Comparison of Three Holding Solutions for Cooled Storage of Equine Embryos

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Equine embryos are traditionally transported in Ham’s F10, a culture medium that requires special handling (bubbling with a specific gas mixture) before it can be used for the conservation of equine embryos. Two media have been tested for the cooled storage of equine embryos (Emcare Holding Solution and Vigro Holding Plus) and appear to be suitable alternatives to Ham’s F10. These media offer the advantage that they are ready-for-use products with a relative long shelf life. Author’s addresses: Equitechnic, Le Mesnil Vicomte, France, peter.daels@ivis.org (Daels); Physiology of Reproduction and Behavior, INRA, 37380 Nouzilly, France (Duchamp); Department of Reproduction, Ecole Vétérinaire de Nantes, BP 40706, 44307 Nantes, France (Bruyas, Moussa); Labogena, Domaine de Vilvert, 78352 Jouy-en-Josas, France (Mahla). © 2002 AAEP.

1. Introduction
Equine embryos can be successfully stored for 24 h at 5°C in Ham’s F-10 media. However, Ham’s F-10 contains a bicarbonate-based buffer and requires a specific atmosphere to maintain a correct pH. Embryo-holding media using other types of buffers (zwitterionic buffers) that are stable in the presence of ambient air mixture have been used successfully for the chilled conservation of embryos in other species. CO₂ is not an absolute requirement for embryonic development, because both ovine and bovine embryos will develop to the blastocyst stage in media containing zwitterionic buffers in a CO₂-free atmosphere.1,2 In the present study, two embryo-holding media containing zwitterionic buffers have been tested for cooled storage of equine embryos.

2. In Vitro Evaluation of Equine Embryos Stored for 6 and 24 Hours in Ham’s F10, Emcare Holding Solution® or Vigro Holding Plus®
The viability of 7-day-old, equine embryos after 0 h, 6 h and 24 h cooled storage in Ham’s F-10 (Sigma, St. Louis, MO), Embryo Holding Solution Emcare® (EHS) (ICP, Auckland, NZ) and ViGro Holding Plus (AB Technology, Pullman, WA) was tested in vitro. The viability of stored and fresh embryos was estimated by counting the number of dead cells present in the embryo using a nuclear stain (DAPI) that selectively stains death cells.

1. Material and Methods
Fifty 7-day-old embryos were collected from fertile Welsh ponies. Embryos were assigned to one of five groups (n = 10/group) (Fig. 1). Embryos in group-0 h were evaluated immediately after collection. Embryos stored for 24 h at 5°C were maintained in Ham’s F-10 gassed with 5% CO₂, 5% O₂, and 90% N₂ for 5 min and supplemented with 10% (v/v) fetal calf serum (FCS) plus 1% penicillin/streptomycin (Group-H-24 h), Emcare Holding Solution® (Group-E-24 h) or ViGro Holding Plus® (Group-V-24 h) (Fig. 2).
The embryos were recovered 7 days after ovulation (ovulation = day 0) by uterine lavage using 3 × 0.5 L warm embryo flushing solution containing albumin (0.1% w/v) and kanamycin sulphate (25 mg/l) (Emcare Flushing Solution, ICP, Auckland, NZ). Embryos were washed 10 times in Emcare Flushing Solution, measured, and graded based on morphology. Stored embryos were placed in a 5-ml tube filled with the appropriate holding solution and placed in a 50-ml Corning tube and stored in an Equitainer (Hamilton-Thorn, S. Hamilton, MA) as previously described.3 After storage, embryos were washed in EHS and then placed in EHS with 1 μg/ml 4,6-diaidino-2-phenylindole (DAPI, Sigma Chemical Co., St. Louis, MO) for 15 min at room temperature. The number of dead cells (DAPI positive, fluorescent cells) per embryo was determined using an inverted fluorescence microscope (Olympus, IMT-2) (Fig. 3). Differences between groups were analyzed using the Kruskal & Wallis test.

2. Results
The mean diameter of embryos was not significantly different between groups (p > 0.05). The mean (+SEM) number of fluorescent dead cells per embryo was 0.4 ± 0.2, 2.4 ± 1.1, 14.4 ± 5.2, 19.3 ± 5.1, and 24.5 ± 7.2 for groups 0 h, E-6 h, H-24 h, E-24 h, and V-24 h, respectively. The mean number of dead cells in group 0 h and E-6 h was similar (p > 0.05). The mean number of dead cells at 0 h and 6 h was significantly lower than at 24 h (p < 0.05). However, no significant differences in number of dead cells was observed between group H-24 h, E-24 h, and V-24 h (p > 0.05).

