

Effects of a single dose HCG administration on the induction of ovulation, follicular dynamics, uterine changes and pregnancy outcomes in mares during the breeding season

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Abstract

In this study, 19 mares brought to the clinic at different times with the complaint of not getting pregnant were followed up. For this purpose, changes in the uterine and ovarian dynamics of mares and behavioral changes in estrus were followed and evaluated. In particular, it was attempted to increase the chance and rate of pregnancy by providing ovulation with ovulation follow-up followed by a single dose of hCG administration (Chorulon, 3.000 IU). All manipulations and applications in the study were carried out between March and July, that is, during the breeding season of the mares. During the study, a B-mode real-time ultrasonography device was used for ovary and uterus examinations of the mares. For the insemination of the mares, fresh semen was obtained from breeding stallions by the artificial vagina method and analyzed fresh semen was used. The intracornual insemination method was used as an artificial insemination method. Pregnancy controls were performed twice on the 15th and 30th days after insemination and the pregnancy status was evaluated according to the criteria of the presence of an embryonic sac in cornu uteri. While the average follicle diameter was around 43.7 millimeter (mm) at the stage when estrus symptoms were at their peak, the diameter of the follicle was 36.2 mm in the USG controls performed at the beginning of estrus. Uterine oedema was measured as 1.78 in the initial phase of estrus, 3.84 in the most intense phase of estrus, and 2.36 during insemination during the same periods. The pregnancy rate was found to be 68% (13/19). As a result, it is recommended to administer gonadotropic hormones such as hCG at an appropriate dose (3000 IU) to mares who have trouble conceiving, especially in cases of delayed ovulation.

Keywords: hCG, mare, ovulation induction, pregnancy rate, uterine oedema

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Introduction

The breeding activity of mares in the northern and southern hemispheres is seasonally dependent, when mares are polyestrous, seasonally long-day breeders (Ataman *et al.*, 2000; Gerlach and Aurich, 2000). The endocrinological control of the estrus cycle is governed by the hypothalamic-pituitary-gonadal axis and represents the basis for the study of reproductive physiology that produces ovulatory estrus cycles in the mares (Malpaux *et al.*, 2001; McKinnon, 2011; Busato *et al.*, 2017). As is known, the breeding of horses is unique in that it has long behavioral estrus and variations in ovarian follicular dynamics, which makes it difficult to standardize breeding time in mares. Accurate prediction of ovulation timing has become a critical component of breeding management in order to maximize the efficiency of a breeding operation and ultimately the pregnancy rate. Reducing the number of breedings or inseminations per cycle maximizes stallion or semen usage and reduces mare contamination and farm labor with its associated costs. The ideal number of matings or inseminations per cycle when a healthy mare is bred using a fertile stallion or good quality semen is one. However, well-managed breeding operations can allow up to 10% rebreeds in the same cycle. Current systems to determine the timing of breeding rely on several of the following: (a) teasing by a stallion, (b) cervical relaxation determined by rectal palpation or vaginal speculum examination, (c) the presence of a "large" follicle detected by rectal palpation and ultrasonography, (d) the appearance of the follicle on ultrasound examination, (e) timing from treatment with an inducing agent, and (f) the presence and pattern of uterine oedema.

In general, when the pre-ovulatory follicle reaches 35 mm, ovulation can occur within approximately 36-48h (Patricia, 2018). The time of ovulation may be influenced by factors such as breed, age, size of pre-ovulatory follicles and score of the uterine oedema, which might subsequently affect pregnancy success (Ginther, 1982). It has been suggested that external factors such as month of the year, side of ovulation and number of follicles ovulated might also affect pregnancy rates (Morris and Allen, 2002). The uterus of the transitional mare will be characteristic because of the presence of endometrial oedema, which is characterized by the visualization of the endometrial folds giving the characteristic appearance of a "cart wheel" or "sliced orange". A distinguishing characteristic of the uterus of transitional mares, particularly in mid to late transition, is the persistence of the endometrial oedema for several days or even weeks with no significant change. In many instances a small amount of fluid accumulation in the uterus can be detected. Because the uterine folds increase in thickness, there is also a significant increase in the surface area of the uterus and small fluid accumulations can easily dissipate within the uterus. The presence of multiple medium to large follicles 25-35 mm or larger is typical of the transitional period. Predicting the day of ovulation will be of considerable use for coordinating the time of breeding and

pregnancy success (Samper, 1997; Cuervo-Arango and Newcombe, 2008).

