The first ovulation of the breeding season in the mare: the effect of progesterone priming on pregnancy rate and breeding management (hCG-response rate and number of services per cycle and mare)

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Con formato: Inglés (Reino Unido)

Abstract

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The mare is a seasonally polyestrous breeder. In early spring, the mare enters a "transition period" between the anovulatory season and the first ovulation of the year. This period is characterized by irregular estrous cycles and high incidence of regressing dominant follicles. There is a belief that pregnancy rates resulting from the first ovulation of the season is lower than in subsequent ovulations, however this has never been studied critically. There is the belief of that pregnancy rate in the first ovulation of the season is lower than in subsequent ovulations, however this has never been studied critically. Progestagens are often used as an aid to manage this difficultthe transition period. The objective of this study was to compare pregnancy rates of mares in from the first ovulation of the year with: a) mares in on their second or subsequent ovulations; and b) mares with progesterone-primed first ovulations. A total of 136 Thoroughbred mares were used in the study. The mares were classified into four groups: 1) mares

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mated ~~in~~ at the first ovulation of the year (n = 46); 2) mares mated in the first ovulation of the year after removal of a previously inserted progesterone device (CIDR) (n = 29); 3) mares mated at the second or more ovulations of the year after prostaglandin-induced estrus, (n = 50); and 4) mares mated after spontaneous return to estrus (n = 11). Pregnancy rates were not different in any of the groups studied: 65.2, 75.9, 76 and 72.7 for groups 1 to 4 respectively ($p > 0.05$). Group 1 mares had the lowest response to hCG treatment ~~and highest cross-cover rate~~ which resulted in the highest number of services per cycle ($p < 0.05$). In conclusion, although the use of progesterone priming~~, although~~ did not affect pregnancy rates, it did improve the breeding management of transitional mares by enhancing the hCG response rate and consequently decreasing the number of ~~covers~~ services per cycle.

Keywords: Transition; Mare; Pregnancy rate; First ovulation; Progesterone

1. Introduction

The mare is a seasonally polyestrous breeder characterized by cessation of cyclicity in the autumn as daylight decreases. At the beginning of the breeding season, in early spring, the mare enters a "transition period" between the anovulatory season and the first ovulation of the year characterized by a resurgence of follicular activity, irregular exhibition of estrous behaviour and resumption of secretion of gonadotrophins and ovarian steroids (Ginther, 1992a).

In practice this transition period is difficult to manage for the veterinarian and the stud manager. ~~Often clients~~Clients may pressure the stud manager to produce early foals, especially in the Thoroughbred industry. ~~Likewise~~ Consequently veterinarians are pressured to have mares cycling, ~~covered~~ mated and ovulated early in spring which is not yet the physiological breeding time. This pressure for early covers results in higher number of services per mare and cycle (cross-cover rate) since it is difficult to predict whether a follicle will ovulate or regress even for an experienced practitioner with or without the use of ~~ovulatory~~ ovulation-inducing drugs. In a study on the efficacy of hCG to hasten ovulation in transitional mares, the average time from hCG administration to ovulation was 67.2 hours (Carnevale et al., 1989), ~~this is far from the average time~~ which is nearly twice as long in cyclic mares (36 ± 4 h) (Samper, 2008). The increase in ~~cross-cover rate~~the number of services per cycle and mare results in higher labo~~u~~r-cost for the ~~stud staff~~farm and veterinarians and may reduce the availability of busy stallions~~ with busy stud books~~.

Progestagens have been largely used to manage more reliably the transition period and to advance the first ovulation of the year. Amongst others, progesterone-releasing intravaginal devices have been ~~proved~~ shown to be reliable in both advancing the first ovulation of the year (Newcombe, 2002) and reducing the number of ~~cross-covers~~services per cycle and ~~per~~ mare in the transition period (Newcombe et al., 2002).

