Investigating the effect of progestrone (Cue-Mate™) administration on ovulation induction in photostimulated anestrus mares

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Abstract:

BACKGROUND: In mare, different methods of earlier cyclicity induction and hastening first ovulation of the year have been reported. **OBJECTIVES:** The goal of present study was to investigate the effect of progesterone (Cue-mate®) on synchronization and acceleration of the first ovulation of the year in winter anestrous mares under artificial lighting system. **METHODS:** After detection of anestrus mares, artificial lighting regimen of 16h light was conducted from early December for both treatment and control groups mares. After detecting of 25 mm follicle in the ovary, treatment group mares received Cue-Mate and examined were through rectal ultrasound until follicle reached the size of 35mm, when cue-mate was removed. Daily ultrasound examination and teasing were done until detection of ovulation. **RESULTS:** Based on our results, no significant difference in rate of ovulation (66% vs 60%) and size of ovulatory follicle existed between treatment and control group. In addition, despite of decreasing time interval of follicular growth from 25 mm to 35 mm (8.33±0.88 vs 10.75±2.28 in treatment and control groups respectively) and from each of these sizes to ovulation size, no significant difference existed between treatment and control groups. **CONCLUSIONS:** This was the first preliminary report of investigating the effect of progesterone plus artificial lighting on hastening first ovulation of the year in early winter anestrous. It seems more studies considering the greater number of mares in different ages are needed.

Introduction

The mare is seasonally polyestrous with regular ovulatory cycles occurring in response to increasing day length (Donadeu and Watson, 2007). In mares that have entered a seasonal anestrous phase during the winter of temperate latitudes, ovulatory cycles do not normally commence until April or early May (October or early November in the Southern Hemisphere) (Ginther, 1974). Most breeder associations use a single date, January 1st for registering all foals born in a calendar year, however, from an economic standpoint production of foals early in the year is essential for performance in the show ring and maximizing prices obtained for yearlings (Squires, 2008). Getting mares pregnant around February 15th is essential and various hormonal and non-hormonal

methods have been described for advancing first ovulatory cycle of mares in both anestrus and transitional phase. Non-hormonal method which is based on photostimulation can be implemented by using various artificial lighting procedures including: Indoor conventional and modified light therapy which requires mares to be kept in stalls during lighting period or mobile light therapy which allows outdoor maintenance of mares by using blue light emitting diode (LED; 468nm) mask (Murphy, et al. 2014). Conventional lighting regimen can be done by 14.5 to 16h daily light (8-9.5h darkness) beginning from December in deep anestrus mares. Modified light regimens use photosensitive phases of the mare's circadian clocks including that of post dusk therapy which uses 2.5h additional light after sunset or skeletal lighting which works based on night interruption through 1 -2 h. lighting, 9.5-10.5 hours after beginning of darkness. Lighting regimens are successful, albeit variable in response time between individuals, but must be started many weeks before ovulation (McKinnon, et al. 2011).

Hormonal treatment methods for induction or hastening earlier cyclicity can be classified into two categories: treatments for inducing onset of cyclicity in anestrus mares including administration of GnRH osmotic pump or implant and recombinant LH and FSH or those whose efficacy has been mainly proved for hastening cyclicity among mares in transition phase like using ovulation inducing agents (GnRH agonists and hCG), dopamine D2-receptor antagonists for increasing prolactin secretion (Panzani, et al. 2011) and progesterone or progestin treatment to suppress GnRH release allowing a surge after cessation of treatment (Squires, 2008). Efficacy of reFSH in stimulating follicular development and advancing the first ovulation of the year in deep anestrous mares maintained under ambient lights was determined. The results of this study indicated that reFSH was effective in stimulating the development of ovarian follicles and advancing the first ovulation of the year in seasonally anestrous mares under ambient lights but was not successful in inducing continued cyclicity (Meyers-Browna, et al. 2013). Diastral level of progesterone inhibit ovulation and cause follicular regress in ruminants, while in equids they just prevent showing of estrus behavior and with regards to the possibility of continuing diastral follicles' development, ovulation is presumable. In one study, short-term preovulatory progestogen (CIDR or altrenogest) treatment in mares could not delay ovulation. Also, treatments had no effect on follicular growth rate or the size of the ovulatory follicle immediately preceding ovulation (Caniso, et al. 2013).