For many years hCG has been used in mares to shorten the period of estrus and induce more predictable ovulation (Meyers, 1997). Administration of 2500 IU hCG to mares in estrus that had dominant ovarian follicles ≥ 35 mm in diameter was followed by ovulation within 48 h of treatment in 70 to 90% of cases (Ginther, 1992; Meyers, 1997). The dose of hCG used to induce ovulation in mares varies from 1500 to 4000 IU, given either by intravenous or intramuscular injection (Voss, 1993). The treatment of mares with 1500-5000 IU hCG has been reported to increase the number of mares which ovulate within a 48 h period following administration (Samper, 2009). This allows the number of matings or inseminations per estrus to be reduced and, as the synchrony between ovulation and mating is improved, results in improved conception rates (Vanderwall *et al.*, 2001). Most success has been achieved when hCG is administered after a follicle has reached 30-35mm in diameter (Barbacini *et al.*, 2000). Samper (2009), reported that 83.3% of hCG treated mares with a follicle greater than 30mm in diameter ovulated within 48 h, and by 96 h 100% of mares had ovulated.

Mares with a clinically normal uterus should not have a significant volume of fluid in the uterus. The presence of a moderate or large volume of fluid in the uterus visible on ultrasound suggests the presence of an active infection, a prolonged non-infectious inflammatory condition, inadequate uterine clearance mechanism or failure of normal cervical function (McKinnon *et al.*, 1993). The incidence of intrauterine fluid retention in mares was indicated as 11- 39% (Reilas *et al.*, 1997; Watson, 2003). The visible amount of intrauterine fluid in the estrus cycle may refer to endometritis and may decrease sperm mobility, which may cause a failure of pregnancy (Griffin and Ginther, 1991; Cuervo-Arango and Newcombe, 2008). The score is 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 oedema and fold, 1 corresponded to a little swelling and endometrial fold, 2 fold endometrial oedema and not very clear, and 3 the mares that had endometrial oedema and fold highlighted. Administration of intrauterine lavage, antibiotics, oxytocin and human Chorionic Gonadotropin (hCG) after breeding has shown to be positively effective on fertility (Kılıçarslan *et al.*, 1996; Kundak and Kılıçarslan, 2018).

In this study, uterine changes, changes in ovarian dynamics and behavioral changes in mares brought to the clinic at different times with the complaint of inability to conceive were monitored with the support of USG and rectal palpation. It was attempted to determine the effectiveness of a single dose of HCG (Chorulon, 3000 IU) administration to increase the pregnancy rate after ovulation and artificial insemination.

Materials and Methods

Animals: A total of 19 mares with the complaint of inability to conceive were included in the study during

the breeding season of March-July 2019. The ages of the animals ranged from 5 to 15 years, and their body weights ranged from 350 to 550 kg.

Study design: In this study, a single dose of gonadotropin-derived hormone injection was administered to mares with estrus irregularity and that were especially thought to have problems with ovulation. For this purpose, Chorulon (3000 IU intramuscular injection), whose active ingredient is synthetic hCG, was used. During the estrus cycle transrectal ultrasonographic examinations were carried out to measure ovarian follicular growth and other developmental changes in the uterus. All mares were examined with the same ultrasound device (B-Mode Real-Time 5-10 MHz linear probe, SonoSite, Holand) used by the same operator. The day of ovulation was considered to be day 0. The diameter of the preovulatory follicle, oedema of the uterus and intrauterine fluid accumulation were evaluated ultrasonographically. Human chorionic gonadotropin 3000 IU (Chorulon) was administered I.V. to estrus mares when at least one follicle \geq 25-30 mm was detected. Only one injection of hCG 3000 IU per estrus cycle was given.

Further reproductive ultrasonographic studies were performed at 12 hour intervals after hCG injection and until ovulation occurred. The ovulation time was recorded as: \leq 24, $>$ 24, \leq 48 and $>$ 48 hours. Mares were considered to have had an inappropriate response to treatment when ovulation did not occur within 48 hours of hCG administration (according to Ginther 1982). Follicle control was performed every 12 hours until the pre-ovulation follicle (POF; \geq 25-30 mm) was determined and thereafter until ovulation occurred. Follicle diameters were measured in all examinations performed since the beginning of ultrasonographic examinations. Follicle diameters were recorded regularly in order to clearly evaluate the change and the response to hormone administration. After the POF was determined, artificial insemination was performed with fresh semen every other day. In the study, routine applications such as intrauterine infusion or antibiotics were also performed to support post-mating pregnancy and implantation when necessary.