About 50 % of the mare population develop~~s~~ one to three anovulatory waves with large follicles (> 40 mm) that regress before the ovulatory wave (Ginther, 1990). Anovulatory large follicles are different from similar-sized preovulatory follicles ~~at several levels:~~for several reasons: First, the magnitude of LH surge is lower during the anovulatory waves and the first ovulation than in subsequent ovulations (Fitgzerald et al., 1984). Anovulatory follicles grow at a slower rate (about 1 mm/day) and may reach larger diameters than ovulatory follicles. Histologically, anovulatory large follicles express fewer growth factors (IGF-I and II, Watson et al., 2004; VEGF, Watson and Al-ziabi,

2002) necessary for the ovulatory process. All these differences might account for the lower response rate to ~~ovulatory~~ ovulation-inducing drugs during this period.

There is no scientific evidence in the mare whether pregnancy rate ~~in the~~following the first ovulation of the year is lower than in subsequent ovulations. In other species ~~of~~ that possess reproductive seasonality, such as small ruminants, pregnancy rates following first estrus and ovulation are considerably lower than in later ovulations especially early in the anovulatory season, with subsequent shortened luteal phases in non-pregnant ewes and goats (Dawson, 2007; Keisler, 2007). Similarly, the first ovulation of cows following parturition in which ~~there was~~the ~~basal~~ preceding progesterone concentration was basal, is less fertile than subsequent ovulations (Shaham-albalancy et al., 1997).

The objective of this study was to compare pregnancy rates of mares ~~in~~ with the first ovulation of the year with: a) mares in their second or subsequent ovulations; and b) mares with progesterone-primed first ovulations.

2. Materials and methods

2.1. Animals

All mares included in the study were resident to a stud farm in the southern hemisphere (Victoria, 39 º S). All animals were Thoroughbred and had no foal at foot (barren or maiden mares). Mares were kept ~~on~~ in large paddocks and were fed ~~on~~ hay, grass and cereal grain. Age was known for each mare. Mating within the same farm was by natural ~~cover~~ service to stallions of known fertility chosen by the owner.

2.2. Reproductive data

Ultrasonography of the reproductive tract was performed by transrectal examination with an ultrasound scanner equipped with a 7.5 MHz linear array probe. Examinations were performed ~~with~~ on a daily basis starting 20th August. Reproductive data was used from 1st September to 1st December 2008. The following parameters were recorded:

- Stage of estrous cycle: a) anestrus: ~~frequent ultrasound examinations revealed absence of ovulations with subsequent CL formation and follicles < 15 mm for more than 15 days~~absence of a CL or follicles > 15 mm in diameter for more than 15 days. Anestrous mares could either enter a transition period with development of one or more anovulatory follicular waves before the first ovulation of the season or develop directly an ovulatory follicular wave; b) cyclinge: spontaneous regression of a previously functional CL followed by estrus and ovulation (2nd or more ovulation of the season); c) PG: prostaglandin-F2alpha-induced regression of a previously functional CL followed by estrus and ovulation (2nd orf more ovulations of the year).
- Insertion of progesterone intravaginal devices: mares in anestrus with follicles ≥ 25 mm (with or without endometrial edema) ~~but that~~ failed to progress to > 35 mm within 4 days were administered 1.55 g of progesterone inas an intravaginal device (CIDR-B®, Pfizer Australia Pty Ltd. West Ryde, NSW, Australia) for 8 to 12 days. Treatment failure was determined when no new follicle > 35 mm, estrus and endometrial edema developed within 5 days of device removal.~~No response to treatment was considered when no new follicle > 35 mm, estrus and endometrial edema developed within 5 days of device removal.~~

