos Pinhais - PR - Brasil. [2]Haras LM; Salvador - BA - Brasil. [3]Faculdade de Medicina Veterinária; Pontifícia Universidade Católica do Paraná; Curitiba - PR - Brasil. [4]Departamento de Medicina Veterinária; Universidade Federal do Paraná; Curitiba - PR - Brasil. [5]Departamento de Medicina Veterinária; Universidade Federal do Paraná; Palotina - PR - Brasil. [6]Programa em Processos Biotecnológicos; Universidade Federal do Paraná; Curitiba - PR - Brasil*

## ABSTRACT

*The aim this study was to compare two protocols of induction for ovulation by desloreline acetate and hCG in Quarter Horse mares. The choice of the animals was based on the observations by the estrus, by rectal palpation of the ovaries and by ultrassonography of the follicular dynamics. After estrus detection and follicle control, the measurement of the follicles and the classification of uterus were carried out. The animals that had dominant follicle (diameter more than 35 mm) and swollen uterus were used. In these conditions, the mares received hCG or desloreline acetate. Once ovulation occurred, the artificial insemination was carried. Two groups were performed: G1 (20 animals) received 1.5 mg desloreline acetate and G2 (20 animals) received 1700 IU of hCG. Following 6h intervals, the control follicular was performed by ultrasonography. The follicular average diameter was 42.6 cm for the groups and set up a score of 0 to 3 of uterine edema displayed by the device as well as the time of ovulation. In conclusion, the desloreline acetate showed better performance than hCG, because the ovulation was induced in less time (nine hours than hCG) ($p<0.05$).The pregnancy rate was 80 and 75 %, respectively in G1 and G2.*

**Key words:** ovulation, deslorelin acetate, human chorionic gonadotrophin, quarter horse mares

## INTRODUCTION

The estrous synchronization in the mares has been little used in biotechnology due to the physiological characteristics and peculiarities of the species (Almeida et al., 2001). The knowledge and manipulation of the estrous cycle in equine is very important with the increasing use of the techniques of artificial insemination (AI) and embryo transfer (ET) (Romano et al., 1998). The reproductive fitness is verified by the production of a viable foal (Morel et al., 2009). The horse is a polyestric seasonal species (Mari et al., 2009) and according to Valle et al. (2000), the breeding activity in horses depends heavily on the daily light, which has most significant effects- the farther are the animals from the equatorial line, the reproductive seasonality intensifies. However, even during the breeding season, there are variations in the reproductive activity of the

*Author for correspondence: kozicki.l@pucpr.br

animals, capable of causing changes in the reproductive efficiency. The breeding season of the mares varies by month. The incidence of ovulation is minimal or almost nil during the winter months, increasing during the spring, reaching a peak in the summer and declining again in the fall (Nunes et al., 2005).

Several associations of reproductive hormones (progestin and estrogen), prostaglandin $F_2$ alpha ($PGF_2$alpha) or its analogs, human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH) have been used to monitor the follicular development and ovulation time (Bergfelt et al., 2007). In order to maximize the reproductive performance equine, many protocols of hormonal treatments have been tested in recent years. The changes in the concentration of circulating reproductive hormones during the estrous cycle was first studied by Sullivan et al.(1973) and has been still increasingly described. In parallel, the ultrasound imaging of the reproductive tract in the mares procedure has proved the basis to monitor the reproduction in the animals (Samper, 2008). As a result and in accordance with Gastal (2009), some studies have correlated the follicular diameter with the hormonal concentration during the pre- ovulation period in the mares. There are two models of follicular waves in the mares: the larger waves, characterized by the dominant and subordinate follicles and smaller waves, where the largest follicle does not reach the diameter of a dominant follicle. The follicular waves refer to the first follicles that emerge and grow in the synchrony, developing in the pre-ovulatory waves and several types of follicular waves (Ginther et al., 2004). The ovarian follicle is the fundamental unit of the ovary, which contains the oocyte, once ovulated can be fertilized and can produce an embryo. Furthermore, the follicular unit produces steroids and protein that are needed to maintain the ovarian cycle and secondary sexual characteristics, preparing the endometrium for embryo implantation. After the ovulation, the corpus luteum produces hormones for the stabilization and development of pregnancy (Findlay et al., 2009).

