Influence of period between breeding and ovulation on foal sex ratio

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Abstract

Objective was to determine the effect of period between breeding and ovulation (PBBO) on the sex of the foal. Foaling records (n = 288) from 2015 - 2018 breeding seasons and from 2 veterinary hospitals were used. There were 122 (42%) fillies and 166 (58%) colts. Foal sex records were categorized into 3 groups based on PBBO < 24 hours, 24 - 48 hours, and > 48 hours. There was an effect ($p < 0.0001$) of PBBO on the probability of the foal being a colt. If the PBBO was < 24 hours, the odds for a colt were 23.3 times higher compared to > 48 hours group (OR = 23.6, 95% CI: 3.0, 185, $p = 0.003$). Additionally, if the PBBO was < 24 hours, the odds for a colt were 4.6 times higher compared to 24 - 48 hours group (OR = 4.6, 95% CI: 2.0, 10.5, $p = 0.0003$). There were no differences ($p = 0.14$) between odds for a colt between 24 - 48 and > 48 hours groups. Confounding variables such as sire, age of dam, size of ovulated primary follicle, and type of semen (fresh or chilled) were not significant. Results indicated that the closer a mare is bred to ovulation with fresh semen, it is more likely that she will have a colt.

Keywords: Ovulation, sex-ratio, mare, stallion, natural breeding

Introduction

For most livestock species, there is an economical influence to have a certain sex of offspring. Livestock producers may increase the probability for progeny of the most desired sex by various means of manipulating the breeding processes. There are many options to preselect sex of offspring, including embryo transfer, sex-sorted sperm, and preimplantation genetic testing.[1,2] In equine industry, desired sex of offspring is dependent on the intended sport. For example, majority of top performing (polo) mares can be inseminated and capable of producing embryos concurrent to their sport careers for transfer to broodmare recipients, thereby maintaining financial productivity.[3] Colts are more desirable among racing Thoroughbreds because of higher sale prices and earning potential, and ability to be used as a breeding stallion after a successful career; a Thoroughbred mare can only produce 1 foal per year, whereas a stallion can breed many mares in a year.[4] Breeding restrictions placed on Thoroughbreds registered in Jockey Club also influence the desire for colts. Jockey Club does not approve artificial insemination (AI), cloning, and embryo transfer for Thoroughbreds.[5] Registered Thoroughbreds are required to be conceived only by natural breeding ('live cover'), making it difficult to manipulate the chances for a particular sex of a resulting foal.

Given breeding restrictions like those placed on Thoroughbreds, breeders consider other methods to increase the probability of desired sex offspring. Period between breeding and ovulation (PBBO) had an influence on resulting foal sex.[6] This approach can be a useful tool for breeders of horses such as the Thoroughbred where natural breeding is necessary. This can also be useful as a more economical method in preselecting foal sex. This retrospective study tested the merit behind the theory that the PBBO has an influence on resulting foal's sex. We hypothesized that closer a mare is bred to ovulation, the more likely that she will have a colt.

Materials and methods

Data from 2 veterinary hospitals for 2015 - 2018 breeding seasons were used. Electronic medical records included name of the mare, breed, date of birth, reproductive status, information regarding ovulation induction medications (type and date), hormones used, date of breeding, date ovulation detected, estimated period in hours from breeding to ovulation, number of ovulations, size of largest primary follicle, type of semen (fresh or chilled) used, stallion name, postbreeding treatments, fetal sex, sex of foal, and veterinarian. Breeding records of twin pregnancies were not used.

Animals

Two-hundred-eighty-eight foaling records were analyzed. There were 279 Thoroughbreds, 5 American Quarter Horses, 2 Paso Finos, 1 Arabian, and 1 Appaloosa mare. Mares bred were between 3 and 22 years. Every Thoroughbred mare was inseminated by natural breeding. Nine mares represented other breeds and were artificially inseminated (fresh or chilled semen). One hundred seven stallions were used to breed 288 mares. Stallions were between 3 and 24 years. There were 122 (42%) fillies and 166 (58%) colts.