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- Number of services: only data ~~of mares mated first time in the season~~from the first breeding cycle in the season (1st service pregnancy rate) was used in the study.
- Follicular diameter: diameter of the largest follicle at each examination was recorded.
- Interval from mating to ovulation (IMO): interval in days from the day of mating to the day of detected ovulation~~ovulation~~ was recorded for each mare. If ~~the interval~~this was longer than 3 days~~ before ovulation was detected~~, the mare was mated again.~~ (cross-cover).~~
- Pregnancy rate (PR): pregnancy diagnosis was performed ~~in~~ on 3 occasions: 13, 28 and 45 days post-ovulation. Pregnancy rate refers to whether the mare was diagnosed positive at the first examination; whereas embryo loss rate (ELR) refers to whether a pregnancy was lost at any of the subsequent examinations (within 45 days).
- Response to ovulation~~ induction ~~ inducing drugs: All mares received 1500 IU hCG (Chorulon®, Intervet Australia Pty. Limited, Bendigo East, VIC, Australia) subcutaneously on the day of mating. Criteria for the use of hCG was based on follicular diameter (> 35 mm) and moderate to heavy endometrial edema. The percentage of mares ovulating within 48 h of hCG treatment was recorded. ~~If~~Those mares which did not ovulate within 3 days~~, they~~ were mated again and a short acting implant containing 2.1 mg Deslorelin (Ovuplant, Peptech Animal Health, Sydney, Australia) was implanted subcutaneously in the neck. ~~administered~~.

2.3. Post-mating protocol

All mares were routinely infused with a combination of intra-uterine antibiotics (6 ml of gentamicin and 6 ml of benzyl-penicillin) 24 h post-mating. Oxytocin (25 IU) was administered once intravenously 24 h after antibiotic infusion. Anestrous mares treated with progesterone were mated ~~at least~~no earlier than 48 h after device removal. All PG-induced mares were mated at least 6 days after prostaglandin F2alpha treatment.

2.4. Experimental design

For data analysis, mares were classified into 4 groups: 1) mares mated ~~in~~ on the first ovulation of the year (non progesterone-primed; n = 46); 2) mares mated ~~in~~ on the first ovulation of the year after progesterone device removal (progesterone-primed; n = 29); and 3) mares mated ~~in~~ on the second or ~~more~~ later ovulations of the year after prostaglandin-F2alpha-induced estrus, (PG-induced, n = 50); and 4) mares mated after spontaneous return to estrus (Cycle, n = 11). For these four groups, statistical differences in PR, ELR, IMO, age and ~~cross-cover rate~~ number of services per cycle and mare w~~as~~ere ~~tested~~ analyzed (Table 1).

In addition, for groups 1 and 2, differences in the interval from follicular diameter of 35 mm to ovulation, follicular diameter of largest follicle at hCG treatment and percentage of mares ovulating within 48 h of hCG treatment were also statistically ~~tested~~ determined (Table 2).

Finally, within group 1, differences in the factors stated above for pregnant and non-pregnant mares were ~~analysed~~analyzed (Table 3).

2.5. Statistical analysis

Categorical data such as PR, ELR, % of mares ovulating within 48 h of hCG treatment and ~~cross cover rate~~% of mares mated more than once at the same estrus were ~~analysed~~analyzed by Chi-square or Fisher's exact test accordingly. Numerical data (age, IMO, interval 35 mm to ovulation and follicular diameter at hCG) were tested for normality and ~~analysed~~analyzed with non-parametric (Mann-Whitney and Kruskal-Wallis) or parametric (one way ANOVA and two samples t-test) tests accordingly. All data were computed in the statistical software Minitab15®.

3. Results

First service pregnancy rate was not different ($P > 0.05$) for any of the groups ~~analysed~~analyzed: 65.2, 75.9, 76 and 72.7 % for groups 1 (1^{st} ovulation), 2 (1^{st} ovulation progesterone-primed), 3 (2^{nd} or more ovulations of the season) and 4 (spontaneous return to estrus) respectively. The percentage of mares mated more than once on the same estrus ~~The number of covers per mare and cycle (cross cover rate)~~ was higher ($P = 0.04$) in group 1 (21.7 %, 10 mares out of 46 had to be mated more than once on the same cycle) than in the rest: groups 2 (3.4 %, 1/29), 3 (4 %, 2/50) and 4 (0 %, 0/11). The rest of reproductive parameters for groups 1 to 4 are shown in Table 1. Progesterone-primed transitional follicles (group 2) had a higher ($P < 0.01$) response rate to hCG than non-primed follicles (group 1): 93.1 % of group 2 mares ovulated within 48 h of hCG treatment as opposed to only 58.7 % in mares from group 1. Follicles of group 1 mares took longer ($P < 0.01$) to ovulate once they reached 35 mm (median interval 5 and 4 days for groups 1 and 2 respectively). ~~In spite of~~ Despite the use of ovulatory drugs at the time of mating, 3.3 % (1/30) and 8 % (4/50) of mares from groups 1 and 2 respectively regressed their dominant follicles and entered again a variable period of follicular activity. One mare from group 2 did not respond to progesterone treatment. It was notice that all mares inserted with CIDR presented at the time of device removal some degree of vaginitis as evidenced by purulent vaginal discharge around the used device. The vaginal discharge disappeared quickly which was no longer evident by the day of mating 2 to 3 days later. All reproductive data for groups 1 and 2 are~~is~~ shown in Table 2.

