Growth of the dominant follicle and endometrial folding after administration of hCG in mares during oestrus

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ABSTRACT: The purpose of the trial was evaluation of follicular growth and endometrial folding in mares after human chorionic gonadotropin (hCG) treatment in comparison with untreated mares during oestrus. In addition, the influence of follicle size at the time of hCG treatment on these parameters was evaluated. HCG (3000 IU) was administered intravenously in 17 mares bearing dominant follicles 35–40 mm in diameter (Group A) and in 13 mares with larger follicles (Group B). Ten mares with follicles ≥ 35 mm were untreated (Group C). Ultrasonographical examination of the mares continued in 6 h intervals until ovulation. Growth of the dominant follicle was faster in Group A than in Groups B and C (1.3 vs. 0.3 and 0.7 mm/6h, $P < 0.05$) but diameters of the preovulatory follicles were similar – 44, 48 and 44 mm in Groups A, B and C, respectively. Similarly, reduction of endometrial folding (on a three point scale) during observation was higher in Group A than in B and C (2.1 vs. 1.2 and 1.8, A : B $P < 0.05$) but endometrial folding values in the term before ovulation were not different (0.6, 0.9 and 0.6 in Groups A, B and C). A positive correlation between the speed of follicular growth and reduction of endometrial folding was found (r_s – 0.479, $P = 0.003$). Irregularity in follicle shape (the difference between the longest axis and its perpendicular axis) at the beginning of observation (3.3, 4.0 and 3.2 mm) was lower than before ovulation (7.4, 10.4 and 9.2 mm) in all groups ($P < 0.01$). The interval from the beginning of observation until ovulation was significantly shorter in Groups A and B versus C (37 and 31 vs. 103 h, $P < 0.01$). The results show that growth of dominant follicles after hCG is influenced by the size of the follicles at the time of treatment and correlates with reduction in endometrial folding as well as irregularity of follicle shape. Nevertheless, hCG treatment does not influence the size and shape of preovulatory follicles or endometrial folding immediately before ovulation.

Keywords: mare; induction of ovulation; ultrasonographical examination; follicle growth; follicle shape; ovulation

The importance of ovulation timing increases simultaneously with the spread of artificial insemination of mares with conserved semen because in this case precious synchronization of insemination with ovulation limits conception. The optimum time for artificial insemination is 6–12 h before ovulation and the interval from 48 h before to 6 h after ovulation is considered to be a period with a good chance of successful fertilisation (Pace and Sulivan, 1975; Woods et al., 1990). Nevertheless, prediction of spontaneous ovulation is inaccurate. Oestrus lasts several days and ovulation usually occurs within 48 h before disappearance of oestrus signs. Some internal symptoms such as diameter, shape, and fluctuation of the dominant follicle and endometrial folding are can be used for the prediction of ovulation (Ginther and Pierson, 1989; Towson and Ginther, 1989), but these symptoms are neither sufficiently accurate nor standard. For this reason hormonal induction/synchronization of ovulation, which makes possible the determination of the suitable term for insemination in advance, is desirable.

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Human chorionic gonadotropin (hCG) administration represents the most frequent method of treatment for this purpose. Induction of ovulation using hCG was described by Day as early as 1939 and Davidson (1947) observed ovulation between 24 and 48 h after this treatment. Nishikawa (1959) found ovulation after day three when hCG was administered on the first day of oestrus. Loy and Hughes (1966) administered 2500 IU of hCG on the second day of oestrus and the average term of ovulation was 4.09 days after the treatment. Recently, administration of hCG in the presence of external signs of oestrus and a dominant follicle ≥ 35 mm in diameter on ovaries is considered to be a reliable method of induction/synchronization of ovulation because ovulation usually occurs between 24 and 48 h after treatment (Harrison et al., 1991; Bruyas et al., 1992; Aurich et al., 1993; Johnson and Becker, 1993; Chavatte and Palmer, 1998; Ataman et al., 2000; Berezowski et al., 2004; Samper, 2008). Thus the treatment interferes in the dynamics of follicle maturation as well as accompanying changes in the uterus during the preovulatory period compared to natural ovulation. The object of this trial was to evaluate the dominant follicle growth and changes in endometrial folding in mares after hCG treatment in comparison with untreated mares. In addition, we evaluated the influence of follicle size at the time of the treatment on these parameters.