According Ishida et al. (1999) and Gigli et al. (2006), GnRH in response to adequate stimuli, takes the message to the pituitary gland and regulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the horses. This stimulation may be due to melatonin, stimulated by the light, increasing the synthesis of FSH and LH. In the species where the pregnancy is long, melatonin interferes with the hypothalamic-pituitary-gonadal axis by inhibiting the synthesis of these hormones. HCG has been used to induce the ovulation with a view to synchronize it, as its biological activity is similar to LH (Caldas-Bussiere et al., 2005). The dosage to induce the ovulation is between 1500 and 3000 IU of hCG and can be administered intravenously or intramuscularly, when the mare forward follicle with a diameter of 35 to 40 mm. This helps the ovulation within 48 to 56 h (Ley, 2006). When hCG is administered in the mare with follicle with a minimum diameter of 30mm, ovulation could occur within 48 h in 80% cases, which could vary from 12 to 72 h. GnRH has been intensively studied by referring to the control of the estrus cycle in mares (Ley, 2006). The administration of GnRH during the estrus cycle stimulates the release of LH, reducing the duration of estrus (Irvine et al., 1975). The development of agonists and GnRH analogues increase its half life by structural changes in the natural GnRH that induces the release of LH within 12 to 24 h (Bergfelt, 2000). Relatively to the ovulation inductors there is the development of deslorelin acetate, a GnRH analogue. It increases the concentrations of LH and induces ovulation in the mares (McKinnon and Voss, 1993; Squires et al., 1994; Munford et al., 1995). Some studies have compared the effects of deslorelin acetate and hCG, influencing the treatment interval / ovulation in the mares. In this respect, there is conflicting data. McKinnon and Voss (1993), Meinert et al. (1993), Vanderwall et al. (2001) and Melo et al. (2005) obtained a range of 47.5 and 45.1 h, 46.9 and 43.0 h, 52.8 and 52.8 h and 38.9 and 34.7 h, respectively, between the treatment and ovulation when used hCG and deslorelin. Ley (2006) and Samper et al. (2002) compared the use of hCG with deslorelin to check the interval of ovulation in the mares, and obtained 26 to 96 h and 36 to 42 h, respectively. Protocol treatment by deslorelin helps stimulating the secretion of gonadotrophin by the pituitary while the hCG regulates the hypothalamic-pituitary-ovary (Raz et al., 2009).

The aim of this study was to compare the effectiveness of the treatment protocols of hCG and deslorelin acetate in Quarter Horse race mares, based on the time of induced ovulation and pregnancy rates.

## MATERIAL AND METHODS

The study was conducted in 40 Quarter Horse mares, aged between 3 and 17 years, body condition score 4 (ECC - scale 1-5) in Bahia, Brazil, between September 2008 to February 2009. The animals were kept in paddocks of six acres, receiving four kilograms of concentrate (ration of 2,000 kcal and 13% crude protein) divided into two daily meals, one in the morning (5:30 am) and another in the afternoon (5:30 pm) and green forage species Pangola available *ad libitum* as well as water and chelated mineral salt. The choice of the animals was based on the observations of the mares by teasing, signs of heat such as the static behavior of acceptance of the male, blink of vulva and spontaneous urination, also performed with rectal palpation of the ovaries, to monitor the evolution of follicular dynamics and the wave growth. After estrus detection, the DF was measured and the uterine lumen changes were observed. The animals used had DF preovulatory diameter more than 35 mm and swollen uterus.

