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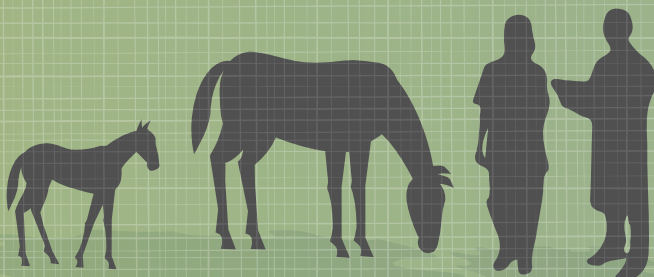
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### A parentage conundrum: delayed embryonic development or long sperm survival in embryo donor mares

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An embryo collection procedure is usually performed 7–8 days post-ovulation in mares. Embryos are subsequently transferred into a synchronised recipient mare that is expected to carry the pregnancy to term. Submission of a blood or hair root sample to an approved genetics laboratory for DNA parentage verification is a prerequisite for foal registration for most horse breed organisations. The goal of this report is to document two clinical cases in which the DNA parentage test of the foals produced by embryo transfer excluded the stallions whose semen was used on the oestrous cycle yielding the embryo and identified as the genetic sire the stallion whose semen was utilised on the previous oestrous cycle.

#### Clinical case 1

A 21-year-old Arabian mare was inseminated with frozen-thawed semen from a deceased stallion (Stallion A) immediately after detection of a single ovulation. No embryo was recovered following uterine lavage 8 days later. Cooled-transported semen from a different stallion (Stallion B) was used on the subsequent cycle. An exceptionally large (2466 µm in diameter) expanded blastocyst stage embryo was recovered 8 days post-ovulation and transferred into a recipient mare. The recipient mare carried the pregnancy to term. Genetic testing of the foal excluded Stallion B as the sire and confirmed Stallion A as the genetic sire of the foal.

#### Clinical case 2

An 11-year-old Quarter Horse mare was inseminated with cooled-transported semen from Stallion A and subsequently

ovulated two follicles. Uterine lavage 6.5 days later yielded one embryo. Cooled-transported semen from a different stallion (Stallion B), housed at a different reproductive centre, was used on the second cycle. The mare ovulated one follicle and uterine lavage 8 days post-ovulation yielded two embryos, one of which was substantially larger than expected. The recipient mare receiving the larger embryo carried the pregnancy to term. DNA parentage testing excluded Stallion B as the genetic sire and confirmed Stallion A as the genetic sire. The intervals from the dates of the original insemination with the genetic sire to the first cycle ovulation, second cycle ovulation and recovery of the embryos in question were 0 days (post-ovulation insemination), 18 days, and 26 days for the Arabian mare and 2 days, 16 days and 26 days for the Quarter Horse mare.

#### Summary

The potential explanations for how an equine embryo could be recovered following an insemination 26 days previously include marked delayed fertilisation or marked delayed embryonic development. Equine DNA parentage testing relies on the principle of exclusion, with the inheritance of a series of short tandem repeat (STR or microsatellite) markers evaluated in the foal, sire, and embryo donor mare. Parentage assignment based solely on breeding records would have been incorrect.

#### Further reading

Bellone, R.R. and Avila, F. (2020) Genetic testing in the horse. *Vet. Clin. North Am. Equine Pract.* **36**, 211–234.

McCue, P.M. and Squires, E.L. (2015) *Equine Embryo Transfer*. Teton New Media, Jackson.