MATERIAL AND METHODS

Experimental animals, treatment and examination

Visual detection and transrectal ultrasonographical examination of the ovaries and uterus were performed daily in Czech Warmblood mares (age 3–18 years) in order to detect oestrus during the breeding seasons of 2009 and 2010. The largest follicle on ovaries with a diameter ≥ 30 mm was considered to be the dominant follicle. Forty mares showing clinical symptoms of oestrus and single dominant follicles ≥ 35 mm in diameter were used in the study. HCG (Pregnyl, N.V. Organon, Oss, Netherlands, 3000 IU) was administered intravenously in 17 mares bearing dominant follicles 35–40 mm in diameter (Group A) and in 13 mares with dominant follicles > 40 mm in diameter (Group B). The treatment represented the first application of hCG in the season. Ten mares were untreated (Group C). Following ultrasonographical examination of ovaries and uterus (Dynamic Imaging, Concept 500, Scotland 5 MHz rectal probe) was performed in experimental mares at 6 h intervals from treatment or achievement of follicle diameter ≥ 35 mm in untreated mares (hour 0) until disappearance of the typical dominant follicle (ovulation). Dominant follicles ovulated in all observed mares and any double ovulation was not included in the trial.

Evaluated parameters

Diameters of the dominant follicles (mm), endometrial folding (scale zero to three points), speed of follicle growth (mm/6 h), reduction of endometrial folding during observation (zero to three points), irregularity of follicle shape (mm) and time of ovulation (hours) were evaluated.

Double measurement of the dominant follicle was performed during each ultrasonographical examination. Only anechogenic antrum without the follicle wall was measured. The longest axis and its perpendicular axis were measured separately during a single measurement. Thus, four values of follicle diameter were obtained during each examination and the average value was taken as the final follicle diameter.

Endometrial folding was evaluated on a scale of zero to three points according to subjective estimation. The result was the average from partial values of endometrial folding in both uterine horns and uterine body (three values).

The speed of follicular growth was calculated as the difference between diameters of the dominant follicle at hour 0 (beginning of regular observation at 6 h intervals) and diameter of the preovulatory follicle divided by the number of examinations. Generally, the growth was slower close to ovulation but variability in the speed of follicle growth during observation was not precisely evaluated.

Reduction in endometrial folding represented the difference between values at the beginning of observation (hour 0) and just before ovulation. The term "reduction" is used because a higher value of endometrial folding was found in most observed mares at the beginning of observation compared to the time just before ovulation. An opposite trend was not found in any mare.

Irregularity of follicle shape was represented by the difference between the longest axis and its perpendicular axis. The final value represented the average

of two partial values because double measurement of the dominant follicles was being performed.

The term of the first examination when no dominant follicle was found was considered to be the term of ovulation and the time of ovulation represented the number of hours from hour 0 until ovulation. Thus, possible inaccuracies in the time of ovulation exist from zero to six hours. The dominant follicle was, before its disappearance, considered to be a preovulatory follicle and accordingly the time of the last examination before ovulation was indicated as the time before ovulation.

Statistical analysis

Differences between follicle diameters as well as endometrial folding at hour 0 and just before ovulation were evaluated using a paired *t*-test within the frame of each experimental group. Parameters including follicle growth, diameter of the preovulatory follicle, reduction of endometrial folding during observation, endometrial folding before ovulation and time of ovulation were compared among the experimental groups using the Steel-Dwass test. Irregularity of the follicle shape at hour 0 was compared with the irregularity at the time before ovulation using the Wilcoxon test in the individual experimental groups. In addition, irregularity of follicle shape was compared among the groups separately at hour 0 and at the time before ovulation using the Kruskal-Wallis test. The relationship of the follicle diameter and the speed of follicular growth to the reduction of endometrial folding during the interval from hour 0 to the time before ovulation were evaluated using a non-parametric correlation test (Spearman Rank Correlation).

