

# Ovulation induction with human chorionic gonadotropin in criollo mares

## *Indução da ovulação com gonadotrofina coriônica humana em éguas Crioulas*

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### Abstract

The effect of age, follicular diameter and month of the breeding season (September to January) on the hCG induction of ovulation was evaluated using 123 Criollo mares. Age varied between two and 24 years and the animals were examined daily by rectal palpation and ultrasonography with a 5 MHz linear transducer. When ovarian follicles reached a diameter of 30 to 35 mm, ovulation was induced with an i.v. injection of 1000 IU (n = 39); 1500 IU (n = 41) or 2000 IU (n = 43) of hCG. The mares were bred the next day and examined daily until ovulation was detected. The percentage of mares ovulating before 24 h of hCG injection was 10.3%, 7.3% and 4.7%; until 48 h after injection 92.3%, 85.3% and 86.0% of the mares treated with 1000, 1500 and 2000 IU of hCG, respectively, ovulated. The month of the breeding season, age of the mares and follicular diameter had no influence on ovulatory response. The three hCG doses used in Criollo mares (P > 0.05) result in the induction of ovulation within 48 h after injection when a pre-ovulatory follicle with a 30 to 35 mm diameter was identified. A single dose of 1000 IU of hCG is efficient to induce ovulation in Criollo mares.

**Keywords:** Criollo. Follicular diameter. Equine. hCG.

### Resumo

O efeito da idade, diâmetro folicular e mês da estação de monta (setembro a janeiro) na indução da ovulação com hCG foi avaliado em 123 éguas Crioulas. A idade das éguas variou entre dois e 24 anos e os animais foram examinados diariamente por palpação retal e ultrassonografia com transdutor linear de 5 MHz. Quando os folículos ovarianos atingiram diâmetro de 30 a 35 milímetros aplicou-se uma injeção intravenosa com 1000 UI (n = 39); 1500 UI (n = 41) ou 2000 UI (n = 43) de hCG. As éguas foram cobertas no dia seguinte e examinadas diariamente até a detecção da ovulação. O percentual de éguas que ovularam antes de 24 h da injeção de hCG foi de 10,3%, 7,3% e 4,7%, até 48h após a injeção foi de 92,3%, 85,3% e 86,0%, nos grupos com 1000, 1500 e 2000 UI de hCG, respectivamente. O mês da estação de monta, a idade das éguas ou o diâmetro folicular não influenciaram a resposta ovulatória. As três doses de hCG utilizadas em éguas Crioulas (P > 0,05) resultaram na indução da ovulação dentro de 48h após a aplicação, quando foi identificado um folículo pré-ovulatório de 30 a 35 mm de diâmetro. Uma única dose de 1000 UI de hCG é eficiente para induzir a ovulação em éguas Crioulas.

**Palavras-chave:** Crioulo. Diâmetro folicular. Equinos. hCG.

In Brazil, the Criollo horse represents the third largest horse breed with more than 200 thousand registered animals. The increasing presence of Criollo horses in competitions has led to growing investments by their owners. However, the reproductive management of these animals needs more investigation. The use of gonadotropin to induce ovulation in mares was first reported by Day<sup>1</sup>. Currently, human chorionic gonadotropin (hCG) is probably the most common hormone used in equine reproduction<sup>2</sup>. This

hormone binds to luteinizing hormone (LH) receptors, inducing maturation and ovulation of the dominant follicle from mares in oestrous<sup>3</sup>.

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According to Webel et al.<sup>4</sup>, ovulation synchronization is more likely when hCG is injected in mares whose follicles have already reached 35 mm of diameter, while the minimum diameter for a follicle to respond successfully to LH stimulation is 33 mm in ponies<sup>5</sup>. There are no standard doses of hCG to induce ovulation, but the most common dose used in mares with follicles larger than 35 mm is 2500 IU of hCG<sup>1,6,7</sup>. Barbacini et al.<sup>8</sup> evaluated the effect of season and age in Warmblood mares on the ovulatory response to hCG s in the northern hemisphere. Ovulation occurred later in mares older than 16 years than in younger ones. Moreover, the ovulation distribution of mares bred in the second half of the breeding season varied significantly in the first 24 hours after hCG induction.

