

# Acupuncture-mediated ovulation induction in mares: Evaluating the efficacy of subdose of histrelin at *Bai-hui* and Governing Vessel-1 acupoints

*Indução da ovulação mediada por acupuntura em éguas: Avaliação da eficácia da subdose de histrelin nos acupontos *Bai-hui* e *Vaso Governador-1**

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**Received:** 2025 May 19 | **Accepted:** 2025 July 9

DOI: <http://dx.doi.org/10.7213/acad.2025.23302>

Rev. Acad. Ciênc. Anim. 2025;23:e23302

## Abstract

Pharmacopuncture has been established for estrus induction in mares using hormonal subdoses, but studies for ovulation induction are scarce. This study aimed to assess the impact of acupuncture on ovulation in

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mares, utilizing subdoses of histrelin at *Bai-hui* (BH) and Governing Vessel-1 (GV-1) acupoints. Three groups were designated for ovulation induction: BH ( $n = 17$ ) and GV1 ( $n = 19$ ) received 75 µg histrelin at the acupoints, while control group (CG,  $n = 38$ ) received 250 µg intramuscularly; histrelin was administered when follicles reached 35 mm. The ovulation rate within 48 hours was investigated over two estrus cycles. Data were analyzed using one-way ANOVA test with significance set at  $p < 0.05$ . Initially, ovulation rates within 48 hours were 80.6%, 88.2%, and 73.7% for CG, BH, and GV1, respectively, with no significant difference among the groups ( $p > 0.05$ ). In the second estrus cycle, ovulation rates within 48 hours were 80.6%, 90.9%, and 91.7% for CG, BH, and GV1, respectively. In conclusion, this study demonstrates that a subdose of histrelin at 75 µg is sufficient to induce ovulation in mares when administered at the *Bai-hui* or GV-1 acupoints.

**Keywords:** Acupuncture. Animal reproduction. Equine. Horse.

## Resumo

A farmacopuntura foi estabelecida para indução de estro em éguas usando subdoses hormonais, mas estudos para indução de ovulação são escassos. Este estudo teve como objetivo avaliar o impacto da acupuntura na ovulação em éguas, utilizando subdoses de histrelin nos pontos de acupuntura *Bai-hui* (BH) e *Vaso Governador-1* (GV-1). Três grupos foram designados para indução de ovulação: BH ( $n = 17$ ) e GV1 ( $n = 19$ ) receberam 75 µg de histrelin nos pontos de acupuntura,

enquanto o grupo controle (GC,  $n = 38$ ) recebeu 250 µg intramuscularmente; a histrelina foi administrada quando os folículos atingiram 35 mm. A taxa de ovulação em 48 horas foi investigada ao longo de dois ciclos de estro. Os dados foram analisados usando o teste ANOVA unidirecional com significância definida em  $p < 0,05$ . Inicialmente, as taxas de ovulação em 48 horas foram de 80,6%, 88,2% e 73,7% para GC, BH e GV1, respectivamente, sem diferença significativa entre os grupos ( $p > 0,05$ ). No segundo ciclo estral, as taxas de ovulação em 48 horas foram de 80,6%, 90,9% e 91,7% para GC, BH e GV1, respectivamente. Em conclusão, este estudo demonstra que uma subdose de histrelina de 75 µg é suficiente para induzir a ovulação em éguas quando administrada nos acupontos Bai-hui ou GV-1.

**Palavras-chave:** Acupuntura. Reprodução animal. Equino. Cavalo.

## Introduction

Pharmacopuncture, a technique involving the injection of substances at specific acupoints to enhance their effects using subdoses, has gained attention in animal studies. It is suggested that pharmacopuncture not only helps maintain drug effectiveness but also reduces the occurrence of side effects and lowers treatment costs (Alvarenga et al., 1998). For instance, administering a subdose of acepromazine (0.01 mg/kg) at the Governing Vessel-1 (GV-1) acupoint induced sedation comparable to the conventional dose (0.1 mg/kg) without causing respiratory depression, as demonstrated in horses (Luna et al., 2008). Similarly, using just 1/10th of the standard dose of flunixin meglumine effectively provided analgesia following horse castration (Reginato et al., 2020).