RESULTS

The diameters of the dominant follicles were higher and endometrial folding was lower in each experimental group at the time before ovulation in comparison with the beginning of observation (hour 0), (Figure 1 and 2). Follicular growth was faster in Group A than in Groups B and C (1.3 vs. 0.3 and 0.7 mm/6h, $P < 0.05$) but the diameters of the preovulatory follicles were similar – 44, 48 and 44 mm in Groups A, B and C, respectively. Similarly, the reduction in endometrial folding during observation was higher in Group A than in Groups B and C (2.1 vs. 1.2 and 1.8, A : B $P < 0.05$) but values of endometrial folding at the time before ovulation were not different (0.6, 0.9 and 0.6 in Groups A, B and C). A correlation between follicle diameter and endometrial folding at hour 0 and at the time before ovulation was not proved but a positive correlation was found between the speed of the follicular growth and reduction in endometrial folding during the interval from hour 0 to the time before ovulation (r_s – 0.479, $P = 0.003$). Irregularity in follicle shape was significantly ($P < 0.01$) higher at the time before ovulation compared to the beginning of observation (hour 0) in all experimental groups (Group A – 7.4 : 3.3 mm, Group B – 10.4 : 4.0 mm and Group C – 9.2 : 3.2 mm). Nevertheless, a comparison of values of irregularity among the experimental Groups A, B and C did not show any differences at hour 0 (3.3, 4.0 and 3.2 mm, respectively) nor at the time before ovulation (7.4, 10.4 and 9.2 mm, respectively). Real average sizes and shapes of the dominant follicles in the experimental groups at hour 0 and at the time before ovulation are shown in Figure 3. The time of ovulation was significantly lower in Groups A and B versus C (37 and 31 vs. 103 h, $P < 0.01$).

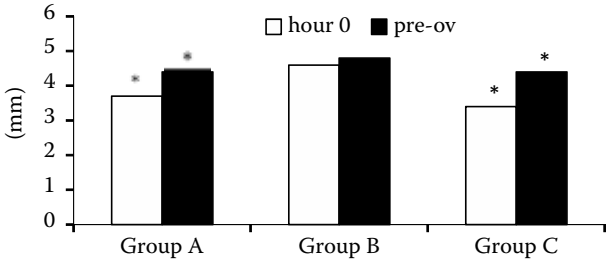

Figure 1. Diameters of the dominant follicles in mares treated by hCG

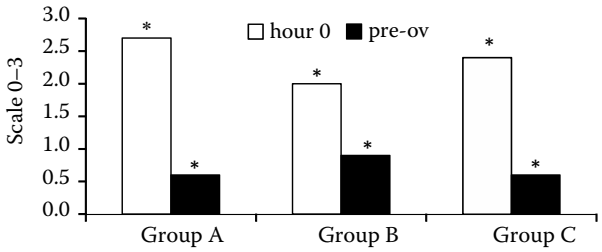

Figure 2. Endometrial folding in mares treated by hCG

(Group A – initial follicle 35–40mm, $n = 17$; Group B – initial follicle > 40 mm, $n = 13$) and in untreated mares (Group C – initial follicle ≥ 35 mm, $n = 10$) at hour 0 and before ovulation (pre-ov)

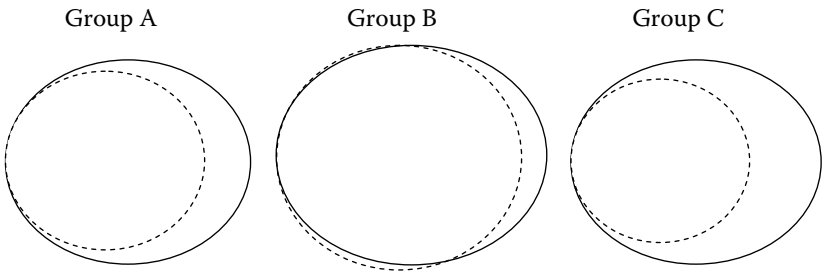

Figure 3. Design of the dominant follicles in mares treated by hCG (Group A – initial follicle 35–40 mm, n = 17; Group B – initial follicle > 40 mm, n = 13) and untreated mares (Group C – initial follicle ≥ 35 mm, n = 10) at hour 0 (----) and before ovulation (–)