Selected animals received one dose of hCG or deslorelin, pending ovulation within 36 to 48 h. The mares were divided into two groups (G): G1 (20 animals) received 1.5 mg of deslorelin (IM) and G2 (20 animals) 1700 IU of hCG (IV). Tll the animals were examined for follicular transrectal control with an ultrasound device (transducer of 7.5 MHz linear probe) at intervals of six hours after detection of estrus by the signs described above. In this study, the follicular diameter of 42.6 cm was used for the study groups and established a scoring system for uterine edema visualized by transrectal ultrasound of the uterine horns and uterine body. The score was represented on a scale of 0 to 3, depending on the size and prominence of endometrial folds where 0 corresponded to the absence of ultrasound endometrial edema and fold, 1 corresponded to a little swelling and endometrial fold, 2 fold endometrial edema and not very clear, and 3 the mares that had endometrial edema and fold highlighted. The animals used had uterine scores between 2 to 3. For data analysis, the Student t test and the Chi-Square were used.

## RESULTS

Among the 40 mares submitted to the ovarian follicular ultrasonography control, the animals of G1 (deslorelin acetate) ovulated an average of 36.6 h and G2 (hCG) 45.6 h after the treatment. Both of these values were significantly different ($p<0.05$) for the animals treated with deslorelin (Table 1).

**Table 1 -** The use of deslorelin acetate and human chorionic gonadotropin (hCG) and the interval treatment to ovulation (hours) for Quarter Horse race mares. 2009. (n = 40).

| | **Diameter of dominant follicle at estrus (mm) (x ± s)** | **Interval treatment to ovulation (hours) (x ± s)** | **Pregnancy rate (n)** | **( % )** |
|---|---|---|---|---|
| Group 1 (deslorelin acetate) | 42.6±3.5[a] | 36.6± 7.0[a] | 16/20 | 80.0[a] |
| Group 2 (hCG) | 42.6 ± 3.5[a] | 45.6 ± 14.9[b] | 15/20 | 75.0[a] |

Different letters in same column indicates significance ($p<0.05$).

## DISCUSSION

Table 1 showed the uniformity of the experimental sample on the ovarian follicular diameter between the two groups of treated animals (42.6 mm). In both the groups, the size of follicles ranged from 35to 50mm. The animals bearing follicles from 35 mm were inserted in the treated groups in order to obtain high rates of the ovulation. Cuervo-Arango and Newcombe (2008) reported that an ideal measurement of the diameter of pre-ovulatory follicles in the mares within 24 h prior to ovulation was in the range of 34 to 70 mm, which was an excellent tool to estimate and optimize the time of coverage. It is common in practice to use the hCG when a follicular diameter of the largest follicle of the wave is over 35mm, resulting in the subsequent ovulation in most mares after two days. There is evidence indicating that when the diameter of the dominant follicle (DF) have measured between 31 and 34 mm, ovulation occurs two days after the treatment with hCG, indicating that this does not differ significantly from two days of ovulation to apply to hCG, when the DF is between 35 to 50 mm. In the present work, DF was 35 mm (Bergfelt et al., 2007). The treatment in the mares with dose from 1500 to 5000 IU hCG induced the ovulation of the animals within 48 h, detecting ovulation 45.6 h after the treatment. The interval of application of the

treatment with ovulation in groups 1 and 2 showed that the acetate deslorelin optimized the ovulation within 36.6 h, significantly ($p<0.05$) earlier than the treatment with hCG to render the same effect with 45.6 h. Samper et al. (2002) observed a long time interval between the application and the ovulation with hCG (28 to 96 h) and a short interval of 36 and 42 h by the use of deslorelin, corroborating data from the present study, showing that deslorelin was more effective to establish a short period between the administration and its real effect. The present data showed a range of 45.6 h with the use of hCG and 36.6h with the use of deslorelin corroborating the report of Hemberg et al. (2006) about the best efficiency of deslorelin. In this experiment, in the G2 (hCG) the ovulations occurred between 24 and 48 h after the treatment, extending the range slightly lower and higher reported by Hemberg et al. (2006). Comparing the two treatments of this work, it was concluded that the administration of deslorelin acetate showed better performance than the hCG due to less waiting time between the ovulation induction, with the difference of 9.0 h in favor of group 1, and was more accurate in ovulation, which was very important in artificial insemination. This difference indicated that this protocol was more suitable, providing a better pregnancy rate (while not significant) compared with hCG administration. Although it was not the objective of this study, the results of pregnancy rate indicated that the protocol with the deslorelin acetate was cheaper than that hCG.