Clinical and Laboratory Findings:

Estrus behavior was graded as follows (Pycocck, 2020):

- Aggressive to the teaser: 0
- Indifferent to the teaser: 1
- Stands with the teaser and everts clitoris (winking): 2
- Stands with the teaser, winking and passing urine: 3

The number of days between ovulations was used to determine the length of the estrus cycle (interovulatory interval). The day of ovulation was called Day 0 because it was the first day that a large follicle seen the day before had vanished and a corpus luteum was visible. Interovulatory intervals were not calculated using diestrus ovulations.

The following scheme was used to grade the uterine echotexture (Pycocck, 2020):

- No oedema with a typical homogeneous echotexture characteristic of diestrus: 1
- Smallest amount of readily detectable uterine oedema, no free fluid: 2
- Moderate amount of oedema, throughout the whole uterus: 3
- Most obvious oedema throughout the whole uterus, sometimes free fluid also noted: 4

Artificial insemination and pregnancy diagnosis: After the perineal areas of the mares prepared for insemination were cleaned with clean water and dried, intracornual artificial insemination was performed. For artificial insemination, 20 ml of semen was taken into the collection container at 37 degrees and passed through the cervix with a suitable intrauterine catheter and inseminated as deep into the cornu as possible. After artificial insemination, ovulation and uterus structure were examined and possible ovulation status within 0-48 hours was monitored. In cases where ovulation did not occur, if the follicles and uterus structure were suitable, re-seeding was performed and ovulation was observed between 0-48 hours. Uterine controls were continued after ovulation and intrauterine lavage was applied in the presence of hydrouterium. The purpose of this application is to provide the appropriate environment for the formation of the embryo. The pregnancy status of mares was determined by transrectal ultrasonographic examination performed on the 15th day after spawning. On the 15th day after ovulation, the diagnosis of pregnancy was made by looking at the criteria for the appearance of spherical embryonic sac(s) in the uterus. The ultrasound examination was carefully performed by imaging the whole uterus. A mare was diagnosed as pregnant when an embryonic vesicle was identified within the uterus. A second ultrasound examination was carried out at 32-35 days post AI to confirm the pregnancy and the rate of pregnancy in mares was calculated by dividing the number of pregnant females by the total of the inseminated ones.

Statistical analyses: SAS software (SAS Institute Inc.) was used to perform a data analysis of variance using the General Linear Model procedure (GLM). Duncan's Multiple Range Test was used to compare the percentage of pregnant mares on hormone therapy, the percentage of pregnancies from left or right ovary ovulation and groups of mares with different pre-ovulation follicle diameters. The presence of correlations between uterine oedema, estrus length, ovulation time, estrus behavior and body condition with follicular activities in the ovaries was assessed using the Pearson correlation. P0.05 was chosen as the significance level for all data analysis.

Results

In this study, the Chorulon application was applied to a total of 19 mares and it was determined that 13 mares became pregnant after the insemination (68%). In pregnant mares, after the ovary follow-up in which the pregnancy was formed, it was determined that

pregnancies occurred in 6 mares from the right ovary and in 7 mares after ovulations originating from the left ovary. In USG controls performed at the initial stage of estrus, it was determined that the average follicle diameter was around 36.20 ± 0.11 mm in diameter and the average follicle diameter was around 43.70 ± 0.06 mm in the phase when the estrus was detected at the maximum level (Table 1). While uterine oedema was 1.78 ± 0.09 on average in mares measured in the initial stages of estrus, it increased to 3.84 ± 0.08 at the stage when estrus intensified with all its indicators at the

maximum level. In the measurements made at the time of artificial insemination, it was determined that the presence of uterine oedema decreased to an average of 2.36 ± 0.1 (Table 1). In correlation analyses, in which the interactions between some reproductive parameters are controlled, a moderate correlation was found between the increase in estrus behavior and changes in ovarian activity and uterine oedema; Correlations have also been found between changes in uterine oedema and changes in dynamics in the ovaries.

Table 1 Mean values of some reproductive parameters in mares based on ultrasonography and observation.

