



Pregnancy outcomes in thoroughbred mares inseminated with fresh semen from either stallion or jack at foal heat or subsequent estrus

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ABSTRACT

The current study investigated the effect of different mating (stallion vs jack) and breeding patterns (foal heat vs subsequent estrus) on the outcome of conception and foaling rates in thoroughbred mares. A retrospective study on the reproductive performance of 6,040 artificially inseminated thoroughbred mares during seven consecutive breeding seasons (2010–2017) has been taken into consideration for the current study. Mares were inseminated with fresh semen obtained from eight of each fertile horse stallions (Thoroughbred) and jacks (Poitou breed). The pregnancy status was examined at 30 (P30), 60 (P60) and 90 (P90) days post breeding and pregnancy rate was calculated as number of mares pregnant at 30, 60 and 90 day post-mating, out of total mares mated. Pregnancy Rates (PR) of mares inseminated at the foal heat were higher, but non-significantly different from PR of the subsequent estrus mares which did not become pregnant after artificial insemination (AI) at foal heat and inseminated again at the subsequent estrous cycle. There was also a significant difference in the PR and Fertility rate (FR) in mares inseminated either with stallion semen or with jack semen to produce horse and mule foals respectively. From the data on mares mated with horse stallion semen, higher PR as well as FR was found in comparison to the mares that were inseminated with jack semen. In conclusion, the present study found a significant effect of breeding pattern and mating pattern affecting both PR and FR in thoroughbred mares.

Keywords: Fertility, Foal heat, Foaling rate, Mule, Pregnancy rate, Reproductive performance, Ultrasonography

For obtaining the optimum economic return from broodmares, the number of foals produced per mare in her lifetime must be maximized (Blanchard *et al.* 2011, Ishii *et al.* 2001). The goal of producing one foal/ mare/year is achievable only if broodmares conceive within first 25d postpartum. This necessitates breeding mares during the three weeks after foaling to produce a foal each year (Malschitzky *et al.* 2015, Ricardo *et al.* 2020).

The mare is the only domestic animal showing ovulatory estrus shortly after foaling (Malschitzky *et al.* 2002, Camargo *et al.* 2017). The involution of the uterus in post-foaling mares is rapid, in comparison to other domestic livestock species (Camargo *et al.* 2017). Within 14 days, the uterus returns to the pre-pregnancy state, allowing for manifestation of fertile estrus (Blanchard and Macpherson 2011, Scoggin 2015). Most equine breeders would like foaling mares to become pregnant as soon as possible after foaling because, foals born early in the season are physically more advanced at time of sale and also have a greater likelihood of realizing their performance potential as draft or race horses (Ishii *et al.* 2001, Morris and Allen 2003). Improvement in reproductive performance at the foal heat

is, therefore, an important consideration in horse breeding tracts. Pregnancy rates are lower for postpartum mares served at the first postpartum estrus than for those served at a subsequent estrus (Macpherson and Blanchard 2005, Lane *et al.* 2016). Boeta and Zarco recorded a pregnancy loss rate of 36.8% in mares with interspecies pregnancies (jackass × mare) which was significantly higher than intra-species pregnancies (horse stallion × mare) with a loss rate of 21.4%.

Most of the studies were conducted on equine post-partum reproductive performance have reported pregnancy rates, but not foaling rates, of mares conceiving at the foal heat or at following post-partum cycles without a comparison with the reproductive performances (Ishii *et al.* 2001, Morris and Allen 2003) of mares bred under similar uniform conditions. Therefore, the current study was conducted to analyse and compare the effects of different breeding patterns, i.e. foal heat breeding vs subsequent estrus heat breeding and different mating patterns i.e. inseminations stallion semen vs jack semen on the pregnancy rate as well as foaling rate in thoroughbred mares.

MATERIALS AND METHODS

The mating history, pregnancy status and foaling details were obtained from the breeding records of the Government stud farm located in Hisar. All the thoroughbred mares,

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stallions and jacks were maintained on pasture and had free access to hay (Lucerne/hay and paddock grass *ad lib.* plus approximately 5 kg of concentrate fed twice or three times per day), water and trace mineralized salt. All the animals had uniform housing facilities and management conditions and no special light provision was made.

Teasing was carried out on a daily basis from the day of foaling until the end of the first detected estrus period and at varying times thereafter until 21st day. Any estrus displayed after Day 21 could not be guaranteed to be the foal heat (FH) and hence day 21 was considered as the deadline for FH detection. Mares were considered to be in estrus based on their behaviour at teasing with either stallion or jack as determined by experienced farm managers. The reproductive management decisions for all mares, such as whether or and monitor for ovulation, breed on FH, uterine therapy, etc. were all made by the experienced farm veterinarians with the ultimate aim of maximizing conception rates. All the mares were inseminated transvaginally with fresh extended stallion or jack semen. All the mares in the current study were inseminated during the breeding season (March–November).