## REFERENCES


Almeida, H.B., Viana, W.G., Arruda, R.P., Oliveira, C.A. (2001), Sincronização de estro e dinâmica folicular de éguas Crioulas submetidas a tratamentos com norgestomet, acetato de melengestrol e altrenogest. *Braz. J. Vet. Res.Anim. Sci.*, **38**, 267-272

Bergfelt, D.R, (2000), Estrous synchronization in the mare. In: *Equine Breeding Management and Artificial Insemination*. Philadelphia: Saunders, pp.195-228.

Bergfelt, D.R., Meira,C., Fleury, J.J., Fleury, P.D.C., Dell'aqua, J.A., Adams, G.P. (2007), Ovulation synchronization following commercial application of ultrasound-guided follicle ablation during the estrous cycle in mares. *Anim. Reprod. Sci.*, **68**, 1183-1191.

Caldas-Bussiere, M.C., Garcia, J.M., Barbosa, J.C., Rosa, E., Silva, A.A.M. (2005), Testosterona e gonadotrofina coriônica humana estimulam a esteroidogênese em células da granulosa de folículo pré-ovulatório de égua? *Arq. Bras. Med. Vet. Zoot.* **57**, 62-69.

Cuervo-Arango, J., Newcombe, J.R. (2008), Repeatability of preovulatory follicular diameter and uterine edema patter in two consecutive cycles in the mare and how they are influenced by ovulation inductors. *Anim. Reprod. Sci.*, **69**, 681-687.

Duchamp, G., Bour, B., Combarnous, Y., Palmer, E. (1987), Alternative solutions to hCG induction of ovulation in the mare. *J. Reprod. Fertil.* ( Suppl.), **35**, 221-228.

Findlay., J.K., Ker, J.B., Britt, K., Liew, S.H., Simpson, E.R., Rosario, D., Drummond, A. (2009), Ovarian physiology: follicle development, oocyte and hormone relationships. *Anim. Reprod. Sci.*,**6**, 16-19.

Gastal, E.L. (2009), Recent advances and new concepts on follicle and endocrine dynamics during the equine periovulatory period. *Anim. Reprod. Sci.***, 6**, 144-158.

Gigli, I., Russo, A., Aguero, A. (2006), Consideraciones sobre la dinámica ovárica en equino, bovino y camélidos sudamericanos. *In.Vet.*, **8**,183-204.

Ginther, O.J., Beg, M.A., Gastal, M.O., Gastal, E.L., (2004), Follicle dynamics and selection in mares. *Anim. Reprod. Sci.*,**1**, 45-63.

Hemberg, E., Lundeheim, N., Einarsson, S. (2006), Successful timing of ovulation using deslorelin (Ovuplant) is labour-saving in mares aimed for single AI with frozen semen. *Reprod. Dom. Anim.*,**41**, 535-537.

Irvine, D.S., Downey, B. R., Parker, W. G., Sullivan, J. J. (1975), Duration of oestrus and time of ovulation in mares treated with synthetic GnRH (AY-24,031). *J. Reprod. Fertil.*, **23**, 279-283.

Ishida, N., Okada, M., Sebata, K., Minato, M., Fukui, Y. (1999), Effects of GnRH and hCG treatments for enhancing corpus luteum function to increase lambing rate of ewes artificially inseminated during the non-breeding season. *J. Reprod. Dev.*, **45**, 337-343.

Ley, W.B. (2006), Reprodução em éguas para veterinários de eqüinos. São Paulo, Roca, 205 p.

McKinnon, A.O., Voss, J.L. (1993), The estrous cycles. In: Mckinnon,A.O. *Equine Reproduction*, Malvern: Lea and Fabiger : 114-189.

