Effects of sunlight hours and hormones on double ovulation, and singleton and twin pregnancies in mares

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Abstract

Equine twin pregnancies are almost exclusively dizygotic, without the application of advanced reproductive technologies, requiring 2 ovulations in 1 estrous cycle. Breeding records were used to determine the effects of sunlight hours, prostaglandin $F_{2\alpha}$, human chorionic gonadotropin, deslorelin (a gonadotropin releasing hormone agonist), and progesterone and estradiol on double ovulation rates, and singleton and twin pregnancy rates. Breeding records of mares (n = 267) and their estrous cycles (n = 914) were analysed. Double ovulations occurred in 10.5% (96/914) of estrous cycles. Twin pregnancies were observed in 42.7% (38/89) of mares that had double ovulations. Overall, per estrous cycle pregnancy rate was 47.2% (405/858) and twin pregnancies was 4.4% (38/858). Double ovulations had higher ($p < 0.001$) per cycle singleton pregnancy rate (69.7%; 62/89) than 1-ovulation cycles (44.6%; 343/769). Deslorelin increased ($p < 0.05$; OR =1.24 95% CI) double ovulations and human chorionic gonadotropin tended ($p = 0.089$; OR =1.68; 95% CI) to increase double ovulations. Deslorelin use resulted in an odds ratio of 2.47 for a positive pregnancy (either singleton or twin) diagnosis compared to cycles without deslorelin use. None of the factors examined had a substantial impact on twin pregnancy rates.

Keywords: Equine twins, double ovulation, deslorelin, sunlight, pregnancy rate

Introduction

Equids are seasonally polyestrus with most mares in anestrus during winter. Melatonin, present at higher concentrations in long periods of uninterrupted darkness, inhibits gonadotropin releasing hormone (GnRH) secretion from hypothalamus. Consequently, concentrations of luteinizing hormone and follicle stimulating hormone are reduced, resulting in anestrus.[1] In late winter and early spring, ambient daylight lengthens in duration and intensity, initiating spring transition. With an average duration of 45 days, sufficient gonadotropin concentrations are reached at the end of spring transition, for the first ovulation of the year to occur. Regular cyclicity resumes at this point, initiating the physiologic breeding season.[2] Hormone therapy and photoperiod adjustments are used to manipulate estrous cycle by hastening spring transition, with the goal for an earlier physiologic breeding season. A photoperiod length of at least 16 hours in 24 hours at an adequate intensity of light (> 100 lux), is required to advance spring transition.[2] Other reproductive effects of increased photoperiod length include: increased testicular size, sperm production and output in stallions, earlier onset of puberty, shorter pregnancy length, and increased foal weight.[3,4]

Daylength has less influence on double ovulation and twinning rates than other factors (e.g. breed, age of mare, and use of reproductive medications).[5] However, in a population of European Thoroughbreds,[6] increase in twin pregnancy rate was observed in June/July (1.76%; 40/2157) compared to February/March (0.9%; 29/3229); however, double ovulation rate was not provided. Increased double ovulation rates were also observed in August (16.5%) and September (26.5%) compared to other months in a population of predominantly American Standardbreds located in Finland.[7]

Reported double ovulation rates ranged widely (0.83 - 42.8%), depending on the population studied.[6,8] Double ovulation is a prerequisite for dizygotic pregnancies.[9] Survival of sperm in uterine tubes up to 7 days is a reason to have embryonic vesicles that are substantially different in size.[10] Twin pregnancies that survive beyond 40 days of pregnancy often suffer from placental insufficiency. This can lead to abortion, fetal mummification, dystocia and birth of small, weak foals and negatively impact the mare's foaling ability in the following breeding seasons.[9-13]

Ovulation inducing agents increase the efficiency of equine reproduction programs.[14] By inducing ovulation in a predictable time frame, typically within 48 hours of a well timed treatment, less inseminations per estrous cycle were needed and odds of achieving optimal timing of insemination relative to ovulation was higher.[14] Increases in pregnancy and twinning rates were observed with human chorionic gonadotropin (hCG) and deslorelin, and a GnRH analogue.[9,13] In an Australian study, pregnancy rates were 72.0% (147/204), 72.0% (1042/1447), and

64.5% (527/817) for deslorelin, hCG, and control, respectively.[5] Use of hCG in a population of Thoroughbred mares tended to increase pregnancy rates (OR 1.21; p = 0.06) and twinning rates (OR 2.18; p < 0.05).[15] However, these observations could vary with the population of mares studied. In another study, hCG had no effect on double ovulation or twinning rates,[7] but this same population had a substantial increase in double ovulation rates with prostaglandin $F_{2\alpha}$ treatment compared to cycles managed without a hormone (16.2 versus 9.9%).[7] Increased twinning in a Thoroughbred mare population with use of hCG alone was less remarkable than the combined use of hCG and prostaglandin $F_{2\alpha.}$ Combination of medications resulted in an odds ratio of 6.37 for twin pregnancies compared to controls.[15] Effects of a progesterone and estradiol $17_{ß}$ (P&E) protocol on double ovulations and twin pregnancy rates were not extensively studied.