The proportion of dead cells in relation to total number of cells was estimated by calculating the number of dead cells per unit of embryo surface. The same observations were made when using the mean number of dead cells per unit of embryo surface. Mean (+SEM) number of dead cells/mm² was 1.7 ± 0.9, 5.2 ± 1.7, 51.6 ± 18.5, 36.7 ± 9.8, and 45.5 ± 14.4 for groups 0 h, E-6 h, H-24 h, E-24 h, and V-24 h, respectively.

When comparing the number of dead cells/embryo for embryos with diameter <400 μm and >400 μm, no difference was observed: 19.6 ± 3.8 and 19.1 ± 6.6 for embryos less than and greater than 400 μm, respectively (p > 0.05). When comparing the number of dead cells per unit of embryo surface to compensate for size of the embryo, embryos <400 μm

Fig. 1. Distribution of 7-day-old embryos among experimental groups.

Fig. 2. Presentation of Embryo Holding Solution Emcare (EHS) (ICP, Auckland, NZ) and ViGro Holding Plus® (AB Technology, Pullman, WA).

Fig. 3. Microphotograph of part of an equine embryo stained with DAPI. The nuclei of the dead embryonic cells appear as bright light blue spots on the image.
had significantly more dead cells (62.7 ± 11.0/mm²) than embryos >400 μm (13.7 ± 4.2/mm²) (p < 0.05).

3. Conclusions

These results suggest that Embryo Holding Solution Emcare and ViGro Holding Plus offer a good alternative to Ham’s F-10 for 24 h cooled storage of equine embryos and that embryos >400 μm may have a better viability after 24 h of cooled storage than the embryos <400 μm.

3. In Vivo Evaluation of Equine Embryos Stored for 24 Hours in Ham’s F10 and Emcare Holding Solution

1. Introduction

The aim of the present study was to compare pregnancy rates after transfer of embryos stored for 24 h in Ham’s F-10 Nutrient Mixture and Embryo Holding Solution Emcare.

2. Materials and Methods

Forty equine embryos, grade 1 and >300 μm in diameter were recovered 7 days after ovulation (ovulation = day 0) by uterine lavage using 3 × 0.5 l warm embryo flushing solution containing albumin (0.1% w/v) and kanamycine sulphate (25 mg/l) (Emcare Flushing Solution). The embryos were rinsed 10 times in the flushing solution and measured and graded based on morphology. On each collection day, three to six donor mares were flushed, and embryos were assigned to one of two treatment groups in alternating manner.

Embryos were placed in Ham’s F-10 (n = 20; group 1) or EHS (n = 20; group 2) as described for experiment 1. Embryos were assigned sequentially (one from each treatment group) to be transferred together as a pair (Ham’s F10 and EHS) to the same recipient mare. Two 5-ml tubes each containing an embryo of each group were placed together in the same 50-ml Corning tube and stored in an Equitainer for 24 h. Each pair (n = 20) of embryos was transferred by standing flank laparotomy as described previously, and the two embryos were simultaneously placed in the same transfer pipette4,5 (Fig. 4).

Pregnancy examination was done on days 12–16 of gestation. If only one embryo was detected, the origin of the embryo was determined by paternity testing using DNA typing of the recovered embryo.6 One-way analysis of variance was used for comparison of the diameter of the embryos and the morphological grades between the treatment groups.

3. Results

The diameters of embryos (511 ± 33 μm for group 1 and 468 ± 27 μm for group 2) and the quality score at collection did not differ significantly (p > 0.05).

Fifteen of the 20 recipient mares (75%) were pregnant after transfer. Two of 15 pregnant mares (13%) were pregnant with twin embryos, and 13 mares (87%) were pregnant with a single embryo. Of the 17 surviving embryos, 9 (45%) had been stored in Ham’s F-10 and 8 (40%) in Emcare. No significant difference in survival rate was observed (p > 0.05).

4. Conclusions

The results of this experiment combined with the previous in-vitro study suggest that Emcare Embryo Holding Solution offers a good alternative to Ham’s F-10 for 24-h cooled storage of equine embryos. The added benefits of this type of transport solution is that it does not need to be gassed before use and can be stored at 4°C throughout the breeding season.

References