AI was performed after cleaning (with water) and drying of the perineum and vulva (with paper towels) and inseminating the mare by vaginal approach (Reilas *et al.* 2014, Scoggin 2015). Fifteen days after the artificial insemination, pregnancy diagnosis was performed through ultrasonography.

All 1,969 mares in FH was detected (estrus accompanies with ovulation) were mated at FH period, and were first inseminated when follicle diameter ranged from 37 to 42 mm and/or follicle consistency was deemed, by an experienced veterinarian, to indicate imminent ovulation (Day 0). Ovulation was assumed to have occurred on the day of last insemination prior to confirmation that ovulation had occurred. Pregnancy diagnosis was first performed using ultrasonic scanning at Day 30 post ovulation. Pregnancy status of 6,040 mares bred in different years either mated with stallion or jack semen was checked at various pregnancy stages post mating. All mares pregnant at this time were then re-examined, via scanning or rectal palpation, on average on days 30, 60 and 90-post ovulation. The foaling rates of these animals were also obtained from the records. The PR was calculated as number of mare pregnant at 30, 60 and 90 day post mating out of total mares mated. The FR was calculated as the number of mares that foaled out of total mares inseminated.

Pregnancy rate (PR) = Pregnant mares at day 30/60/90/ inseminated mares (%).

Abortion Rate (AR) = Aborted mares/ inseminated mares (%).

Foaling rate (FR) = Foaling mares/ inseminated mares (%).

In order to study the effect of various factors affecting pregnancy rate at different days of pregnancy (30, 60 and 90 as only this data has been presented), abortion rate and foaling rate a linear fix model was used. Data was analyzed

using GLM procedure of SPSS20. The linear model included fix effect of mating type (2 levels-FH and Normal heat), year of breeding (7 level-2011, 2012, 2013, 2014, 2015, 2016 and 2017 for pregnancy rate and 6 level for AR and FR) and breeding methods (2 levels-Horse breeding and Mule breeding).

$$Y_{ijkl} = \mu + A_i + B_j + C_k + e_{ijkl}$$

where, Y_{ijkl} , individual observation; μ , overall mean; A_i , fixed effect of mating type; B_j , year of breeding; C_k , fix effect of breeding method and e_{ijkl} , normally distributed random variable with mean zero and variance σ_e^2 .

RESULTS AND DISCUSSION

The means of PR, AR and FR and effects of various factors on the studied traits are presented in Table 1. The values observed in the present study are in corroboration with the findings of the previous workers' study in thoroughbred and draft mares (Blanchard *et al.* 2004, Sharma *et al.* 2010, Aoki *et al.* 2013).

To achieve the desired reproduction rate of one foal per year per mare and to optimize the reproductive potential of mares, FH should be utilized as a tool for improvement of fertility (Blanchard *et al.* 2001, Ishii *et al.* 2001, Sharma *et al.* 2010, Blanchard *et al.* 2012, deMestre *et al.* 2019). The foal heat has been reported to occur usually from 4 to 14 days after foaling and can occur 11 to 20 days after foaling (Macpherson and Blanchard 2005, Ricardo *et al.* 2020). In another report, 93% of foaling mares came into foal heat 5 to 18 days after parturition, and 77% of foaling mares 7 to 10 days after foaling (Malschitzky *et al.* 2002, Malschitzky *et al.* 2015). The mating in foal heat does not affect the initial pregnancy (up to 60 days) rates significantly. However, effect of mating type was significant ($P \leq 0.01$) on pregnancy rate at day 90 and foaling rate. The pregnancy rate following breeding at the foal heat was reported to be under 50% in most studies (Malschitzky *et al.* 2002, Morris and Allen 2003, Malschitzky *et al.* 2015). The mares mated in foal heat recorded lower pregnancy rate, higher embryonic death and foaling percentage. The differences observed in pregnancy rate (P90), abortion rate and foaling percentage between foal heat and subsequent estrus mated mare was highly significant ($P \leq 0.01$). More than 2% higher mortality and more than 5% higher foaling was seen in mares mated in normal heat. It is not recommended to breed mares on FH when they have problems at parturition (e.g. dystocia, retained placenta, delayed uterine involution, fever) (Scoggin 2015). In these situations, mares should be properly treated and re-evaluated before presenting for breeding (Sharma *et al.* 2010). Several studies reported lower conception rates (Malschitzky *et al.* 2002, Sharma *et al.* 2010, Lane *et al.* 2016) and higher embryonic mortality and abortion rates (Malschitzky *et al.* 2015) in mares bred at the first postpartum estrus, even without any pathological situation. In some studies, pregnancy rates and embryonic losses have been similar in mares bred either on FH or on later

