

Does hCG administration increase the incidence of double and multiple ovulations in Campolina mares with co-dominant follicles?

A administração de hCG incrementa a incidência de ovulações duplas e múltiplas em éguas da raça Campolina apresentando folículos codominantes?

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Resumo

A gonadotrofina coriônica humana (hCG) é rotineiramente empregada na indução de ovulação em éguas. A incidência de duplas ovulações é variável, sendo comum em determinadas raças brasileiras, como Brasileiro de Hipismo e Campolina. O objetivo deste trabalho foi avaliar se a administração de hCG em éguas da raça Campolina incrementa a incidência de ovulações duplas e múltiplas. Através de palpação retal e ultrassonografia, foram avaliados dois ciclos estrais de 104 éguas, divididas em dois grupos. À detecção de um ou mais folículos ≥ 35 mm e edema endometrial grau 2, 2.500 IU de hCG (IV) eram administrados às éguas do grupo I. O grupo II serviu como controle. Os resultados obtidos foram analisados pelo Qui-quadrado. A incidência de ovulações duplas nos grupos I e II foi de, respectivamente, 32,7% (34/104) *versus* 30,8% (32/104). Ambos os grupos obtiveram incidência semelhante de ovulações triplas (1,9% - 2/104). Apenas uma ovulação quádrupla (1,0% - 1/104) foi detectada, no grupo controle. A incidência de duplas e múltiplas ovulações foi similar entre os grupos ($p > 0,05$). Pelo fato das éguas da raça Campolina apresentarem, fisiologicamente, considerável incidência de ovulações duplas e múltiplas, é possível que a administração de hCG não tenha influenciado estes padrões ovulatórios.

Palavras-chave: hCG, dupla ovulação, ovulação simétrica, Campolina, égua.

Abstract

The human chorionic gonadotrophin (hCG) has been routinely used in the equine species to induce ovulations for more than four decades. The incidence of multiple ovulations in horses is variable, being double ovulations a common occurrence in some Brazilian breeds, such as Brasileiro de Hipismo and Campolina. The aim of this work was to evaluate the role of hCG administration to increase, in Campolina mares, the incidence of double and multiple ovulations, which are desirable in embryo transfer programs. One hundred and four (104) mares had their estrous cycles recorded through rectal palpation and ultrasonographic evaluation during the breeding season of 2003 - 2004, in a total of two hundred and eight (208) cycles (two cycles per mare). Animals were allocated in two groups, with fifty two (52) horses each. In Group I mares, once one or more = 35 mm follicles and grade 2 uterine edema were present, 2.500 IU of hCG were intravenously administered. The untreated mares in Group II served as control. The Chi-Square test was used to analyze obtained results. The incidence of double ovulations in groups I and II was, respectively, of 32.7% (34/104) versus 30.8% (32/104). The incidence of triple ovulations was low in both groups, of 1.9% (2/104). Only once in the control group (1.0% - 1/104) was a quadruple ovulation detected. There was no difference in the incidence of double and multiple ovulations between both groups ($p > 0.05$). Possibly, hCG administration did not influence ovulation patterns in this work due to the fact that Campolina breed mares physiologically show a considerable incidence of double and multiple ovulations.

Keywords: hCG, double ovulation, symmetric ovulation, Campolina, mare.

Introduction

The human chorionic gonadotrophin (hCG) has been used in the equine species for induction of ovulations for more than four decades (Loy and Hughes, 1966). Due to its linking capacity to the receptors of the luteotrophic hormone, hCG is able to promote a similar action on follicular and oocyte

maturation, as well as on the ovulation process (Aurich, 2005). Clinically, 1500 to 3000 I.U. of hCG are used to induce a timed ovulation between 24 and 48 hours after it's administration in a majority of mares. Mares are classified as monovulatory animals; however, the incidence of multiple ovulations is variable, depending on breed, family line (Ginther et al., 1982), reproductive status (Ginther, 1983) and age (Davies Morel

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and O'Sullivan, 2001; Davies Morel et al., 2005). Double ovulations are a common occurrence, having a reported incidence that ranges from 4% to 44% of the evaluated cycles (Daels and Hughes, 1993). However, if fertilization occurs, multiple ovulations can result in more than one fetus, which can not be supported inside the mare's uterus (Ginther and Griffin, 1994). Some Brazilian horse breeds, such as Brasileiro de Hipismo and Campolina, have been reported to have high incidences of double ovulations, of, respectively, 43% (Carmo et al., 2004) and 28.6% (Greco et al., 2005). According to literature, the use of hCG in mares can lead to higher incidences of twin pregnancies (Veronesi et al., 2003) and double ovulations (Aurich, 2005), being the later desirable in embryo transfer programs, for they enhance embryo recovery rates (Greco et al., 2005). The aim of the present study was to evaluate the role of hCG administration in increasing the incidence of double ovulations in Campolina breed mares, as for the other ovulation patterns and the overall rate of multiple ovulations.

