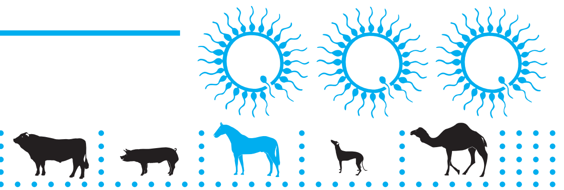

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Cryopreservation of equine embryos with the Equine Embryo Direct Transfer (dt) Vitrification Kit

Introduction

Cryopreservation of equine embryos allows owners to collect an embryo at any time, regardless of performance schedules, and transfer the embryo for a convenient expected foaling date. It also facilitates international shipping of embryos, reduces cost and labor of synchronization of donor and recipient mares, and makes it possible to bank embryos from super-ovulated mares. In addition, cryopreservation is necessary when embryos are submitted for pre-implantation genetic testing for diseases and traits. The success of embryo cryopreservation is dependent on the developmental stage and size of the embryo. Smaller embryos collected 6.5 days after ovulation survive cryopreservation procedures much better than day-7 or day-8 embryos (Seidel, 1996). Reports by Lascombes and Pashen (2000) and Maclellan et al. (2002), indicated that transfer success rate after thawing of small equine embryos can reach levels acceptable for commercial use.

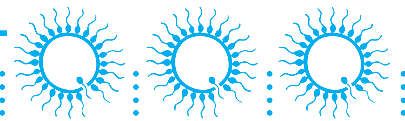
Embryos can either be cryopreserved using a slow-cooling method (Maclellan et al, 2002; Slade et al, 1995), or by vitrification (Eldridge-Paluska et al, 2005). Using the slow cooling method embryos are loaded into a 0.5 mL plastic straw in a cryopreservation media, using glycerol, or ethylene glycol as cryoprotectant. The embryo is cooled to -6°C at a rate of 4°C per minute, seeded and held for 15 minutes, then cooled at 0.3°C per minute to -35°C, and then plunged into liquid nitrogen (Squires et al, 2005). Vitrification is a simple method that does not require sophisticated and expensive equipment. Following washing of the embryo in EquiPRO™ Holding Medium, the embryo is placed in vitrification solutions (see instructions for Equine Embryo Direct Transfer (dt) Vitrification Kit, Minitube). The embryo is then loaded into a 0.25 mL straw, which is sealed (Ultraseal™, Minitube) and placed into a cooled plastic goblet surrounded by liquid nitrogen for 1 minute. The goblet containing the straw is then plunged into liquid nitrogen. Before transfer, the straw is warmed at room temperature for 10 seconds, and then incubated in a water bath at 20-22°C for an additional 10 seconds. This technique allows practitioners to collect an embryo at a convenient location, and ship the embryo to a referral center for cryopreservation.

Clinical data

Study 1: In a clinical study at the Minitube International Center for Biotechnology in 2008, a total of 9 mares were flushed on day 6.5 after ovulation, and 8 excellent to good embryos were recovered. All 8 embryos were vitrified using the Equine Embryo Direct Transfer (dt) Vitrification Kit (Minitube) according to the manufacturer's instruction. The embryos were later warmed and transferred to synchronous recipient mares. The recipient mares were examined for pregnancy by transrectal ultrasonography at day 14. Seven of the eight transferred embryos (87.5%) resulted in pregnancies at this time (figure 1). Because not all presumptive foals were needed, 4 recipient mares were randomly selected for intentional termination of pregnancy by a single injection of 10 mg PGF2α at day 14. The 3 remaining pregnancies developed normally and resulted in healthy foals.

Table 1. *Equine embryos cryopreserved in Minitube dt-Vitrification system.*

Mares flushed	9
Vitrified embryos	8
Transferred embryos	8
Pregnancies (Day 14)	7
Intentional pregnancy termination (Day 14)	4
Pregnancies (Day 120)	3
Live foals	3



Study 2: In a follow-up study at the International Center for Biotechnology, 11 mares produced 12 embryos at 6.5 days after ovulation. A total of 8 excellent embryos were submitted to biopsy, and a small number of cells were removed from each embryo via micromanipulators. After biopsy, embryos were cryopreserved using the Equine Embryo Direct Transfer (dt) Vitrification Kit (Minitube) according to the manufacturers instruction. The embryos were later warmed and transferred to synchronous recipient mares. Ultrasonographic pregnancy examinations were performed on days 5-6 and 7-9 days after transfer. Pregnant recipient mares were examined every 7 to 10 days until ~Day 100 and monthly thereafter. Genomic DNA was extracted from biopsied cells using the PicoPure® DNA Extraction Kit (Molecular Devices), and a positive control gene (18 S) could be amplified in all samples. Six of the eight transferred biopsied and vitrified embryos (75%) resulted in pregnancies at Day 14 (figure 2). Three of the pregnancies were lost between 15 and 45 days. The remaining recipient mares are pregnant late in gestation and due to foal in early 2010. All pregnant recipient mares were maintained on Altrenogest (0.044 mg/kg/day) orally until Day 100.

Table 2. *Biopsied equine embryos cryopreserved in Minitube dt-Vitrification system.*

Biopsied and vitrified embryos	8
Transferred embryos	8
Pregnancies (Day 14)	6
Lost Pregnancies (Days 15-45)	3
Late Gestation (> Day 120)	3

Discussion

Results from these studies demonstrate that the Minitube dt-Vitrification system can be used to successfully produce pregnancies, resulting in healthy live foals. It also shows that morula-early blastocyst stage embryos can successfully be biopsied and vitrified for genetic testing. The advantage of genetic testing of an embryo is that the actual genetics of the offspring is tested rather than the probability of dissemination of a gene.

EQUIPRO®Vit-Kit™
Equine Direct Transfer Vitrification REF.: 19500/1200

References

1. Seidel, G.E. "Cryopreservation of equine embryos." Veterinary Clinics of North America: Equine Practice 12 (1996): 85-99.

2. Lascombes, F.A., and R.L. Pashen. "Results from embryo freezing and post ovulation insemination in a commercial embryo transfer programme." In Havemeyer Foundation Monograph Series No. 3. Proceedings of the 5th International Symposium on Equine Embryo Transfer, Finland, 6th-9th July 2000, edited by T. Katila and J.F. Wade, 95-96. Newmarket: R&W Publications, 2000.

3. Maclellan, L.J., E.M. Carnevale, M.A. Coutinho da Silva, P.M. McCue, G.E. Seidel, Jr., and E.L. Squires. "Cryopreservation of small and large equine embryos pre-treated with cytochalasin-B and/or trypsin." Theriogenology 58(2002): 717-720.

4. Slade, N.P., T. Takeda, E.L. Squires, R.P. Elsden, and G.E. Seidel, Jr. "A new procedure for the cryopreservation of equine embryos." Theriogenology 24 (1985): 45-58.

5. Eldridge-Paluska, W.D., V. Caracciolo di Brienza, G.E. Seidel, Jr., E.L. Squires, and E.M. Carnevale. "Establishment of pregnancies after serial dilution or direct transfer by vitrified equine embryos." Theriogenology 63 (2005): 1308-1319.

6. Squires, E.L., E.M. Carnevale, and P.M. McCue. "Embryo freezing." In Havemeyer Foundation Monograph Series No. 18. Proceedings of a Workshop – International Equine Gamete Group, Kiihlungsborn, 18th-21st September 2005, edited by H. Alm, H. Torner and J.F. Wade, 39-40. Newmarket: R&W Publications, 2005.