DISCUSSION

In our trial the average speeds of follicular growth during observation were 1.3, 0.3, and 0.7 mm/6h (which translate into 5.2, 1.2 and 2.8 mm/day) in Groups A, B and C, respectively. Palmer (1987) described a follicle growth speed of 2.5–3.0 mm per day during the luteal phase of the oestrous cycle. A similar speed of 2–3 mm/day was found in ovulatory follicles up to diameters of 41–45 mm 24–48 h before ovulation (Kahn et al., 1994). Thus the values in the control non-treated mares are comparable with the results of the above mentioned authors which were obtained also in naturally ovulating mares. But the speed of follicle growth was markedly higher after hCG treatment in mares bearing dominant follicles 35–40 mm in diameter at the time of treatment. Follicle growth usually ceases, and the preovulatory follicle even can diminish in size just before ovulation (Carnevale et al., 1988; Bollwein and Braun, 1999). Follicle growth was slower or ceased close to ovulation also in our trial but variability in the speed of growth during observation was not precisely evaluated. We suppose that the speed of follicle growth decreases after achievement of a follicle diameter of 40–45 mm.

Various changes in features of the ripe follicle just before ovulation were described (Pierson and Ginther, 1985; Carnevale et al., 1988). The size of the follicle usually remains constant or decreases before ovulation and the follicle turns flat, soft with a thicker wall and distinct fluctuation. The shape of the follicle can be ovoid or piryform. A hyperechogenic wall and echogenic droplets can be seen in USG picture. These preovulatory changes usually start about 24 h before ovulation, but the changes are neither regular nor uniform (Towson and Ginther, 1989). Our results revealed elongation of preovulatory follicles and a more distinct ovoid shape immediately before ovulation. Even though the dynamics of changes in follicle size were not evaluated in our trial the diameters remained the same or decreased in most mares before ovulation. The diameters of preovulatory follicles in mares usually range from 35 to 60 mm. The diameters can be influenced by season, breed or the number of preovulatory follicles. The follicles are larger in April (46 mm) and May (48 mm) compared to July (40 mm) (Ginther and Pierson, 1989). In cases of double ovulation the diameters are larger at bilateral ovulations (40 mm) compared to unilateral ovulations (35 mm) and also at asynchronous ovulations (40 mm) compared to synchronous ovulations (36 mm) (Ginther and Pierson, 1989). Only single ovulations during spring were evaluated in our trial and the diameters of preovulatory follicles were similar (44, 48 and 44 mm in Groups A, B and C, respectively). Cuervo-Arango and Newcombe (2008) found that induction of ovulation decreases the size of the preovulatory follicles in comparison with natural ovulation. In addition, Gastal et al. (2006) found a relation of hCG doses to the diameter of the preovulatory follicle. Also Kerban et al. (1999) described some differences in follicle development after treatment with hCG. However, in our study we did not establish a relationship between hCG treatment and the size and shape of preovulatory follicles several hours before ovulation as these parameters were similar in all experimental groups.

Ovulation usually occurs between 24 and 48 h after hCG treatment (Duchamp et al., 1987; Bollwein and Braun, 1999; Cetin et al., 2003; Bouakkaz et al., 2005; Bulbul and Sonmez, 2007). Accordingly, ovulations occurred between 12 and 48 hours after the treatment in our trial. Gastal et al. (2006) and Moreli and Newcombe (2008) described the effect of different doses of hCG on the interval to ovulation. Cox et al. (2009) observed ovulation up to 48 h after hCG treatment in 100%, 58% and 29% of mares bearing follicles with diameters 35, 28 and 24 mm at the time of treatment. But Bollwein and Braun (1999) did not establish any relationship between follicle size at the time of hCG administration and the term of ovulation. We also did not observe in our trial that

the diameter of the follicles at the time of treatment would significantly influence the term of ovulation. Nevertheless, mares with smaller follicles (Group A) ovulated between 12 and 48 hours and mares with larger follicles ovulated between 12 and 42 h and most ovulations occurred in Group A (11/17, 65%) between 30 and 36 h while in Group B (6/13, 46%) between 18 and 24 h after the treatment.