Materials and methods

One hundred and four (104) Campolina breed mares, which were donors in a commercial embryo transfer program, had their estrous cycles recorded through daily rectal palpation and ultrasonographic evaluation during the breeding season of 2003 - 2004, in a total of 208 cycles resulting in ovulation (two cycles per mare). A subsequent estrous cycle was analyzed only when the mare failed to ovulate. The evaluations began when the first signs of estrous behavior were detected or, in those mares which were already in the embryo transfer process, four days after an uterine flush was performed. Mares were monitored 24 hours after the first ovulation of the cycle was recorded, in order to detect possible synchronic ovulations. Animals were randomly assigned in two different groups, with 52 animals each. Once one or more ≥ 35 mm follicles and grade 2 uterine edema (Gastal et al., 1998) were present, group I mares received 2.500 IU of hCG (VETECOR®) intravenously. The untreated mares in group II served as control animals. Group I was composed of mares aging 6-18 years, average 12 years, while group II mares aged 5-19 years, average 11 years. The ovulatory patterns of every mare were recorded in only two estrous cycles, for the efficacy of hCG is presumably reduced when administered more than twice during a single breeding season (McCue et al., 2003). Obtained results were analyzed through the Chi-Square test.

Results

In group I, 79.8% (83/104) of the cycles had their first ovulation occurring between 24 and 48 hours after the hCG administration, and 11.5% (12/104) resulted in ovulations within the first 24 hours. Only 4.6% (10/218) of all estrous cycles evaluated in both groups did not result in an ovulation, being the incidence of anovulatory follicles formed equally low in both groups (4.6%). The incidence of the ovulatory patterns in groups I and II was respectively of: 32.7% (34/104) versus 30.8% (32/104) of double ovulations and 65.4% (68/104) versus 66.3% (69/104) of single ovulations. The incidence of triple ovulations was equally low in both groups, of 1.9% (2/104). Only once (1.0% - 1/104), in group II, was a quadruple ovulation detected. The incidence of multiple

ovulations in group I was 34.6%, against 33.7% in group II. The incidence of double, triple and quadruple ovulations between groups I and II did not differ, as for the overall incidence of multiple ovulations ($p > 0.05$). In both groups, although not statistically relevant, most single ovulations occurred on the left ovary: 51.5% (35/68) in group I and 52.2% (36/69) in group II, against 48.5% (33/68) and 47.8% (33/69) on the right ovary, respectively. In the thirty-four (34) group I cycles in which a double ovulation occurred, 52.9% (18/34) were unilateral double ovulations, and 47.1% (16/34) bilateral double ovulations. As for the thirty-two (32) group II cycles, the incidence of unilateral and bilateral double ovulations was 50.0% (16/32). The incidence of unilateral and bilateral double ovulations was similar ($p > 0.05$) when separately analyzed in both groups, as for the incidence of unilateral and bilateral double ovulations between mares who received hCG (group I) and control animals (group II).

Discussion and conclusion

The overall percentage of group I mares in which ovulation was induced between 24 and 48 hours (79.8%) in this study was similar to that reported by other investigators (Barbacini et al., 2000), as for the percentage of mares induced to ovulate within 48 hours (91.3%). In both studies, hCG administration was highly effective in inducing and synchronizing ovulations in the equine species. Although only 218 cycles were analyzed, the equally low incidence of anovulatory follicles in both groups (4.6%) indicates that the administration of hCG, when the follicle is already measuring 35mm, is probably not able to prevent the occurrence of these structures. Acosta et al. (2004) could distinguish between future anovulatory and ovulatory follicles using color Doppler ultrasound technology, demonstrating that these anovulatory structures develop in a different way before they reach 35mm. In order to sustain such hypothesis, a higher number of cycles should have been evaluated. However, in the present study, only two estrous cycles were recorded per mare. This was due to the fact that the efficacy of hCG is presumably reduced when administered more than twice during a single breeding season (Sullivan et al., 1973; McCue et al., 2003). Moreover, we were unable to analyze the repeatability of the ovulation patterns within these mares.

In this study, hCG administration was not able to lead to higher incidences of double, triple and quadruple ovulations ($p > 0.05$), as for the overall incidence of multiple ovulations. This does not agree with previously published data by Veronesi et al. (2003). Probably, the results obtained from their work, in which the use of hCG in Thoroughbred mares before mating resulted in higher incidences of twin pregnancies, can be due to the fact that the ovulations, which would be presumably synchronized, could lead to higher fertilizations rates. These rates could have been also influenced by concomitant factors, such as mare breed, age and nutrition. Although the following data was not analyzed nor published, recorded cycles from groups I and II had similar embryo recovery rates (81.7% against 76.9%). These were considerably high, possibly due to the frequent incidence of multiple ovulations and the use of fresh semen from fertile stallions. Nonetheless, we assume that the incidence of multiple ovulations in the present work was not enhanced by the use of hCG because Campolina breed mares, as detected through the examination of group

II, have a considerable physiological incidence of synchronic multiple ovulations (33.7%).