For the treatment of reproductive dysfunctions, several key acupoints are commonly indicated, including *Bai-hui*, Bladder 23 (BL-23), *Guan Yuan Yu*, GV-1, *Hou San Li*, *Ming Men*, *Shen Shu*, *Shen Peng*, *Tian Ping*, *Wei Gen*, *Yan-chi*, *Yang Guan*, and *Yao Qian* (Shmalberg and Xie, 2009; Zuo et al., 2016). Among these, the most extensively studied in production animals are *Bai-hui*, GV-1, and *Shen Peng* (Nie et al., 2001). In mares, a luteolytic effect has been reported following administration of a subdose of 0.5 mg of

PGF2 $\alpha$  – representing only 10% of the conventional dose – at the *Bai-hui* acupoint (Alvarenga et al., 1998).

*Bai-hui* is a frequently used acupoint located at the dorsal midline, over the lumbosacral space, in close anatomical proximity to the reproductive organs. While it is commonly indicated for conditions affecting the back and hind limbs (Xie and Yamagiwa, 2007), its therapeutic potential in reproductive disorders has also been demonstrated (Alvarenga et al., 1998).

While anatomically advantageous for practical field implementation, studies on hormonal protocols involving the use of GV-1 acupoint to control the estrous cycle are limited. Results demonstrated that the use of a subdose of human chorionic gonadotropin (hCG) administered at the GV-1 acupoint efficiently induced ovulation in mares (Ribeiro et al., 2020). Histrelin acetate, a gonadotropin-releasing hormone (GnRH) analog, has been reported to efficiently induce ovulation in jennies, similar to hCG, in less than 48 hours, while 100% of the control subjects receiving a saline injection ovulated in more than 48 hours (Ribeiro et al., 2020). However, studies using a subdose of histrelin acetate in acupoints, specifically in mares, are not described.

The present study formulates the hypothesis that subdoses of 30% of the standard dose of histrelin acetate at the acupoints *Bai-hui* and GV-1 is effective for inducing ovulation in mares.

## Material and methods

This cohort crossover study was approved by the Committee on Ethics in the Use of Animals of Pontifical Catholic University of Paraná (PUCPR) in 2021, under number 02053, and conducted at the Experimental Farm of the PUCPR, located in the municipality of Fazenda Rio Grande, Paraná, Brazil.

A group of 19 mixed-breed mares, aged 5 to 12 years, from a teaching herd were included in the experimental group, and historical information from 38 mares enrolled in a previous study was utilized here as a positive control. All the mares were managed extensively in paddocks, receiving a daily ration of 12.5 kg of Tifton hay, 1.0 kg of a commercial concentrated feed, and had access to water *ad libitum*. To be eligible for inclusion in the study, mares needed to meet specific criteria, including having