Because endometrial folding in mares correlates with oestrogen production in the dominant follicle it can be used as an indicator of follicle development. Endometrial folding usually starts to be noticeable on day seven before ovulation, it culminates one or two days before ovulation, and subsequently diminishes and disappears by day two or three after ovulation (McKinnon et al., 1987; Kahn et al., 1994; Pycock, 2002). Endometrial folding started to decrease in all mares immediately after hCG treatment in our trial. Thus, our results correspond with the above mentioned studies because ovulation occurred in these mares within 48 h after treatment. But the dynamics of endometrial folding was not in accordance with the studies in untreated mares because the folding persisted and decreased in these mares from the beginning of observation even though ovulation occurred after several days. Endometrial folding increased only in one control mare during the first 12 h of observation. Samper (1997) used a five point scale and found an average value of endometrial folding of 1.3 immediately before ovulation. According to McKinnon et al. (1987) a reduction in endometrial folding from three to zero indicates approaching ovulation when a three point scale is used. We also used a three point scale in our trial and in the experimental Groups A, B and C we found average values of 2.7, 2.0 and 2.4 at the beginning of observation and 0.6, 0.9 and 0.6 immediately before ovulation.

In conclusion, our results demonstrate that intravenous administration of 3000 IU of hCG during oestrus in mares bearing a dominant follicle ≥ 35 mm in diameter represents a reliable method of induction/synchronization of ovulation when ovulations occur between 12 and 48 h after treatment. Growth of dominant follicles after hCG was influenced by the size of the follicles at the time of treatment and follicle growth correlated with the reduction in endometrial folding and the irregularity of follicle shape. Nevertheless, hCG treatment did not influence the size and shape of preovulatory follicles nor endometrial folding immediately before ovulation.

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References

Ataman MB, Gunay A, Uzman M, Gunay U (2000): Ovulation induction with human chorionic gonadotropin (hCG) and gonadotropin releasing hormone (GnRH) in mares. Indian Journal of Animal Science 70, 810–812.

Aurich C, Aurich JE, Klug E (1993): Possibilities of controlling the oestrous cycle in mares. Praktische Tierarztliche 74, 1001–1008.

Berezovski CJ, Stitch KL, Wendt KM, Vest DJ (2004): Clinical comparison of 3 products available to hasten ovulation in cyclic mares. Journal of Equine Veterinary Science 24, 231–233.

Bollwein H, Braun J (1999): Follicular dynamics in mares treated with HCG for induction of ovulation. Tierarztliche Praxis Ausgabe Grosstiere Nutztiere 27, 47–51.

Bouakkaz A, Ouzrout R, Camillo F, Vannozzi I, Rota A, Romagnoli S, Aria G (2005): Time of ovulation after treatment with hCG in the mare. Assiut Veterinary Medical Journal 51, 331–335.

Bruyas JF, Fieni F, Allaire F, Tainturier D (1992): Control of the oestrous cycle in mares. A review. Recueil de Medecine Veterinaire 168, 937–946.

Bulbul B, Sonmez M (2007): The effect of various doses of hCG on induction of ovulation in mares. Indian Veterinary Journal 84, 712–714.

Carnevale EM, McKinnon AO, Squires EL, Voss JL (1988): Ultrasonographic characteristics of the preovulatory follicle preceding and during ovulation in mares. Journal of Equine Veterinary Science 8, 428–431.

Cetin H, Korkmaz O, Atli MO (2003): The effect of human chorionic gonadotropin administration at the time of mating on induction of ovulation and pregnancy rates in the purebred Arabian mares (in Turkish). Saglik Bilimleri Dergisi, Firat Universitesi (Veteriner) 17, 179–182.

Chavatte P, Palmer E (1998): Induction of ovulation in the mare. Equine Veterinary Education 10, 26–30.

Cox TJ, Squires EL, Carnevale EM (2009): Effect of follicle size and follicle-stimulating hormone on ovulation induction and embryo recovery in the mare. Journal of Equine Veterinary Science 29, 213–218.

Cuervo-Arango J, Newcombe JR (2008): Repeatability of preovulatory follicular diameter and uterine edema pattern in two consecutive cycles in mare and how they are influenced by ovulation inductors. In: Pro-

ceedings of the European Equine Meeting – XIV SIVE-FEEVA Congress, Venice, 357.

