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## Main factors affecting an artificial insemination program in Lusitano horses (Puro Sangue Lusitano): a retrospective study

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There are many factors that can impact the success of an artificial insemination program in horses. In this study, we focused on year, month of AI, age of mare, type of heat (foal heat vs normal), reproductive status (maiden, barren, foaled), ovulation, presence of oedema, uterine inflammation, degree of inbreeding in both mare and stallion, and type of semen (fresh, refrigerated, frozen). We analysed 447 breeding records from Lusitano mares at the Alter Real stud from the years 2016 to 2023. The year did not have any statistically relevant influence on the fertility at 14 days ( $p=0.7$ ) or the breeding season ( $p=0.3$ ). The month of AI influenced fertility at 14 days ( $p=0.015$ ) with March and May being the most "fertile" months (57% and 54%, 14 days). The mare's age did not affect fertility at 14 days ( $p=0.275$ ), but for the breeding season ( $p<0.001$ ): the most fertile mares were of ages 15 and younger. The fertility at 14 days is increased if the foal heat is used compared to normal heat (98% vs 45%,  $p<0.001$ ); the same applies for end of season fertility (85% vs 71%,  $p<0.009$ ). The reproductive status of the mare (barren, maiden or foaled) did not affect fertility, either at 14 days ( $p=0.6$ ) or for the season ( $p=0.216$ ). The size and type of ovulation affected fertility at 14 days ( $p=0.003$ ), with maximal fertility in mares with double ovulations (100%), and lowest fertility

(47%) if the ovulation occurred at the left ovary; when determined for the breeding season, ovulation did not affect fertility ( $p=0.597$ ). The presence of oedema before ( $p=0.075$ ) or after ( $p=0.723$ ) AI did not affect fertility at 14 days, while the presence of endometritis did influence fertility at 14 days ( $p=0.003$ ): animals without endometritis were more fertile (53%) than animals showing signs of inflammation (40%). Inbreeding had a statistically relevant effect in stallion fertility at 14 days ( $p=0.003$ ), and our data supports that stallions with level of inbreeding below 10% were the most fertile. The degree of inbreeding in mares did not influence the breeding season fertility ( $p=0.111$ ). The stallion used for AI had an impact on the fertility at 14 days ( $p<0.001$ ) but not for the breeding season ( $p=0.09$ ). Finally, the type of semen used for AI (fresh, refrigerated, frozen) affected fertility at 14 days ( $p=0.007$ ) but not for the breeding season ( $p=0.296$ ); the most "fertile" semen is fresh or refrigerated (56% for both), followed by frozen (39%). In conclusion, whereas year, reproductive status of the mare, ovulation side and presence of oedema before or after was not relevant for fertility, the month of AI and the mare's age, breeding in foal heat, presence of endometritis, degree of inbreeding, the stallion and type of semen used must be considered to influence fertility.