Follicles of group 1 mares that ended in fertile ovulations with subsequent conceptions took significantly shorter (P = 0.04) from 35 mm to ovulation (4 days) than those of non-pregnant mares (6 days). The rest of parameters ~~analysed~~analyzed for pregnant and non-pregnant mares within group 1 were not significantly different (Table 3).

4. Discussion

The objectives of this study were to compare pregnancy rates of cyclic mares with those of transitional mares in their first ovulation of the year from both spontaneous and progesterone-induced ovulations. No significant difference was found amongst any group; however, while the pregnancy rates in groups 2, 3 and 4 were very similar at about 2.5 % difference, in the spontaneously ovulated group this was noticeably high by anther 10 %. This difference may well have been significant had it been maintained in a larger number of mares. ~~nonetheless~~In addition, mares with spontaneous 1st ovulations had ~~some~~ other differences with progesterone-primed mares that might have relevant implications to fertility and breeding management. Such differences were lower response rate to hCG, delayed ovulation and higher ~~cross-cover rate~~number of services required per cycle and mare.

4.1. Response rate to hCG

It is widely assumed that following administration of 1500-3000 IU of hCG in mares with a dominant follicle > 35 mm, ovulation will occur within 36 to 48 h (on average 36 h) post treatment in about > 80 % of treated cyclic mares. Although there is a certain variation in the response rate attributable to individual mare factors such as age, stage of estrus, ~~and~~ follicular diameter (Barbaccini, 2007; Samper, 2008) and~~,~~ the most significant factor affecting the response rate to hCG, ~~is~~ the presence of anti-hCG interfering antibodies (Siddiquie et al., 2008). Apparently none of these factors seemed to be the reason why the response rate was significantly lower in group 1 (Table 2). Neither age nor follicular diameter were different in group 1 and 2, nor was the criterion for when to administer hCG. The barren/maiden ratio was not different either, so similar hCG ~~antibodies~~ antibody level should be assumed. The decision on whether a mare was allocated to group 1 or 2 was entirely dependent on follicular activity: in most occasions, progesterone was given to mares with follicles of 25 to 30 mm that failed to progress to larger follicles or show prominent endometrial folding and estrus. In this regard it could be argued that this group of mares was ~~in~~ at a less advanced stage within the transition phase than mares of group 1 which spontaneously developed larger follicles (35 to 40 mm) and showed clinical signs of estrus. ~~and therefore~~Therefore, even a lower response rate would have been expected in progesterone-primed mares.

~~Peripheral LH concentration increases immediately after hCG administration in a similar way that the pre-ovulatory LH surge observed in non-treated mares.~~ The LH surge, either in spontaneous ~~ovulations~~ or hCG-treated ovulations, triggers the cascade of events-by binding to the follicular wall LH-receptors- necessary for the ovulatory process including the resumption of development of the oocyte from an arrested stage (Meiosis I) to the secondary oocyte (Meiosis II) (Ginther, 1992b).