We hypothesized that progesterone supplementation increase efficiency of lighting therapy and results in earlier ovulation induction compared with control group.

The goal of present study was to investigate the effect of progesterone on synchronization and acceleration of the first ovulation of the year in winter anestrous mares under artificial lighting system.

Material and Methods

Animals: Crossbred and mixed bred barren mares (n=10), aged from 5 to 10 years old, body weight averaged of 500 Kg and good body condition were used in this study.) in research and training hospital of Faculty of Veterinary Medicine, University of Tehran-Iran located in Mohammad Abad

(in Alborz province-Iran). They were fed with hay, alfalfa and cereals concentrate in standard rations. Because of stallion exposure effect on hastening onset of cyclicity, we were careful not to be allowed the mares to have contacted with stallions. Mares were weekly examined by rectal palpation and ultrasonography from late November of 2014. Mares with signs of anestrus condition, were characterized by inconclusive or no estrous behavior, a relatively relaxed cervical canal, little or no uterine tone, absence of ultrasonically visible endometrial edema or a corpus luteum, and usually follicles of less than 20 mm. They were examined at least 2 weeks apart and were divided randomly in two groups (each group = 5 mares).

Treatments: All of enrolled mares were exposed daily to artificial lighting regimens of 16h light (8h darkness) from early December by using incandescent 200 W bulb and daily programmed analog time switcher (theben Co.). The status of mares' reproductive system in terms of follicular growth and uterine edema was evaluated by ultrasonography examination, twice a week. Unfortunately, 2 mares in treatment group were eliminated from the study after about one month due to some problems. So, we continued with 3 and 5 mares in study and control group respectively. Treatment group mares also received Cue-mate (Bayer Animal Health, New zealand), which is a type of progesterone releasing intravaginal device (PRID) containing Progesterone 1.56g within two silicone pods attached to a flexible 'wishbone' for insertion into the vagina (Fig. 1). The device was originally designed for use in cattle to induce and synchronize estrus. In the mare, sufficient progesterone is absorbed through the vaginal wall to give peripheral plasma concentrations above 10.5 nm/l for the first 4 days. Concentrations diminish to about 7 nm/l by the 8 day and to 5.3 nm/l or lower by day 16.

Study design: If the follicle with a diameter of 25 mm was observed, mares were divided randomly into two groups:

Treatment group: Mares treated by insertion of a Cue-Mate in vaginal region. They were re-examined ultrasonographically about 6 days after insertion of the Cue-Mate and then daily according to the level of follicular activity found. When a follicle of 35 mm or greater had developed, the Cue-Mate was removed. If such a follicle had not developed after 12 to 16 days, the Cue-Mate was also removed. Daily teasing began on the day after Cue-Mate removal until estrus was detected. Then, reproductive examination for detection of reproductive signs of estrus and ovulation was done by ultrasonography.

Control group: Mares were not treated using insertion of a Cue-Mate, but they were re-examined daily according to the level of follicular activity. When a follicle with ≥35 mm diameter had developed, teasing and ultrasonography was performed daily until ovulation.

Statistical analysis: The obtained data such as rate of estrous, rate of ovulation, and rate of antral hemorrhagic follicles occurrence were analyzed by Chi-square statistical test. The numerical data included time interval from size of deviation (≈ 20 mm diameter) to becoming inducible follicle (≈ 35 mm), time interval from gaining capability of ovulation (≈ 35 mm) to ovulation, and diameter of follicles at ovulation and day of ovulation following photostimulation program were tested by T-test. All

of the data were analyzed by SAS statistical software version 9.2 through PROC FREQ and PROC T-TEST, respectively.

Results

Mares in treatment group were treated by Cue-Mate for a mean of 11.6±0.6 days. During the treatment period, none of the intravaginal devices (Cue-Mate) was expelled. Mild vaginitis was seen in just one mare and resolved spontaneously after Cue-Mate removal. One of the mares in control group did not respond to lighting regimen and her ovaries remained inactive during the study. All mares in both groups ovulated after Cue-Mate removal except one in treatment group.

In spite of shortened duration observed in follicular growth from ≈25 mm to ≈35 mm (8.33±0.88 days vs 10.75±2.28 days in treatment and control groups respectively) and from each of these sizes to ovulatory size, no significant difference existed between treatment and control groups (Table 1). It was found that mean diameter of follicles at the time of ovulation (44.7 ± 3.3 mm and 43.1 ± 3.3 mm in treatment and control groups respectively) did not show any difference between two groups (Table 2). Also, there was no significant difference in time interval from the beginning of lighting program to ovulation (Table 2). No significant difference was found in ovulation rate and proportion of mares showing estrus between treatment and control group (Figure 2).