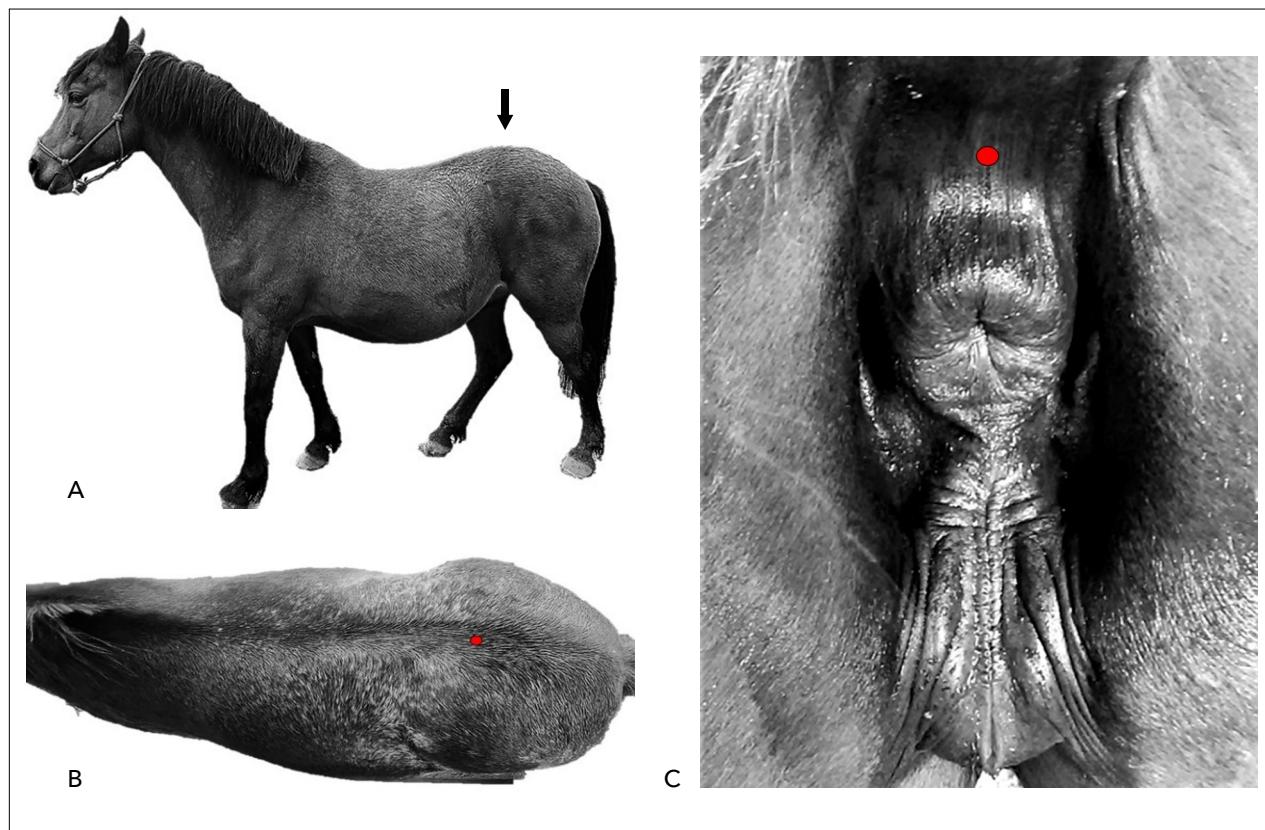
a *corpus luteum* and negative uterine cytology (indicating the absence of polymorphonuclear cells) at the beginning of the study.

The study commenced with the monitoring of ovarian follicular dynamics using a 5.0 MHz linear transducer (A5V, SonoScape®, China). Upon confirmation of the first (natural) ovulation within five days, the animals were randomly divided into two groups: *Bai-hui* (BH) and GV-1 (GV1), which received 6.71 mg of PGF2 $\alpha$  (Lutalyse®; Zoetis, São Paulo, Brazil) intramuscularly. Mares meeting eligibility criteria, including the presence of follicles with a diameter of  $\geq 35$  mm and grade 3 uterine edema (Rasmussen et al., 2015), were considered for induction. Once the follicles reached a minimum diameter of 35 mm, 75  $\mu$ g of histrelin was administered at *Bai-hui* (located on the dorsal midline at the lumbosacral space, in the depression between the spinous processes of the last lumbar and the first sacral vertebrae) or GV-1

(in the depression between the ventral base of the tail and the dorsal boundary of the anus) (Figure 1) (Xie and Yamagiwa, 2007). Before any interventions, acupuncture sites underwent antisepsis using 70% alcohol. Then a 0.8 x 40 mm (21G x 1 1/2) hypodermic needle was inserted into the designated acupoint to a depth of approximately 3 cm, and subsequently, a 1 mL syringe was attached to deliver the hormonal subdose.

Ovulations within less than 48 hours were investigated over two estrous cycles in a standard 2 x 2 crossover design. Mares in the control group (CG) received 250  $\mu$ g of histrelin intramuscularly.

The mean among groups was compared using the one-way ANOVA test followed by the Tukey test of multiple comparisons, considering a significance level of  $p < 0.05$ . The GraphPad Prism program, version 8.0.2 (GraphPad Prism, San Diego, California, USA), was used.