Reproductive hormones are extensively used in mares that have a short breeding season (delayed spring transition) due to their geographic location. Combined impacts of reproductive hormones and daylight hours on double ovulation rates and pregnancy rates for these regions is not known. We hypothesized that both reproductive manipulations and increasing daylight hours would increase double ovulation rate. Objective was to analyze the effects of daylight hours and use of prostaglandin $F_{2\alpha,}$ deslorelin, hCG, and P&E on the rates of double ovulation and resulting singleton and twin pregnancy rates

Materials and methods

Breeding records (2011 - 2019) maintained at the institution for client-owned mares (n = 267) and their estrous cycles (n = 914) were used. Mares were located at 46.50N longitude and 63.40W latitude. Over 50% of the broodmares were Standardbreds, with warmbloods, Quarter Horses, and draft breeds represented to a lesser degree. Mare age was not recorded in the records. Mares in the study carried their own pregnancies. Breeding records included: date of examination, ovarian activity, uterine abnormalities, presence of embryonic vesicle(s), endometrial cysts, cervical tone, stallion name, and reproductive treatments. Fresh semen was the predominant type of semen used in these cycles in addition to cooled, shipped, and frozen semen.

Mares were examined at 12 - 16, 24 - 28, and 60 - 70 days postovulation. A mare was classified as pregnant if ultrasonographic evidence of pregnancy was observed. Diagnosis of singleton or twin pregnancies was made at the initial pregnancy examination and this pregnancy status was used for analysis. Twin pregnancies that were manually reduced to singleton pregnancies remained classified as twin pregnancies for the purpose of this study. Pregnancy outcomes were unknown in 49 single ovulation cycles and seven double ovulation cycles. These cycles were therefore not included in the pregnancy rate calculations.

Values for daylight hours were rounded to the nearest half decimal to create uniformity throughout the dataset and an interval of a half hour within the statistical analyses. Medications used include: dinoprost 5 - 10 mg IM (Lutalyze®, Zoetis Canada Inc. Kirkland, QC, Canada), cloprostenol 0.25 - 0.5 mg IM (Estrumate®, Intervet Canada Corp., Kirkland, QC, Canada), deslorelin 1.5 mg IM (Trutina Pharmacy Inc., Ancaster, ON, Canada) and hCG 2500 - 5000 IU IV (Chorulon®, Intervet Canada Corp., Kirkland, QC, Canada). Protocol for P&E (Chiron Compounding Pharmacy Inc, Guelph, ON, Canada) treatment was: daily 150 mg progesterone and 10 mg estradiol $17_{ß}$ IM for 10 days, with 0.25 - 0.5 mg cloprostenol IM on the 10th day. After cloprostenol treatment, 2500 - 5000 IU of hCG was given when a 35 mm follicle was present (~ 7 - 9 days).

Data analyses

Data were analyzed using a statistical software program (Minitab 19, Minitab LLC, State College, PA). Singleton pregnancy rates with single and double ovulations cycles were compared using a two-sample test of proportions. Odds ratio was used to compare odds of a pregnancy in a cycle that used deslorelin with odds of a pregnancy that did not use deslorelin. Sunlight hours, prostaglandin $F_{2\alpha,}$ deslorelin, and hCG treatment in relation to double ovulation rates were evaluated using multivariable analysis to determine odds ratios with a high and low CI of 95%. Use of P&E and twin pregnancy rate was evaluated using Fisher's exact test. Significance was established at $p \leq 0.05$. A tendency was established at p = 0.06 - 0.10. Generalized estimating equation (GEE) method (Stata, StataCorp) was used to account for dependence of multiple records per mare.

Results

Per cycle double ovulation rate was 10.5% (96/914), pregnancy rate was 47.2% (405/858), and twin pregnancy rate was 4.4% (38/858). Double ovulations had a higher (p < 0.001) per cycle singleton pregnancy rate (69.7%; 62/89 [95% CI: 60.1 - 79.2%]) than single ovulations (44.6%; 343/769 [95% CI: 41.1 - 48.1%]). Mares with double ovulations had a twin pregnancy rate of 42.7% (38/89).

Deslorelin used cycles (n = 330) had 2.47 times more positive pregnancy examination, either singleton or twin, ([95%; CI:1.85 - 3.32] p < 0.001) compared to cycles (n = 528) that did not use deslorelin. Per cycle pregnancy rate of cycles that used deslorelin was 60.9% (201/330) and was 38.6% (204/528) in cycles that did not use deslorelin.