Davidson DF (1947): The control of ovulation in the mare with reference to insemination with stored sperm. Journal of Agricultural Science 37, 287–290.

Day FT (1939): Ovulation and descent of the ovum in the fallopian tube of the mare after treatment with gonadotropic hormones. Journal of Agricultural Science, Cambridge 29, 459–469.

Duchamp G, Bour B, Combarnous Y, Palmer E (1987): Alternative solutions to hCG induction of ovulation in the mare. Journal of Reproduction and Fertility 35 (Suppl.), 221–228.

Gastal EL, Silva LA, Gastal MO, Evans MJ (2006): Effect of different doses of hCG on diameter of the preovulatory follicle and interval to ovulation in mares. Animal Reproduction Science 94, 186–190.

Ginther OJ, Pierson RA (1989): Regular and irregular characteristics of ovulation and the interovulatory interval in mares. Journal of Equine Veterinary Science 9, 4–12.

Harrison LA, Squires EL, Mc Kinnon AO (1991): Comparison of HCG, buserelin and luprostiol for induction of ovulation in cyclic mares. Journal of Equine Veterinary Science 11, 163–166.

Johnson AL, Becker SE (1993): Hormonal control of ovulation in the mare. Animal Reproduction Science 54, 1735–1745.

Kahn W, Wolkmann D, Kenney RM (1994): Veterinary Reproductive Ultrasonography. Schlütersche Verlagsantalt und Druckerei GmbH & Co., Hannover. 256 pp.

Kerban A, Dore M, Sirois J (1999): Characterisation of cellular and vascular changes in equine follicles during hCG-induced ovulation. Journal of Reproduction and Fertility 177, 115–123.

Loy RG, Hughes JP (1966): The effect of human chorionic gonadotropin on ovulation, length of estrus, and fertility in the mare. Cornell Veterinary 56, 41–50.

McKinnon AO, Squires EL, Carnevale EM (1987): Diagnostic ultrasonography of uterine patology in the mare. In: Proceedings of the 33rd Annual Conference of American Association of Equine Practitioners, 605–622.

Moreli MCGD, Newcombe JR (2008): The efficacy of different hCG dose rates and the effect of hCG treatment on ovarian activity: ovulation, multiple ovulation, pregnancy, multiple pregnancy, synchrony of multiple ovulation; in the mare. Animal Reproduction Science 109, 189–199.

Nishikawa, Y (1959): Studies on reproduction in horses. Japan Racing Association, Tokyo. 340 pp.

Pace MM, Sulivan JJ (1975): Effect of timing of insemination, numbers of spermatozoa, and extender components on the pregnancy rate in mares inseminated with frozen stallion semen. Journal of Reproduction and Fertility 23 (Suppl.), 115–121.

Palmer E (1987): New results on follicular growth and ovulation in the mare. In: Roche JF, O'Calagan D (eds.): Follicular Growth and Ovulation Rate in Farm Animals. Martinus Nijhof Publishers, Dordrecht. 237–255.

Pierson RA, Ginther OJ (1985): Ultrasonic evaluation of the preovulatory follicle in the mare. Theriogenology 24, 359–368.

Pycock JF (2002): Ultrasound characteristics of the uterus in the cycling mare and their correlation with steroid hormones and timing of ovulation. http://www.equine-reproduction.com/articles/ultrasound-steroids.shtml.

Samper JC (1997): Ultrasonographic appearance and the pattern of uterine edema to time ovulation in mare. In: Proceedings of the 43th Annual Convention of American Association of Equine Practitioners, 189.

Samper JC (2008): Induction of estrus and ovulation: why same mares respond and other do not. Theriogenology 70, 445–447.

Towson DH, Ginther OJ (1989): Size and shape changes in the preovulatory follicle in mares based on the digital analysis of ultrasonic images. Animal Reproduction Science 21, 63–71.

Woods J, Bergfelt DR, Ginther OJ (1990): Effect of time of insemination relative to ovulation on pregnancy rate and embryonic loss rate in mares. Equine Veterinary Journal 22, 410–415.

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