Information selected

Periods between ovulation and breeding, sire, semen type (fresh or chilled), size of primary follicle, and mare's age. PBBO was categorized into 3 groups: mares that ovulated < 24 hours after breeding (Group 1), mares that ovulated 24 - 48 hours after breeding (Group 2), and mares that ovulated > 48 hours after breeding (Group 3). Sires were compared within each group regardless of breeding method (natural breeding or AI).

Data analyses

FREQ procedure was utilized to descriptively analyze frequencies of foal sex in relation to other possible explanatory variables. Additionally, FREQ procedure was utilized to assess number of each stallion's breeding. Each stallion was assigned to groups to test if frequently used stallions tended to yield more male or female foals. Finally, for inferential statistical analyses, separate, single variable generalized linear mixed models with binary distribution and logit link were used in the GLIMMIX procedure of SAS 9.4 (SAS Institute, Cary, NC) to model the probability of a foal being a colt. If variables were individually associated with foal sex, they were added into a model and manually eliminated in a backward fashion to elicit the most parsimonious explanatory model. The ILINK function was used to convert logits to mean probabilities. Model adjusted probability to yield a colt and standard errors are presented. Significance was set at alpha = 0.05. Records for mares that conceived twins were not used for statistical analysis due to difficulty in identifying those that were fertilized first.

Results

Period between breeding to ovulation

Descriptive statistics for the number of colts and fillies born to dams with each PBBO are provided (**Table**). There was an effect ($p < 0.0001$) of PBBO on the probability of having a foal colt. If the PBBO was < 24 hours, the odds for having a colt were 23.6 times higher compared to the odds for a colt in the > 48 hours group (OR=23.6, 95% CI: 3.0, 185, $p = 0.003$). Additionally, if the PBBO was < 24 hours, the odds for a colt birth were 4.7 times higher compared to the odds for a colt in the 24 - 48 hours group (OR = 4.7, 95% CI: 2.0, 10.5, $p = 0.0003$). There was no difference ($p = 0.14$) between odds for a colt in 24 - 48 hours and > 48 hours PBBO groups (**Figure**).

Table. Descriptive statistics reporting the number (percentages) of colts and fillies born to dams bred within PBBO categories

PBBO (hours)	Colts	Fillies	Total
< 24	156 (64%)	65 (36%)	242
24 - 48	9 (28%)	23 (72%)	32
> 48	1 (7%)	13 (93%)	14