Based on the results obtained in the present study, we can conclude that the Campolina breed has a high incidence of synchronic double ovulations (30.8% obtained in the control group), since it averages around 16.0% in horses (Blanchard et al., 2003). This is in agreement with previously published data (Greco et al., 2005), in which Campolina mares had a 28.6% chance of having a synchronic double ovulation in their estrous cycle. Obtained incidence of double, triple and quadruple ovulations could have been higher in both groups if evaluations persisted for more than 24 hours after the first ovulation was detected. This was not analyzed, though, for double ovulations are particularly useful only when the mare is not to become pregnant and is an embryo donor. In this case, asynchronous ovulations less often result in more than one embryo being recovered after a uterine flush. The high incidence of recorded multiple ovulations (34.6% and 33.7% in groups I and II, respectively) can be, partly, due to the age of studied mares (average 12 and 11 years). According to Davies Morel and O'Sullivan (2001) and Davies Morel et al. (2005), there is a consistent association between age and incidence of multiple ovulations. In the latter study, mares whose age ranged from 11-13 years showed a multiple ovulation incidence of 32.5%, when compared to animals 2-4 years old (20.7%). The explanation to this fact still remains unclear. A previously published study showed that mare age is associated with lower quality oocytes and embryos (Carnevale et al., 1999). It has been suggested in humans that multiple ovulations act as a "fertilization insurance" against possible failures, and as a compensation mechanism for defective embryos (Ball and Hill, 1999). This association explains the low incidence of double ovulations (12.1%) recorded by Watson et al. (1994) in their work using maiden Thoroughbred mares, in spite of the fact that this horse breed shows this ovulation pattern frequently (Ginther et al., 1982). Similarly high incidences of multiple ovulations in mares have been detected in many previously published works (Ginther et al., 1982; Newcombe, 1995; Davies Morel and O'Sullivan, 2001; Carmo et al., 2004; Davies Morel et al., 2005; Greco et al., 2005).

In both groups, the incidence of single ovulations on the left and right ovaries was statistically similar ($p > 0.05$). Cows have been shown to have a significant tendency in the site of ovulation in favor of the right ovary (Scaramuzzi and Downing, 1997), being considered as asymmetrical ovulating animals. On the other hand, mares have already been classified in literature as having the asymmetrical ovulation pattern, favoring the ovulation in the left ovary (Arthur, 1958; Ginther, 1983) and

the symmetrical ovulation pattern, occurring in a randomic way (Bain and Howey, 1975; Butterfield and Mathews, 1979; Davies Morel and O'Sullivan, 2001). The results in the present work were similar to the ones obtained by the latter authors. Moreover, in the experiment published by Ginther (1983), ovulation from the left ovary occurred more frequently only in maiden mares, with equal frequency from either ovary in lactific and barren mares. No such hypothesis that maiden mares have different ovulation patterns was evaluated in the present study, for only a very small percentage of studied animals were maiden.

In this experiment, the incidence of triple ovulations was equally low (1.9%) in both groups. It is known that double ovulations occur more frequently than triple ovulations (Meadows et al., 1995). When analyzing the obtained results from the control group, the lower triple ovulation (1.9%) rate is in contrast to the double ovulation one (30.8%), agreeing with previously published data (Wesson and Ginther, 1981; Ginther, 1983; Davies Morel and O'Sullivan, 2001). Contrasting results were obtained by Carmo et al. (2004), in whose work triple ovulations achieved the percentage of 10% of all eight hundred and twenty nine (829) recorded cycles. Thus, only once, in the control group, was a quadruple ovulation detected (1.0%).

Finally, the incidence of unilateral and bilateral ovulations obtained in the present work was similar in both groups when separately analyzed, as for the incidence of unilateral and bilateral ovulations between them ($p > 0.05$). One can conclude that hCG administration was not able, in any way, to modify the incidence of the different ovulatory patterns in Campolina breed mares. Moreover, the current work indicates that there is an equal distribution between double ovulations occurring from a single ovary (unilateral) and from both ovaries (bilateral). In literature, previously published experiments have achieved similar results (Wesson and Ginther, 1981; Squires et al., 1987). However, this distribution is still obscure. Ginther (1983) concluded that significantly fewer multiple ovulations are unilateral. Thus, in an evaluation during three consecutive years, Davies Morel and O'Sullivan (2001) found out that this difference was only statistically relevant in the first year of their experiment, being the distribution of unilateral and bilateral multiple ovulations equal in the following years. According to these authors, however, when analyzing the results as a whole, multiple ovulations occur more frequently in a bilateral fashion. The reason for these results, as discussed in their work, remains unknown.

Through the analysis of the results obtained from the present study, one can conclude that hCG administration did not enhance the incidence of double and multiple ovulations of Campolina breed mares.

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