Discussion

Various types of progesterone therapy, including long act injectable (Lopez-Bayghen, et al. 2008) or orally administered progestins (Squires, et al. 1983) and intravaginal releasing progesterone (Handler, et al. 2007) has been used for synchronizing and hastening of ovulation in the mares. Intravaginal route offers a relatively cheap and convenient method of delivering progesterone to mares which does not require daily administration. Treatment of transitional mares with an intravaginal progesterone containing device specifically designed for mares (Cue-Mare) for 10 days, resulted in follicular growth and ovulation within 4 days of device removal and it was associated with minimal discomfort and vaginitis (Grimmett, et al. 2002). Induction of ovulation of mare using hormonal methods did not receive as much attention in the past as dairy cattle and sheep (Squires, 2008, Talebkhan Garoussi, and Golzar, 2012a). It was associated with earlier pregnancy

Table 1. Comparison mean time interval from Cue-mate administration (≈ 20 mm) to ovulation capacity (≈ 35mm), from this time to ovulation and from beginning of treatment to ovulation between treatment and control groups.

Variable (Mean±SEM)	Control	Treatment	P-value
Time interval to ovulation capacity (day)	10.75±2.28	8.33±0.88	0.4
Time interval of inducible follicle ovulation (day)	8±1.78	5±2	0.3
Time interval to ovulation from 25mm (day)	18.2±6.7	12±2.82	0.1

Table 2. Comparison mean diameter of follicles at the time of ovulation and time interval from the beginning of lighting program to ovulation between treatment and control groups.

Variable (Mean±SEM)	Control	Treatment	P-value
Diameter of follicle at ovulation (mm)	3.3 ± 44.7	3.3 ± 43.1	0.9
Day of ovulation following lightening (day)	8.8 ± 58.3	6.2 ± 53.3	0.6

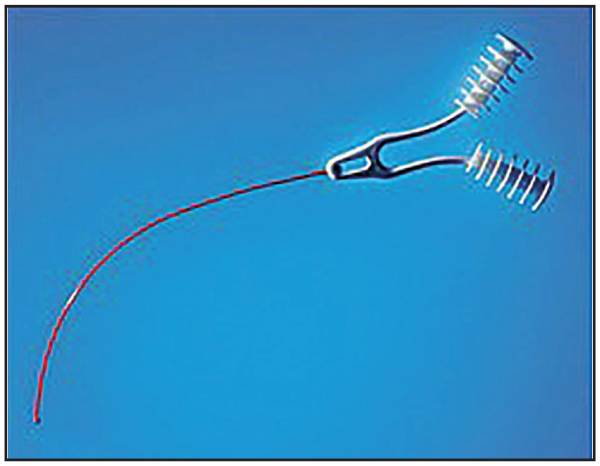

Figure 1. Cue-Mate.

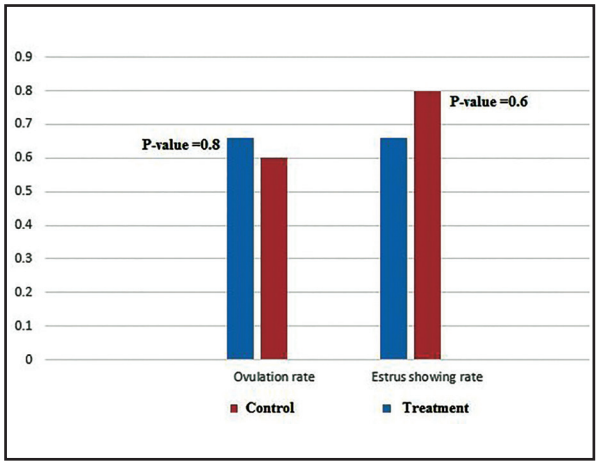

Figure 2. Comparison of proportion of mares ovulated or showed estrus behavior between treatment and control group.

during breeding season and increased risk of pregnancy at the end of it (Hanlon and Firth, 2012). Moreover, intravaginal devices designed for cattle, including CIDR (1.38 g P4), PRID (1.55 g P4) and cue-mate (1.56g P4) have been used off-label in mares and are effective in stimulating follicle growth in transitional mares (Klug and Jochle 2001). Regardless of shape and a little difference in amount of progesterone content, all of these devices have a silicone layer which slowly releases progesterone and maintains its plasma level over 1 ng/ml during 7 to 10 days of treatment period (Klug and Jochle 2001).