Measurements	Pregnant		Non-Pregnant		Overall average	
	Mean \pm SEM	Range	Mean \pm SEM	Range	Mean \pm SEM	Range
Changes in follicle diameter (mm)						
Beginning of the estrus	37.4 ± 0.08	35.0 - 44.0	33.6 ± 0.31	25.0 - 44.0	36.2 ± 0.11	25.0 - 44.0
Preovulatory follicle	43.6 ± 0.05	40.0 - 48.0	44.0 ± 0.17	37.0 - 48.0	43.7 ± 0.06	37.0 - 48.0
Changes in uterine oedema						
Beginning of the estrus	1.84 ± 0.10	1.00 - 2.00	1.66 ± 0.21	1.00 - 2.00	1.78 ± 0.09	1.00 - 2.00
In the most intense heat	3.84 ± 0.10	3.00 - 4.00	3.83 ± 0.16	3.00 - 4.00	3.84 ± 0.08	3.00 - 4.00
At the time of insemination	2.30 ± 0.13	2.00 - 3.00	2.50 ± 0.22	2.00 - 3.00	2.36 ± 0.11	2.00 - 3.00
Follicle diameter at ovulation (mm)						
Beginning of the estrus	30.0 ± 0.17^a	20.0 - 40.4	37.6 ± 0.24^b	30.0 - 40.5	32.4 ± 0.16^a	20.0 - 45.0
At the time of ovulation	48.8 ± 0.10	40.4 - 50.6	52.3 ± 0.15	48.0 - 57.0	49.9 ± 0.09	44.0 - 57.0
Side of the ovary with ovulation (%)						
Right	0.46 ± 0.14	0.0 - 1.00	0.16 ± 0.16	0.0 - 1.00	0.36 ± 0.11	0.0 - 1.00
Left	0.53 ± 0.14	0.0 - 1.00	0.83 ± 0.16	0.0 - 1.00	0.63 ± 0.11	0.0 - 1.00
Ovulation formation hours after hCG administration (hours)						
24 h	0.84 ± 0.10	0.00 - 1.00	0.33 ± 0.21	0.0 - 1.00	0.68 ± 0.10	0.0 - 1.00
48 h	0.15 ± 0.10	0.00 - 1.00	0.66 ± 0.21	0.0 - 1.00	0.31 ± 0.10	0.0 - 1.00
Pregnancy rate (%)	100.0 ± 0.00	0.00 - 100.0	0.00 ± 0.00	0.0 - 100.0	0.68 ± 0.10	0.0 - 100.0

Values with different superscripts (a, b) in the same row are significantly different ($P < 0.05$). SEM: Standard error of the mean.

In this study, mares' follicles were treated with hCG when measured between 25 mm and 30 mm. In the group treated with 3000 IU hCG, the mean preovulatory follicle diameter was 49.90 ± 0.09 mm. A regular ovulation response to hCG administration was obtained in all mares. In the evaluations made in terms of the time of ovulation after 3000 IU hCG administration, ovulation occurred in 24 hours in 84% and in 48 hours in 16% of pregnant mares (Table 1). According to Chavatte and Palmer (1998), ovulation induction occurs 32 to 42 hours after hCG injection. Ovulations that occur before 24 hours are not caused by hCG but are induced by an endogenous LH peak. In the study, oxytocin hormone and intrauterine lavage were applied to the mares who were found to have fluid together with oedema in the uterus by rectal examination and rectal ultrasonography. Thus, the possible problems for pregnancy and possible implantation are eliminated. As a result, out of 7 mares with oedema and treatment, 5 became pregnant and 2 of them were not pregnant. The fertility rate was determined as 68% (13/19) in mares exposed to rapid dose hCG application. As mentioned before, according to the findings obtained from ultrasonographic and rectal examination findings performed at frequent intervals throughout the cycle, significant changes were detected in uterine oedema and ovarian follicle dynamics in this process (Table 1). The duration of

estrus, which is one of the important signs of reproduction in mares, was evaluated separately for pregnant and non-pregnant mares and the duration of estrus was determined as 6.93 ± 0.19 and 5.84 ± 0.15 days in pregnant and non-pregnant mares, respectively (Table 2). When the relationships between some reproductive parameters and ovarian dynamics during the breeding season in mares were evaluated, it was determined that there were positive correlations between uterine oedema and the intensity of estrus behavior, as well as increased follicular activation in the right and left ovaries ($r: 0.769^{**}$; $r: 0.412^{**}$ and $r: 0.410^{**}$). In addition, a high level of positive correlation was found between the time of ovulation and the intensity of estrus behavior ($r: 0.438^{**}$) (Table 3).