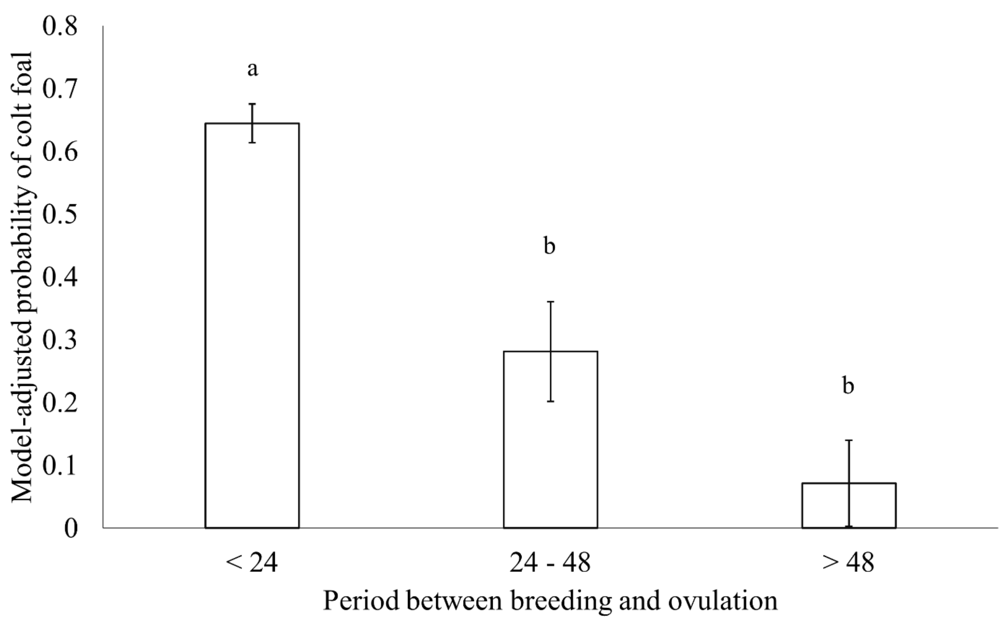

Figure. Model-adjusted probabilities for the outcome of a colt foal were different ($p < 0.0001$) for PBBOs of < 24 hours, 24 - 48 hours, and > 48 hours. Error bars represent 1 SE of the mean, and bars with similar letter are not different at $\alpha = 0.05$.

Sire

For the 288 foaling records, there were 107 exceptional stallions used as sires. Of the 107 sires, 19 were utilized to sire 5 or more foals. These 19 stallions were evaluated to determine if there was an association between sire and foal sex. Sire was not associated ($p = 0.87$) with the probability for a foal to be a colt.

Age of dam

Age of dam ranged from 3 to 22 years (mean = 8.56, median = 7.98). Age was considered a possible predictor of foal sex. For the purpose of analysis, mares' age was categorized into 4 groups: mares ≤ 4 years (category 1); mares 4.1 - 7.9 years (category 2); 8 - 11.9 years (category 3); and ≥ 12 years (category 4). There were 90, 93, 62, and 45 foals born to mares within the age categories 1, 2, 3, and 4, respectively. There was no relationship ($p = 0.76$) between mare age and foal sex outcomes.

Type of semen/method of insemination

There were 2 types of semen utilized in the dataset: fresh and chilled. There were 9 foals conceived using chilled semen compared to 279 conceived with fresh semen via natural breeding. There was no effect ($p = 0.15$) of semen type on foal sex. Although there was likely insufficient power to detect a difference in proportion of male or female foals to semen type, numerically 6 of 9 (67%) foals conceived with chilled semen were fillies, compared to 116 (42%) fillies of 279 conceived via natural breeding.

Size of ovulated primary follicle

Size of primary follicle ranged from 30 to 52 mm (mean = 40.08 mm, median = 40.0 mm). For analyses, follicle size was categorized into 3 groups based upon distribution of values. Categories 1, 2, and 3 corresponded to sizes 30 - 35, 35.1 - 42.9, > 43 mm, respectively. There was no association ($p = 0.28$) between primary follicle size category and foal sex.

Use of ovulation inducing hormones

Ovulation inducing hormones were utilized in 68 conceptions. These hormones included human chorionic gonadotropin (hCG), (2,500 IU; IV hCG, Chorulon: IntervetInc, Unterschleissheim, Germany), deslorelin acetate (1ml IM; SucroMate, Thorn BioScience LLC, Louisville, KY) and the combination of hCG and deslorelin acetate (SucroMate, Thorn BioScience LLC) at the same dose. Number of pregnancies after use of each hormone or hormone combination was 8, 58, and 2, respectively. We analyzed the binary variable (yes or no) of hormone used with regard to foal sex outcomes. There was no association ($p = 0.50$) of hormone use and foal sex.

Full model

Although the only variable associated with foal sex was PBBO, we ran the full model including age of dam, size of primary follicle, use of ovulation inducing hormones, and PBBO to determine if there were any major confounding variables. In this full model, only PBBO was importantly associated ($p < 0.0001$) with foal sex. Other variables did not affect foal sex, nor did they largely impact or confound the effect of PBBO.