Our study aimed to test the efficacy of different hCG (Intervet Schering-Plough) doses (1000 IU, 1500 IU or 2000 IU) to induce ovulation in Criollo mares, as well as the influence of the mare's age, follicular diameter and month of the reproductive season after hCG injection. This experiment was carried out on a Criollo horse farm (Cabanha do Infinito) in southern Brazil during a breeding season from September of 2007 until January of 2008. Lactating or barren Criollo mares (n = 123), between two and 24 years of age, kept on natural grass pasture combined with white clover, and weighing an average of 415 Kg ( $\pm$  25.4) were used in the study.

After estrous detection, the mares were examined daily by rectal palpation and ultrasonography with a 5 MHz trans-rectal transducer. When the follicles had reached a diameter between 30 and 35 mm, the mares were randomly allocated into one of three treatment groups as follows: each mare received one i.v. injection of 1000 IU (n = 39), 1500 IU (n = 41), or 2000 IU (n = 43) hCG to induce ovulation. The mares were bred the next day with a stallion of proven fertility every 48 hours until ovulation and examined daily until ovulation was detected.

The hCG effect on the ovarian response was evaluated considering age, season and follicle diameter at different treatment doses. The mares were examined daily, and it was possible to observe a higher incidence of ovulations (79.7%; 98/123) between 24 and 48 hours after the hCG injection. Only 7.3% (9/123) mares ovulated between 0 and 24h after hCG injection. After hCG injection of 1000, 1500 or 2000 IU, ovulation occurred within 48h in 92.3% (36/39), 83.7% (35/41) and 86% (37/43) of the mares, respectively, corresponding to 87.8% of the animals used in this study. Table 1 shows no statistical difference among the dosage groups.

In the present study mares were treated with hCG when follicles measured between 30 mm and 35 mm. The average follicle diameter measured  $32.6 \pm 0.34$  mm in the group treated with 1000 IU of hCG,  $32.9 \pm 0.31$  mm in the mares that received 1500 IU, and  $33.4 \pm 0.26$  mm in mares treated with 2000 IU of hCG. Due to the uniform ovulatory response to hCG treatment in all three groups, one can say that if the follicular diameter at induction is between 30 and 35 mm, any of the three doses used give a satisfactory response and the small variation in follicular diameter does not influence the ovarian response. The mares were categorized by age as young (2 - 9 years, n = 71), mature (10 - 16 years, n = 37) and old mares (greater than 16 years, n = 15). Ovulations occurred until 48 hours after hCG injection in 87.4% of the young mares, 84%

Table 1 - Ovulation induction in Criollo mares after use of different doses of human chorionic gonadotropin (hCG), mean and standard deviation of follicular diameter at injection time and mare's age during one breeding season from September/2007 to January/2008 in São Sepé/RS, Brazil located at 30°09'38" South

hCG dose (IU)	Ovulation within 48 h n (%)	Follicular diameter (mm)	Age (years)
1000	36/39 (92.3)	$32.6 \pm 0.34$	$10.4 \pm 0.97$
1500	35/41 (85.3)	$32.9 \pm 0.31$	$8.6 \pm 0.77$
2000	37/43 (86.0)	$33.4 \pm 0.26$	$8.6 \pm 0.82$

P > 0.05

of mature mares and 95% of the old mares. After hCG injection, 2.5% (2 - 9 years), 8% (10 - 16 years) and 5% (> 16 years) of the mares did not ovulate.

The reproductive season was separated into two periods: first (September to October) and second half (November to January). Since mares treated with hCG at the beginning of the breeding season had a similar response to the mares that received hCG at the end of the breeding season. We concluded that

the month of the season where the ovulation induction occurred in this experiment also did not influence the ovulatory response (Table 2). The age of the mares, dosage of hCG (1000 IU to 2000 IU), and month of the breeding season had no influence on the induction of ovulation. These findings indicate that 1000 IU or higher doses of hCG can successfully induce ovulation in Criollo mares of different ages with follicles measuring 30 to 35 mm diameter. In

Table 2 - Monthly ovulation distribution after human chorionic gonadotropin (hCG) injection in Criollo mares during one breeding season from September/2007 to January/2008 in a stud farm located in São Sepé/RS, Brazil at 30°09'38" South

Month	Number of ovulated mares according to time (hours) after hCG injection				Non-ovulated mares n (%)
	0 - 24 n (%)	24 - 48 n (%)	48 - 72 n (%)	> 72 n (%)	
<b>Beginning of the breeding season</b> (September-October)	1/39 (2.56)	32/39 (82.5)	1/39 (2.5)	1/39 (2.5)	4/39 (10.2)
<b>End of the breeding season</b> (November, December and January)	9/84 (26.4)	66/84 (78.5)	5/84 (5.95)	3/84 (3.5)	1/84 (1.1)