Even though artifcial photoperiod has been shown to be quite effective in hastening the frst ovulation of the year, mares still experience a transition period from winter anestrus to normal cyclicity. Conversely, administration of PRID to anestrus mares without sufficient pre-treatment artifcial lighting has limited value and generally results in the follicular growth without ovulation, hence combination of artifcial photoperiod for approximately 30 to 60 days and then 12 to 15 day exposure to exogenous progesterone after some follicular development on the ovary can be used to synchronize and accelerate the frst ovulation of the year, resembling applied procedure during vernal transition period (Squires, et al. 1979).

Most experiments about the use of PRID are confned to vernal transition mares. It has been reported that using PRID together with ovulation inducing agents (GnRH analogs and hCG) enhanced effcacy of both treatments in comparison with administration of each of them alone (Newcombe, et al. 2002). It should be mentioned that we did not use any ovulation agent in this study. Newcombe (2002) reported that PRID administration to photostimulated anestrus mares resulted in ovulation in 92% of mares, 6.6 days after PRID removal, our fnding about ovulation of 66% of mares, 5±2 days after removal is comparable with this result. Although positive effect of PRID for induction of estrus in transition mares has been reported (Hanlon and Firth, 2012, Kumar, 2011), based on our fnding no signifcant difference in rate of showing of estrus existed between treatment and control group. Sufficient effect of lighting therapy for induction of follicular growth in control group mares can justify our results. Lack of difference in time interval to ovulation from

beginning of lighting therapy in treatment and control groups mares also imply this point.

Regarding the size of ovulatory follicles, it has been reported that administration of CIDR in transitional mares stimulates follicular growth (Handler, et al. 2006), possibly due to mimicking changes of luteal phase progesterone level and enhancing LH concentration after day 6 of its insertion (Hanlon, et al. 2010). However, our results did not show significant differences in size of ovulatory follicles between treatment and control groups. The good results may arise from different study designs and time of transition phase in which treatment has been applied.

It has been reported that priming the follicles by progesterone decreased time interval of 20 mm follicle to 35 mm follicle (Staempfli, et al. 2011) and 35 mm follicle to ovulation (Newcombe, et al. 2002). According to our results, time interval from beginning of treatment at the time of follicular growth to 35mm, from 35 mm follicle to ovulation and from beginning of treatment to ovulation, were all lower in treatment group mares compared with control group. Lack of significance is probably due to low number of mares in the experiment. It seems that additional studies are needed to evaluate the effect of progesterone and lighting on induction of ovulation and hastening the first ovulation of the year in early winter anestrous. Also, ovulation induction agents such as hCG can also be applied in a discrete group to evaluate their effect on these criteria.

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References

Caniso, I.F., Gallacher, K., Gilbert, M-A., Korn, A., Schweizer, C.M., Bedford-Guaus, S., Gilbert, R.O. (2013) Preovulatory progestagen treatment in mares fails to delay ovulation. Vet J. 197: 324-328

Donadeu, F.X., Watson, E.D. (2007) Seasonal changes in ovarian activity: Lessons learnt from the horse. Anim Reprod Sci. 100: 225-242.

Ginther, O.J. (1974) Occurence of anoestrus, oestrus, dioestrus and ovulation over a 12-month period in mares. Am J Vet Res. 35: 1173-1179.

Ginther, O.J. (1979) Reproductive Biology of the Mare: Basic and Applied Aspects. Ginther, O.J. (ed.). (1st ed.) Published by author. Department of Veterinary Science, University of Wisconsin, Madison, Chapters 6 and 7, USA.

Grimmett, J.B., Hanlon, D.W, Duris, G.F., Jochle, W. (2002) A new intravaginal progesterone-releasing device (Cue-Mare (TM)) for controlling the estrous cycle in mares. Theriogenology. 58: 585-587

Handler, J., Schönlieb, S., Hoppen, H.O., Aurich, C. (2006) Seasonal effects on attempts to synchronize estrus and ovulation by intravaginal application of progesterone-releasing device (PRID TM) in mares. Theriogenology. 65: 1145-1158.