Discussion

Influence of PBBO on foal sex support research conducted in humans and many animal species. Other factors tested, such as the sire and age of dam, did not influence sex ratio, although some studies have reported otherwise. Data from several studies in humans have indicated that if PBBO is < 24 hours, there was either an equal sex ratio or a skew towards males.[7]

Compared to Y, X sperm are more resilient, making it more likely for them to succeed in achieving fertilization under stressful environmental conditions.[6] Vulnerability of Y sperm is attributed to the act of early capacitation and consequently, due to prolonged stay in uterus and oviduct.[6] In humans, Y-bearing sperm had a substantially higher motility and swam through cervical mucus more efficiently than X-bearing sperm, and possibly provided more opportunities to fertilize ova around ovulation.[7] In deer, decreases and increases in sex ratio were observed based on early and late natural breeding, respectively. Mating that occurred within 36 hours of the onset of estrus (early), there was a decrease (27%) in number of male fawns. In mating that occurred within 37 - 96 hours after the onset of estrus (late), there was an increase (70%) in number of male fawns. PBBO effect in cattle was inconsistent.[6,8] In dairy cattle, there was an insignificant difference in sex ratio in various insemination-ovulation periods; early: 36 - 20 hours prior to ovulation; intermediate: 20 - 28 hours before ovulation; late: 8 - 12 hours after ovulation resulted in 50, 50, and 55% females respectively.[9] This effect has rarely been studied in the mare, furthermore the sample was not adequate.[10] However, sex ratio was not different even when a larger sample size (n = 433) was used.[11] Embryos (n = 16) derived from ovulations before 48 hours from AI resulted in more male offspring (57% males versus 43% females) than those (n = 10) that ovulated 48 hours after AI (30% males versus 70% females).[12] There was a significant difference among those embryos that were fertilized after 48 hours from AI to those that were fertilized before 24 hours[12] and no significant difference between those that ovulated between 24 and 48 hours. This was speculated to be due to unequal amount of resilience among X and Y sperm postbreeding, a possible reason for conflicting results on the impact of length of PBBO on sex ratios, particularly, in cattle that are synchronized for breeding. Result was also not significant in horses that tested this hypothes.[1] We suggest that PBBO has significant impact on the sex of the resulting foal, possibly due to the early capacitation of the Y sperm.

In horses, there may be an association among individual sires and sire's breed on secondary sex ratio.[13] Foaling records (n = 4,491) from 92 stallions (Thoroughbred, Arabian, and Akhal-Teke) indicated that sire's breed had an influence on offspring sex ratio. Arabian and Thoroughbred stallions were reported to have higher (63%) or lower (46.1%) offspring sex ratio than the expected 50%. Akhal-Teke stallions had 53.9%, not significantly different from the hypothetical expected 50%.[13] Results of the aforementioned study indicated that the probability of conceiving a certain foal sex can be increased with the use of semen from stallions that have a proven skewed ratio of progenies, although our results did not suggest that individual stallions are more likely to produce a specific sex of foal. This was possibly due to the lower number of stallions with multiple breeding records used in this analysis.

Age of the mare did not influence foal sex ratio. However, in a larger a study (n = 59,950) sex ratio decreased with an increase in mare's age.[14] More fillies were born to mares that were > 15 years compared to younger mares.[14] It was suggested that mare's age had a marked influence on offspring sex ratio.[14] Sex ratio of the offspring of the mare have been known to markedly deviate from the 1:1 ratio in situations of decreased body condition of the mare at conception.[10] Age of the mare could be correlated with a decreased condition or nutritional status that would skew resulting sex ratios at conception.[10]

There were not enough numbers of AI in our study to compare among methods of natural breeding and AI on the impact of the resulting sex ratio in horses. However, it has been suggested that sex ratio may not be different between frozen and fresh sperm use in women, and overall, AI appeared to favor male offspring (60%).[7] Evidence is accumulating that estrus synchronization or ovulation induction treatments in cattle may influence sex ratio.[6,8] Similar reports are not available for mares.

Conclusion

Closer a mare is bred to ovulation with fresh semen, the more likely that she will have a colt supporting the theory of resiliency of the X sperm and the long-term vulnerability of the Y sperm within the mare reproductive tract. Other possible factors (sire, age of dam, method of insemination, size of ovulated primary follicle, or use of ovulation inducing hormones) may not impact foal sex ratio.

Conflict of interest

None to declare.

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