

# OptiPrep™ Application Sheets

## **C46 Concentration of equine, boar and gazelle spermatozoa on to a density barrier**

- ◆ **OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml**
- ◆ For links to other relevant files click on the double blue arrow in the following text

### **1. Background**

The maximization of viability, survival and fertility of spermatozoa is necessary for efficient artificial insemination for many species. Prior to cooling or freezing processes it is often necessary to concentrate the viable spermatozoa and at the same time to separate the cells from the seminal plasma since, in a number of instances, it is recognized that some component of the plasma is detrimental to the viable spermatozoa during storage [1]. Stallion semen is a notable and economically important example of this problem. As with many cell types, simple pelleting of the spermatozoa by centrifugation leads to aggregation and loss of viability.

The protocol below is adapted from ref 2.

### **2. Solutions required**

- A.** OptiPrep™ (shake gently before use)
- B.** Diluent: e.g. Genus Equiprep™ Solution A (see Note 1).

### **3. Protocol**

1. Dilute the semen with an equal volume of Solution B at 30°C.
2. Transfer the diluted semen to a conical centrifuge tube and, using a syringe and metal cannula, underlay the semen with 2-4 ml of OptiPrep™ (see Notes 2 and 3).
3. Centrifuge at 1000 g for 20-25 min at room temperature (see Note 4).
4. Aspirate the seminal plasma to within 2-3 mm of the band of sperm cells at the interface and then with a syringe attached to a metal cannula slowly aspirate as much of the cushion as possible (see Note 5).
5. Dilute the spermatozoa remaining in the tube with the freezing extender or any other solution as required.

### **4. Notes**

1. The diluent solution may be any semen compatible solution. Contact Dr Stuart Revell, Genus Freezing Unit, Llanrhydd, Ruthin, Denbighshire, LL15 2UP, UK for more details.
2. Stainless steel metal cannulae 8-12 cm in length (i.d. approx 0.8 mm) can be obtained most surgical instrument supplies companies (e.g. Holborn Surgical, Margate, Kent, UK).
3. The volume of cushion is not particularly critical and will depend on the size of the diluted semen sample and the size of the centrifuge tube. Use of a conical centrifuge tube will facilitate aspiration of the cushion in Step 4. Sieme et al [3] underlaid 46 ml of stallion semen with 5 ml of OptiPrep™ cushion and Loomis [1] noted that some workers used only very small volume (30 µl) in a specially constructed glass tube, which obviated its removal in Step 4 prior to addition of the extender. Matás et al [4] used a 5 ml cushion for boar semen and Saragusty et al [5] employed a 1 ml cushion for gazelle semen.
4. Most methods use approx. 1000 g for approx. 20 min, while for gazelle semen 1500 g was used [5].
5. The conical tip of the centrifuge tube should allow removal of essentially all of the cushion; with practice less than 75 µl of liquid remains in the tube. The alternative of aspirating the band of

sperm cells into a rubber-free syringe can be adopted if preferred but the removal of the cushion avoids is “kinder” to the cells.

## 5. References

◆ To access abstracts of the following refs. (File CA46) click on the double blue arrow. →→

1. Loomis, P.R. (2006) *Advanced methods for handling and preparation of stallion semen* Vet. Clin. Equine **22**, 663-676
2. Revell, S.G., Ford, T., Pettit, M.T. and McGuirk, B. (1999) *Use of centrifugation and iodixanol to reduce damage when processing equine semen for freezing* J. Anim. Breed., **3**, 35-38
3. Sieme, H., Knop, K. and Rath, D. (2006) *Effects of cushioned centrifugation on sperm quality in stallion semen stored cooled at 5°C for 24h, and stored cooled for 2h or 24h and then frozen* Animal Reprod. Sci., **94**, 99-103
4. Matás, C., Decuadro, G., Martínez-Miró, S. and Gadea, J. (2007) *Evaluation of a cushioned method for centrifugation and processing for freezing boar semen* Theriogenology **67**, 1087-1091
5. Saragusty, J., Gacitua, H., King, R. and Arav, A. (2006) *Post-mortem semen cryopreservation and characterization in two different endangered gazelle species (Gazella gazella and Gazella dorcas) and one subspecies (Gazella gazelle acaiae)* Theriogenology, **66**, 775-784