Handler, J., Schönlieb, S., Hoppen, H.O., Aurich, C. (2007) Influence of reproductive stage at PRID TM insertion on synchronization of estrus and ovulation in mares. Anim Reprod Sci. 97: 382-393.

Hanlon, D.W., Evans, M.J., Firth, E.C. (2010) Effects of intravaginal progesterone on follicu-

lar dynamics and FSH, LH, and progesterone concentrations in transitional mares. Anim Reprod Sci. 121: 32-34.

Hanlon, D.W., Firth, E.C. (2012) The reproductive performance of Thoroughbred mares treated with intravaginal progesterone at the start of the breeding season. Theriogenology. 77: 952-958.

Klug, E., Jochle, W. (2001) Advances in synchronizing estrus and ovulations in the mare: A mini review. J Equine Vet Sci. 21: 474-479.

Kumar, R., Phogat, J.B., Mann, J.S., Singh, U., Sharma, A.K. (2011) Estrus induction and fertility response in anestrus mares with exogenous progesterone releasing device (CIDR-B) during late breeding season. Biol Med. 3: 359-364.

Lopez-Bayghen, C., Zozaya, H., Ocampo, L., Brumbaugh, G.W., Sumano, H. (2008) Melengestrol acetate as a tool for inducing early ovulation in transitional mares. Acta Vet Hung. 56: 125-131.

McKinnon, A.O., Squires, E., Vaala, W.E., Varner, D.D. (2011) Equine Reprod. (2nd ed.). Wiley-Blackwell, UK.

Murphy, B.A., Walsh, C.M., Woodward, C.M, Prendergast, R.L., Ryle, J.P., Fallon, L.H., Troedsson, M.H. (2014) Blue light from individual light masks directed at a single eye advances the breeding season in mares. Equine Vet J. 46: 601-5.

Meyers-Browna, G.A., McCue, P.M., Troedsson, M.H.T., Klein, C., Zent, W., Ferris, R.A., Lindholm, A.R., Scofield, D.B., Claes, A.N., Morganti, M., Colgin, M.A., Wetzel, R.L., Peters. (2013) Induction of ovulation in seasonally anestrous mares under ambient lights using recombinant equine FSH (reFSH). Theriogenology. 80: 456-462.

Newcombe, J.R., Handler, J., Klug, E., Meyers, P.J., Jöchle, W. (2002) Treatment of transition phase mares with progesterone intravaginally and with deslorelin or hCG to assist ovulations. Vet Rev. 22: 57-64.

Newcombe, J.R. (2002) Field observation on the use of a progesterone-releasing intravaginal device to induce estrus and ovulation in seasonally anestrus mares. J Equine Vet Sci. 22: 378-382.

Panzani, D., Zicchino, I., Taras, A., Marmorini, P., Crisci, A., Rota, A., Camillo, F. (2011) Clinical use of dopamine antagonist sulpiride to advance first ovulation in transitional mares. Theriogenology. 75: 138-43.

Staempfli, S.A., Clavier, S., Thompson, D.L., Burns, P.J., Lyle, S.K., McKinnon, A.O. (2011) Effect of a single injection of long-acting progesterone on the first ovulation in early and late spring transitional mares. J Equine Vet Sci. 31: 744-748.

Squires, E.L., Stevens, W.B., McGlothlin, D.E., Pickett, B.W. (1979) Effect of an oral progestin on the estrous cycle and fertility of mares. J Anim Sci. 49: 729-735.

Squires, E.L., Heesemann, C.P., Webel, S.K., Shideler, R.K., Voss, J.L. (1983) Relationship of altrenogest to ovarian activity, hormone concentrations and fertility of mares. J Anim Sci. 56: 901-910.

Squires, E.L. (2008) Hormonal Manipulation of the Mare: A Review. J Equine Vet Sci. 28: 627-634.

Talebkhan Garoussi, M., Golzar, E. (2012a). Survey of clinical effects of norgestomet implant and estradiol valerate on conception rate of subfertile high yielding Holstein dairy cows. Iranian Journal of Veterinary Clinical Sciences. 6: 71-78.

Talebkhan Garoussi, M. Gallehdar, E., Farzaneh, N., Mohri, M. (2012b). The effect of Norgestomet on the reproductive performance of out of breeding season of ewes with or without PMSG. Trop Anim Health Prod. 44: 965-968.