Table 2 The relationship of some reproductive parameters with pregnancy status in mares during the breeding season.

Reproductive Parameters	Overall average (n=19)		Non-Pregnant (n= 6)		Pregnant (n=13)		P Value
	N	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	
Body Condition Score	197	3.28 ± 0.04	66	3.34 ± 0.05	131	3.25 ± 0.66	0.346
Estrus behaviour	107	1.98 ± 0.07	32	2.06 ± 0.14	75	1.94 ± 0.09	0.502
Uterine oedema	161	2.28 ± 0.08	56	2.41 ± 0.14	105	2.21 ± 0.10	0.269
Growth rate of dominant follicle left ovary (LOV)	191	2.70 ± 0.13 ^a	65	3.10 ± 0.24 ^b	126	2.49 ± 0.15 ^a	0.028
Growth rate of dominant follicle right ovary (ROV)	188	2.48 ± 0.10 ^a	65	2.81 ± 0.13 ^b	123	2.31 ± 0.13 ^a	0.018
Length of estrus	197	6.57 ± 0.14 ^b	66	5.84 ± 0.15 ^a	131	6.93 ± 0.19 ^b	0.003
Ovulation time	10	23.87 ± 4.98	3	26.63 ± 12.60	7	22.68 ± 5.45	0.739

N: Number of measurements. SEM: Standard error of the mean. Values with different superscripts (a, b) in the same row are significantly different ($P < 0.05$)

Table 3 Correlations between some reproductive parameters in the breeding season in mares.

Parameters	Body Condition Score	Estrus behaviour	Uterine oedema	Growth rate of dominant follicle left ovary	Growth rate of dominant follicle right ovary	Length of estrous	Ovulation time
Body Condition Score	1.000						
Estrus behaviour	0.019	1.000					
Uterine oedema	-0.091	0.769**	1.000				
Growth of follicle left ovary	-0.092	0.343*	0.410**	1.000			
Growth of follicle right ovary	-0.119	0.270*	0.412**	-0.020	1.000		
Length of estrus	-0.000	-0.007	-0.172*	-0.143*	-0.072	1.000	
Ovulation time	0.201*	0.438**	0.074	0.370*	0.133	-0.281*	1.000

*Correlation significant at $P < 0.05$, and **Correlation significant at $P < 0.01$

Discussion

Human chorionic gonadotropin was the first ovulatory agent to be used in mares and remains the hormone of choice by many equine practitioners to this day. It is known that human chorionic gonadotropin improves breeding efficiency as it facilitates prediction of ovulation time and synchronism with breeding. Several studies investigating the effects of hCG in hastening ovulation have demonstrated that the duration of estrus and the interval from the onset of estrus to ovulation are significantly reduced in treated mares (Sullivan *et al.*, 1973; Ginther, 1982; Duchamp *et al.*, 1987; Barbacini *et al.*, 2000). However, in the evaluation made in terms of estrus duration in our study, the duration of estrus (days) (6.93 ± 0.19) in mares with positive pregnancy was compared to the others (5.84 ± 0.15) and the mean (6.57 ± 0.14) was found to be longer. It has been previously reported that hCG is effective to induce ovulation within 48 h of administration in 80% of cases (Ginther, 1982; Barbacini *et al.*, 2000). In similar studies, the majority of researchers reported that ovulation is expected within 25-48 hours after hCG administration in 73-75% of cases (Sullivan *et al.*, 1973; Duchamp *et al.*, 1987; Barbacini *et al.*, 2000). Comparable results have been obtained in the present study since ovulation occurred within 48 h of hCG injection in 99.9% of cycles. In the present retrospective study, ovulation occurred within 24 of hCG administration in 83.6% of cycles.