P > 0.05

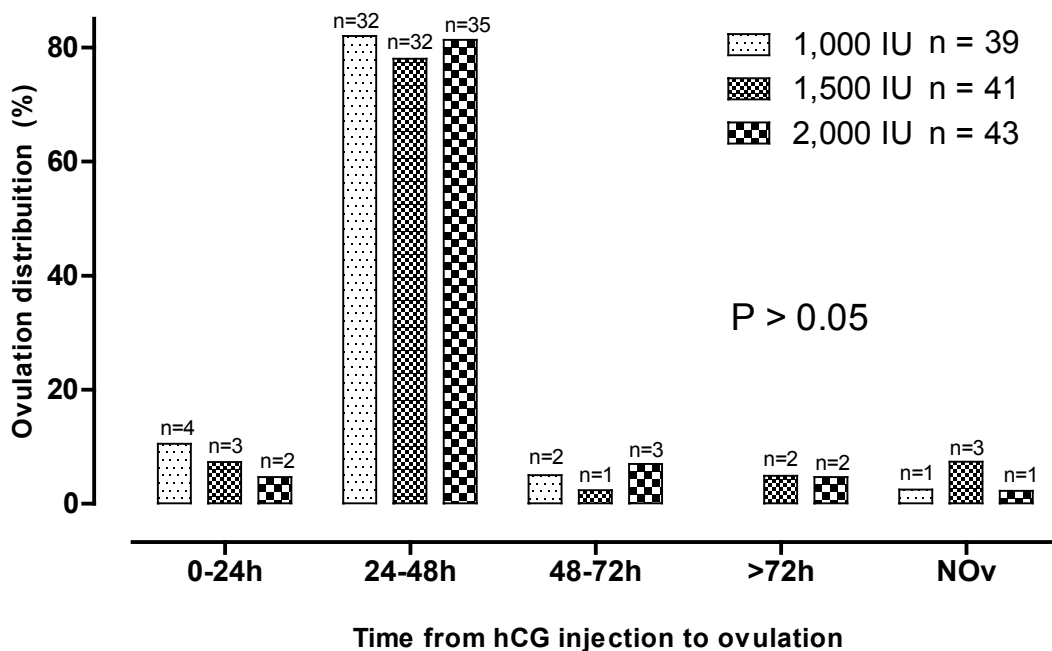


Figure 1 - Distribution (%) of ovulated and non-ovulated Criollo mares at different time intervals after human chorionic gonadotropin (hCG) injection in 123 mares from a stud farm located in São Sepé/RS, Brazil at 30°09'38" South (P > 0.05)

the equine species, studies have been done to evaluate the effect of doses between 500 IU and 6.000 IU of hCG. The ideal dose for ovulation induction in mares has still not been determined and routinely several authors use 2500 IU of hCG<sup>1,7,9,10</sup>.

According to Chavatte and Palmer<sup>7</sup> ovulation induction occurs between 32 and 42 hours after hCG injection. Ovulations that occur before 24 hours are not caused by hCG but are induced by an endogenous peak of LH. In a study carried out on Breton mares<sup>10</sup>, the average time for ovulation occurrence was shorter and ovulation synchrony increased with the injection of 1500 IU and 2500 IU of hCG when compared to mares treated with 500 IU. The results of our study (Figure 1) are similar to those<sup>1, 8,11,12</sup>, where mares ovulated within 48 hours after hCG administration. This is contradictory to previous reports<sup>8</sup> performed between 1994 and 1999 on Warmblood mares and in the 1996 to 2002 breeding seasons on Quarter Horse, Paint and Arabian mares<sup>2</sup>.

Our results indicate that factors such as the longevity of age can favor the response in Criollo mares, which did not happen with the mares of the breeds used by the above mentioned authors. In these studies, the older mares (> 16 years) did not respond adequately to hCG and showed a higher ovulation percentage after 48 hours of treatment, when compared to the younger mares. According to McCue et al.<sup>12</sup> it is possible that this occurred due to the repeated use of the hormone in the recent past. In our study, ovulation induction seemed to be unaffected by anti-hCG antibody production. This could be related to the single use of hCG in each mare during the reproductive season. Voss et al.<sup>6</sup> cited that anti-hCG antibody production can occur after repeated hCG administration in two to five cycles during a breeding season.

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