**Figure 1** - Localization of the acupoints *Bai-hui* (located on the dorsal midline at the lumbosacral space, in the depression between the spinous processes of the last lumbar and the first sacral vertebrae) in the lateral view (A, arrow) and dorsal view (B, red dot), and Governing Vessel-1 (in the depression between the ventral base of the tail and the dorsal boundary of the anus) (C, red dot), in a mare.

## Results and discussion

Data related to the assessment of ovulation induction are summarized in Table 1, showing no statistical difference in the comparisons among groups ( $p > 0.999$ ).

In the present study, the induction of ovulation in crossbred mares using a subdose of histrelin administration at acupoints *Bai-hui* and GV-1 was found to be effective, marking the first-time reporting of such results. The use of subdoses implies lower costs as well as fewer undesirable pharmacologic effects, a facet not explored in the present research and recommended for further investigation to comprehensively understand its implications.

The choice to use the acupoints *Bai-hui* and GV-1 is based on their effects, proximity to the reproductive system, and easy accessibility. The GV-1 acupoint can be readily assessed by the veterinarian working in reproduction due to its localization in the perineal region. According to Traditional Chinese Medicine (TCM), *Bai-hui*, also known as 'hundred meetings,' is selected for its various effects over the lumbar region, including the reproductive tract. The GV-1 acupoint, as the first point of the Governing Vessel, continues over the dorsal midline and is related to the nervous system. It also serves as a connecting point to the Kidney meridian, which plays a significant role in fertility according to TCM (Xie and Yamagiwa, 2007).

**Table 1** - Evaluation of ovulation induction in mares receiving 250 µg of histrelin intramuscular for the control group (CG,  $n = 38$ ) and 75 µg in the acupoints *Bai-hui* (BH,  $n = 17$ ) and Governing Vessel-1 (GV1,  $n = 19$ )

Groups	First ovulation induction			Second ovulation induction		
	TO	Oval.	SDF	TO	Oval.	SDF
CG	43.8 ± 9.1	32/38	36.9 ± 3.0	43.8 ± 9.1	32/38	36.9 ± 3.0
BH	45.7 ± 10.0	15/17	36.9 ± 2.0	46.0 ± 7.6	10/11	37.9 ± 2.6
GV1	46.0 ± 17.6	14/19	37.4 ± 2.1	44.7 ± 7.8	11/12	37.2 ± 2.6

Note: TO = time to ovulation in hours for the first estrus cycle (mean ± standard deviation); Oval. = ovulation in < 48h (number/total); SDF = size of the dominant follicles (mm ± standard deviation).

The measurement of the diameter of the dominant follicle in millimeters before the administration of histrelin aimed to establish a baseline among the groups, ensuring that the results were not influenced. This approach aligns with findings from a previous study (Carnevale et al., 2005), and the establishment of a baseline was crucial for accurately assessing the impact of histrelin administration on ovulation, contributing to the robustness of the study's findings.

The induction of ovulation using hormonal subdoses at acupoints aimed to achieve ovulation in each experimental group within 48 hours. The results obtained in this study are consistent with the findings of Phaneuf et al. (1994), who used GnRH analogs, such as histrelin. The use of these inducing agents facilitates the detection of ovulation by re-

ducing the time the mare remains in estrus. Both deslorelin and histrelin have been observed to induce ovulation within 40 to 44h when administered the full dose intramuscularly (Alonso et al., 2016).

This study presents some limitations and suggestions. Initially, saline could have been injected into the acupoints, and dry needles could've been applied to test the sole effects of the acupoints without hormonal influence. Further investigation into the positive effects of subdoses of hormones to induce estrus and ovulation is necessary, and future studies should aim to demonstrate the reduction of costs as well as welfare indicators. Additionally, other acupoints, such as *Yan-chi*, *BL-23*, *Guan Yuan Yu*, *Ming Men*, *Shen Shu*, and *Shen Peng*, should be tested for similar investigations with indications for reproductive effects.

## Conclusion

In conclusion, the present study demonstrates that one third the dose of histrelin, injected in the acupoints *Bai-hui* or *GV-1*, can be applied to induce ovulation in mares.

## Acknowledgments

This work was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) -Finance code 001.

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