Mari, G., Morganti, M., Merlo, B., Castagnetti, C., Parmeggiani, F., Govoni, N., Galeati, G., Tamanini, C. (2009), Administration of sulpride or domperidone for advancing the first ovulation in deep anestrous mares. *Anim. Reprod. Sci.*, **71**, 959-965.

Meinert, C., Silva, J.F., Kroetz, I., Klug,E., Trigg, T.E., Hoppen, H.O., Jochle. W. (1993), Advancing the time of ovulation in the mare with a short-term implant releasing the GnRH analogue deslorelin. *Eq. Vet.J.*, **25**, 65-68.

Melo, C.M.; Papa, F.O.; Medeiros, A.S.L.; Dell'aqua JR, J.A; Carmo, M.T; Araújo, G.M; Alvarenga, M.A. (2005), Efeito da Deslorelina e do extrato de pituitária equina na indução da ovulação em éguas. In: XIX REUNIÃO ANUAL DA SBTE, *Ac. Sci. Vet.*, **33**,193.

Morel, M.D., Newcombe, J., Hinchliffe, J. (2009), The relationship between consecutive pregnancies in Thoroughbred mares. Does the location of one pregnancy affect the location of the next, is this affected by mare age and foal heat to conception interval or related to pregnancy success. *Theriogenology*. **71**, 1072-1078.

Munford, E. L., Squires, E. L., Johle, W., Harrison, L. A., Nett, T. M., Trigg, T. E. (1995), Use of deslorelin short-term implants to induce ovulation in cycling mares during three consecutive estrous cycles. *Anim. Reprod. Sci.*,**39**, 129-140.

Nunes, M.M., Gastal, E.L., Gastal, M.O., Rocha Filho, A.N., Mellagi, A.P., GInther, O.J. (2005), Follicle and gonadotropin relationships during the beginning of the anovulatory season in Mares. *Anim. Reprod. Sci.*,**2**, 41-49.

Raz, T., Carley, S., Card., C. (2009), Comparison of the effects of eFSH and deslorelin treatment regimes on ovarian stimulation and embryo production of donor mares in early vernal transition. *Anim. Reprod. Sci.*, **71**, 1358-1366.

Romano, M.A., Mucciolo, R.G., Silva, A.E.D.F. (1998), Biologia reprodutiva de éguas: estudo do ciclo estral e momento de ovulação. *Braz. J. Vet. Res. Anim. Sci.*, **35**, 25-28.

Samper, J. C., Jensen, S., Sergenat, J. (2002), Timing of induction of ovulation in mares treated with ovuplant or chorulon. *J. Eq.Vet. Sci.*, **22**, 320-323.

Samper, J.C. (2008), Induction of estrus and ovulation: Why some mares respond and others do not. *Anim. Reprod. Sci.* **70**, 445-447.

Squires, E. L., Moran, D. M., Farlin, M. E., Jasko, D. J., Keefe, T. J., Meyers, S. A., Figueiredo, E., Mccue, P. M., Jochle, W. (1994), Effect of dose of GnRH analog on ovulation in mares. *Theriogenology*. **41**, 5-9.

Sullivan, J.J., Parker, W.G., Larson, L.L. (1973), Duration of estrus and ovulation time in non-lactating mares given human chorionic gonadotropin during three successive estrous periods. *J. Amer. Vet. Med. Assoc.*, **162**, 895-898.

Valle, G.R., Silva Filho, J.M., Palhares, M.S., Oliveira, H.N., Magnago, L.G., Viana, W.S. (2000), Efeito do bimestre dentro da estação de monta sobre a fertilidade de éguas inseminadas com sêmen diluído, resfriado e transportado. *Arq.Bras. Med. Vet. Zoot*., **52**, 14-20.

Vanderwall, D. K., Juergens, T. D., Woods, G. L. (2001), Reproductive performance of commercial broodmares after induction of ovulation with hCG or OvuplantTM (deslorelin). *J. Eq. Vet. Sci*., **21**, 539-542.

Received: February 19, 2010;
Revised: September 14, 2010;
Accepted: January 27, 2011

Página
Em
Branco