Transitional follicles ~~somehow~~ might lack ~~of~~ a sufficient number of LH-receptors to respond to hCG at a similar rate ~~than~~ to progesterone-primed follicles. ~~However, at the moment this is only a theory. T~~at present the hCG response rate ~~for this study~~ (% of ~~ovulated mares~~mares ovulating within 48 h of hCG treatment) was 93.1 % for progesterone-primed mares and 58.7 % for non-progesterone treated mares ($P < 0.01$). In this regard, progesterone-treated mares of group 2 may have developed follicles under more physiological conditions ~~typical of~~similar to those ~~in~~ of cyclic mares. Progesterone releasing devices are designed to mimic the progesterone concentration ~~pattern~~ of the luteal phase. The mechanisms by which progesterone was able to make follicles more responsive to hCG remains to be elucidated.

The difference in hCG response rate between pregnant and non-pregnant mares of group 1, 67.7 and 43.7 % respectively (Table 3) might also indicate some involvement of follicular LH receptors on oocyte quality and pregnancy rate provided that the interval from mating to ovulation and the number of services per cycle and mare in both groups was not statistically different. ~~.~~

In an early study, pregnancy rate after equine pituitary extract (EPE) follicular stimulation and ovulation of mares in late anestrus / early transition was significantly lower than in non-treated mares that took longer to grow follicles and ovulate (Lapin and Ginther, 1977). ~~It seems that pregnancy rate following artificially induced ovulations in mares too far from the physiological breeding season is disappointingly low.~~

4.2. Interval from 35 mm to ovulation

The lower response to hCG of follicles from group 1 ~~as~~ when compared with progesterone-primed follicles may account, at least in part, for the longer interval required by the follicle to ovulate. It is known however that transitional follicles grow ~~slower~~ more slowly (about 1 mm a day) and may reach larger follicular diameters before the 1st ovulation of the year than follicles of subsequent ovulations (Ginther, 1990). This seems to be due to differences in LH concentration. As a result of a longer interval to ovulation and lower response rate to hCG, group 1 mares had to be mated more times per cycle than progesterone-treated or cyclic mares (Table 1). The resultant increased cross-cover rate per cycle is undesirable for the stud management, busy stallions and uterine health of mares susceptible to endometritis.

In addition, delayed ovulation has been linked to reduced fertility and embryonic abnormalities in the cow (for review see Inskeep, 2004). It appears that oocytes from persistent follicles are subject to prolonged exposure of estrogens and LH resulting in aged, infertile oocytes.

4.3. Effect of progesterone levels during the follicular growth phase on pregnancy rate

Very little research ~~if some~~ has been done on the effect of progesterone levels during the preceding follicular development on oocyte quality and pregnancy rate in the mare. The results of the present study show~~ed clearly~~ a better response rate to hCG and shorter interval from 35 mm to ovulation of follicles developed under ~~progestagen~~progesterone~~s~~ influence. In cattle however, this phenomenon has been studied in more depth (Shaham-Albalancy et al., 1997). Experimentally-induced low ~~(Shaham-Albalancy et al., 1997)~~ or basal progesterone concentration (~~as~~ seen ~~during the~~

preceding ~~stage to~~ the 1st ovulation in the post-partum cow) affected fertility in two ways: 1) increasing concentrations of estradiol and LH during early follicular development with a resultant earlier oocyte maturation; and 2) altering the endometrial morphology with subsequent ~~increment~~ increase in secretion of ~~prostaglandin~~ PGF2alpha in response to oxytocin resulting in decreased fertility even though the original oocyte was healthy (Shaham-Albalancy et al., 1997).

In conclusion, treatment of transitional mares with intravaginal progesterone-releasing devices has significant advantages in improving the breeding management during a difficult time of the year for both the veterinarian and the stud manager.~~:~~ ~~this~~ This improvement is mainly due to higher follicular response rate to hCG in progesterone-primed mares. The only apparent disadvantage of using intravaginal progesterone devices appears to be the purulent vaginal discharge found on device removal which however only seems to be aesthetical since it does not negatively affect pregnancy rates. Secondly, the results presented here may be encouraging for researchers willing to study the possible difference in oocyte quality between transitional and cyclic mares, the involvement of circulating progesterone concentrations during the preceding follicular development phase and its implications on fertility.

Acknowledgments

The author Juan Cuervo-Arango thanks Mr. J.R. Newcombe for his guidance on using the protocols of progesterone intravaginal devices in transitional mares.

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