Results of logistic regression analysis (both for double ovulations and twin pregnancies) are presented (Table). Increasing daylight hours had no significant effect. Deslorelin increased (p = 0.045; OR = 1.57) the rate of double ovulation. A tendency (p = 0.089) towards increasing double ovulations occurred with hCG. Prostaglandin $F_{2\alpha}$ and P&E administration had no impact on the rate of double ovulation. None of the factors examined had substantial impact on twin pregnancy rates.

Individual mares repeated for several years and multiple cycles were evaluated in a single year. The assumption of independence could not be held true due to these factors. The mean gap between cycles of the same season was 21.3 days (standard deviation 11.5 days), which is accurate to the biology of equine estrous cycles. Data were then evaluated using the GEE method and results were similar. Original multivariable logistic regression was used for analysis.

Discussion

Effect of daylight hours on double ovulation rates or twin pregnancy rates was not substantial. Study location (eastern Canada) has a large seasonal variation in daylength, with > 15 daylight hours during summer solstice and < 9 hours during winter solstice.[16] Since appreciable variation (northern location) in daylength has not impacted the rate of double ovulation it is reasonable to assume that daylight hours in equator areas might have minimal impact. Further study with a larger number of cycles would be worthwhile to determine if significance could be established between daylight hours and multiple ovulations or multiple pregnancies.

Table. Odds Ratio for variables listed for double ovulations and twin pregnancies

	Double ovulation		Twin pregnancies	
Variable	**Odds ratio (95% CI)**	**p value**	**Odds ratio (95% CI)**	**p value**
Daylight	1.189 (0.956 - 1.478)	0.119	1.203 (0.815 - 1.774)	0.353
Deslorelin	1.569 (1.010 - 2.439)	0.045	0.946 (0.465 - 1.925)	0.878
hCG	1.6848 (0.924 - 3.071)	0.089	1.288 (0.512 - 3.238)	0.590
P&E	0.5053 (0.117 - 2.191)	0.362	*	*
Prostaglandin	1.242 (0.541 - 2.851)	0.609	1.826 (0.380 - 8.764)	0.452

*No significant difference between the use of P&E and the occurrence of twin pregnancies.

Rate of double ovulations (10.5%) was consistent with reports for Standardbreds (13 -15%).[5,7] Deslorelin increased (p = 0.045) double ovulation rate and hCG use had a trend (p = 0.089). Multiple ovulations are a requirement for dizygotic twins or multiple pregnancies. Higher singleton pregnancy rate observed with double ovulations cycles (69.7%; 62/89) compared to single ovulation cycles (44.6%; 343/769) suggested that double ovulation cycles could be desirable, especially, in an embryo transfer program. However, proper management of twin pregnancies resulting from double ovulations, within populations of mares intended to carry their own pregnancy, is essential. Early diagnosis of twins through prompt and repeated pregnancy examinations is essential, particularly, when a second dominant follicle is detected at insemination.

Records analyzed here reflect per cycle pregnancy rates for fresh semen, shipped cooled semen, and frozen semen. Evaluation of combined pregnancy rates, rather than separating pregnancy rate according to type of semen is a limitation of the study. Overall pregnancy rate of 47.2% in our study was lower than generally accepted pregnancy rates for fresh and shipped cooled semen of 50 -70%. Similar mare populations had pregnancy rates of 51.7 - 56.7% for fresh and shipped cooled semen.[7,17] We incorporated frozen semen cycles in our dataset which have generally lower reported per cycle pregnancy rates compared to fresh and cooled semen.[7,17] Combination of pregnancy results from several semen types may be the cause of a lower-than-expected overall pregnancy rate. Higher singleton pregnancy rate with double ovulation cycles than single ovulation cycles reflects that the mare's reproductive status can be as important as the semen type used on resulting pregnancy rates.

Use of deslorelin increased the odds of a pregnancy by 2.47 times. Association of an ovulation induction agent with an increased chance of pregnancy can be carried into clinical practice. Increased odds of pregnancy might be due to the predictable timing of ovulation from the use of deslorelin. Decreased time from insemination to ovulation for fresh and shipped semen and insemination close to ovulation for frozen semen breedings improved the odds of pregnancy. Less resulting inseminations per cycle improved stallion efficiency and minimized uterine inflammatory responses.[15] Deslorelin use increased rate of multiple ovulations that increased opportunities for fertilization in each estrous cycle. Use of deslorelin did not increase twinning rates in this population; however, it might have the benefit of increasing the odds of embryo recovery.

Conclusion

An increase in daylight hours and use of prostaglandin, and P&E had no influence on twin pregnancy rates. Use of hCG had a tendency to increase double ovulation rate. Deslorelin use increased rate of double ovulations per cycle and singleton pregnancy rate. Double ovulation cycles had a higher singleton pregnancy rate than single ovulation cycles.

Acknowldgements
Authors thank Henrik Stryhn and Jason Stull for assistance with statistical analysis.

Conflict of interest
None to declare.

Funding
Supported by 'Summer Canada Student Jobs' and a poster was presented at the 2020 National Veterinary Scholars Symposium.

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