Low uterine oedema (1-2) and the presence of a large follicle (often >40 mm) is a good indication of imminent ovulation. The appearance and disappearance of uterine oedema are related to the onset of estrus in mares (Samper, 1997; Cuervo-Arango

and Newcombe, 2008). Uterine oedema is a powerful tool when controlling the mare to improve the time of ovulation and detect possible uterine infection. However, this tool has to be combined with rectal palpation of follicles and is not a good indicator when used alone (Samper, 1997; 2009). The results obtained from this study, in accordance with the literature, show that uterine oedema may be a reliable parameter to predict pregnancy success in mares and reach similar results. Uterine oedema findings become most clear approximately 3 days before ovulation but decrease 1 to 2 days before ovulation, thus uterine oedema is considered an effective indicator for the timing of ovulation (Cuervo-Arango and Newcombe, 2008; Ginther *et al.*, 2008). However, uterine oedema findings were found in 64% of anovulatory cycles during the breeding transition period (Watson *et al.*, 2003) and significant uterine oedema findings are also observed in endometritis (Samper, 1997). In support of the important literature information given above, in our study, mean uterine oedema was 17.8 ± 0.09 mm in the early stages of estrus in mares during the breeding season, while uterine oedema increased to 38.4 ± 0.08 mm during the peak periods of estrus data. Uterine oedema measured during artificial insemination was measured as 23.6 ± 0.11 mm and showed a decrease supporting the literature data. The diameter of dominant follicles and uterine oedema findings are considered effective indicators to predict ovulation timing in horses (Samper *et al.*, 2007; Cuervo-Arango and Newcombe, 2008; Miyakoshi *et al.*, 2014). The mean maximum diameter of the ovulatory follicle is usually between 40 and 45 mm but there are variations depending on season and type of horse. In this study, preovulatory follicle diameter in mares before

ovulation varied between 44 mm and 57 mm. These values are greatly similar to the mean follicle diameters previously reported for horses. In study of Griffin and Ginther (1991), uterine diameter was increased significantly between 11 and 21 days of estrus cycle. The difference in diameter of preovulatory follicles might be affected by breed, season of the study, the number of ovulations as single or double ovulation per cycle (Ginther *et al.*, 2008), as well as the age of animals as confirmed by Davies Morel *et al.*, (2010). The rate of follicle growth can be influenced by stages of the estrus cycle at which the diameter of follicles are measured, as well as induction of ovulation and estrus in addition to breed differences. As the maximum diameter of preovulatory follicles is indicative of a relative time of ovulation (Samper, 2009); a strong correlation with teasing scores in the present study confirms, implementation of teasing in combination with rectal palpation and ultrasonography is the best method to determine breeding time and cost effective if used alone with good experience. However, growth rate can be affected by age, stage of estrus cycle and induction of ovulation (Gastal *et al.*, 2006).

These research results found different aspects of follicular dynamics of the mare's uterus and ovary, including uterine diameter, ovarian follicular size, number of follicles in each ovary and growth rate of dominant follicles and phases of the estrus cycle from routinely collected transrectal ultrasonography data. The tendency for ovulation to occur within 24 to 48 h may have been due to an increase in LH and FSH caused by Chorulon administration speeding up follicle maturation. We hypothesize that Chorulon administration promoted LH secretion, leading to the desensitization of gonadotropic cells or negative feedback on LH secretion. In this study, the Chorulon application was applied to a total of 19 mares and it was determined that 13 mares became pregnant after the insemination. In our study, it was determined that pregnancy occurred in the right ovary in 6 of the mares and in the left ovary in 7 mares. The overall conception rate of 13/19 (68.42%) in the present study was comparable with previous report of 67.8 % and 69.6 % in the study of Rota *et al.*, (2004) and Crowe *et al.*, (2008) respectively. However, the present result was higher than the rate in a group of Arab bred mares hormonally treated with Chorulon (37%) and the control group (34%) (Najjar *et al.*, 2018). Pregnancy rates reported as 77 % for fresh semen in retrospective study of Squires *et al.*, (2006) was relatively similar with a rate of 13/19 (68.42%) for mares in this study.

In conclusion, detection of the pattern of uterine oedema with ultrasonography, combined with rectal palpation of the follicles and the use of ovulation-inducing agents, helps veterinarians determine the optimal time for breeding of a normal mare. In addition, the routine examination of mares will help veterinarians (a) determine the optimal time for the administration of ovulatory agents such as hCG; (b) determine mares that are in true standing estrus when a teasing stallion is not available; (c) determine when there is estrogenic competence of follicles in transitional mares; and (d) make a prognosis of the possibility of early embryonic death.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics: This article is original and contains unpublished materials. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues were involved.

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