Table 1. Least squares means of PR, AR and FR in thoroughbred mares

Particular	No. of mares	Pregnant day 30 (%)	Pregnant day 60 (%)	Pregnant day 90 (%)	Abortion (%)	Foaling (%)
Overall Mean	6040	50.44±0.83(3005)	45.29±0.96(2698)	42.55±0.96(2539)	2.18±0.30(92)	39.95±1.28(2032)
Year		NS	NS	NS	NS	**
2011	874	51.77±1.91(453)	45.98±1.98(401)	44.24±2.75 (385)	1.89±0.62 (13)	42.24±3.21 ^c (370)
2012	860	51.56±0.94 (441)	46.44±2.58(394)	44.41±3.18 (376)	2.19±0.80 (14)	40.79±3.69 ^c (358)
2013	799	47.87±1.02 (378)	45.08±2.06(351)	43.38±2.82 (337)	2.69±0.94 (18)	39.40±3.37 ^{abc} (308)
2014	829	46.63±1.31(386)	42.72±2.39(353)	40.49±2.84(333)	1.94±0.59 (17)	37.84±3.51 ^{ab} (309)
2015	854	49.09±1.09 (413)	43.31±1.62(368)	40.19±2.21 (341)	2.07±0.82 (16)	37.68±3.05 ^{ab} (321)
2016	896	52.03±2.67 (452)	45.79±2.57(404)	43.38±3.13 (383)	2.30±0.97 (14)	40.75±0.35 ^{bc} (366)
2017	928	54.12±3.76 (482)	47.72±4.73(427)	41.73±1.86 (384)	-	-
Breeding type		**	**	**	**	**
Horse breeding	1969	52.57±1.36 (1038)	48.88±1.21(962)	46.67±0.75 (923)	0.58±0.19 (21)	44.96±6.87(768)
Mule breeding	4071	48.31±0.57 (1967)	41.71±0.64 (1736)	38.42±0.79 (1616)	2.66±0.39 (71)	34.95±1.24(1264)
Mating type		NS	NS	**	**	**
Foal Heat (FH)	1690	50.53±1.47 (846)	44.54±1.72 (732)	41.00±1.54 (668)	3.31±0.32(48)	37.09±1.84(499)
Normal Heat (Subsequent estrus)	4350	50.34±0.34(2159)	46.05±0.89 (1966)	44.09±1.04 (1871)	1.05±0.16(44)	42.81±1.39(1533)

NS, Non-significant; **, indicates significant at $P \leq 0.01$. Values in parenthesis indicate number of females. Post-hoc analysis was done using Tukey test. Means with different superscript differ significantly.

postpartum period (Blanchard *et al.* 2004, Sharma *et al.* 2010). Malschitzky *et al.* (2002) analyzed 214 mares (thoroughbreds) and reported that the pregnancy rate during foal heat does not differ from that during subsequent cycles, suggesting that foal heat must be used to maximize the reproductive capacity of breeding mares.

The foaling rate achieved in different years of current study period differed significantly ($P \leq 0.01$). But there was no significant effect of years of breeding on pregnancy rate at any given period. Several studies reported lower conception rates (Lane *et al.* 2016) and higher embryonic mortality and abortion rates (Malschitzky *et al.* 2015) in mares bred at the first postpartum estrus, even without any pathological situation. In some studies, pregnancy rates and embryonic losses have been similar in mares bred either on FH or on later postpartum period (Blanchard *et al.* 2004, Sharma *et al.* 2010). Malschitzky *et al.* (2002) analyzed records of 214 mares (thoroughbreds) and reported that the pregnancy rate during foal heat does not differ from that during subsequent cycles, suggesting that foal heat must be used to maximize the reproductive capacity of breeding mares.

Effect of breeding type was found to have significant ($P \leq 0.01$) effect on PR at 30d, PR at 60d, PR at 90d, AR and FR. The mares mated with stallion semen recorded higher PR and FR in comparison to that of mares carrying mule pregnancies. Mule production success depends on the crossbreeding between jackasses and mares with high genetic potential, coupled with factors such as age and reproductive capacity (Jordao *et al.* 1954). Jordão *et al.* (1954) also observed higher pregnancy loss rates. Boeta and Zarco (2005) evaluated mares until 150 days of pregnancy resulting from inseminations with jackass semen and found a high pregnancy loss incidence (36.8%), being

all losses observed after 40 days of ovulation and thus, classified as fetal losses. In the current study, abortion percentage was also significantly ($P \leq 0.01$) higher in mares mated with jackass semen. This may be attributed to poor viability for fetuses produced through interspecies mating compared to fetuses produced from same species mating.

The current study evaluated the pregnancy and fertility rates of the mares mated during their either foal heat or normal heat and the breeding with stallion semen and jackass semen. The foal heat breeding recorded lesser foaling rates and higher early embryonic deaths and abortions to that of normal estrus breeding. There is also a significant difference observed in the pregnancy rate and foaling rate in mares mated with stallion semen or jack semen to produce horse and mule foals respectively. The mares with hybrid embryos also recorded lesser pregnancy and fertility rates and incidences of more abortions. Hence, it is recommended that though foal heat is fertile one, the mares should be considered breeding only if the previous foaling is uncomplicated and the mares are devoid of any uterine infections.

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