

## Procedure for in vitro fertilization with sexed bull semen

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This protocol is based on procedures obtained from George Seidel, Colorado State University. Details of medium preparation including Percoll and PHE can be obtained at <http://www.animal.ufl.edu/hansen/ivf> The procedure can also be used for unsexed semen and is particularly useful when amounts of semen are limited.

### Preparation of Media

1. Prepare fertilization plates by placing 50  $\mu$ L drops of IVF-TALP in a 60 x 15 mm petri dish and cover with mineral oil.
2. Prepare a Percoll gradient by layering 0.5 mL 45% Percoll over 0.5 mL 90% Percoll in a 2 mL microcentrifuge tube
3. Prepare one 15 mL conical tube with 4 mL of HEPES-TALP and label WASH
4. Place 15 mL of HEPES-TALP in two 15 mL conical tubes and use for washing the cumulus-oocyte complexes (COCs) prior to fertilization.
5. Place 3 mL of IVF-TALP in a 15 mL conical tube and leave the cap loose.
6. Place fertilization plates and the conical tube with IVF-TALP in an incubator at 38.5°C in an atmosphere of 5% CO<sub>2</sub> and allow to equilibrate for at least 2 hrs.
7. Place the Percoll gradient and conical tubes containing HEPES-TALP in an oven to warm.
8. Plug in citothaw and place 1 aliquot of PHE in the oven to warm.

### COC preparation

1. Remove COCs from maturation drops in groups of 30 and wash once in HEPES-TALP.
2. After washing, place each group of 30 COCs into a fertilization drop in a volume of 10  $\mu$ L.

### Semen preparation

1. Thaw one straw of semen and gently layer on top of the Percoll gradient
2. Place microcentrifuge tube in a microcentrifuge and centrifuge for 20 min at 300 x g
3. After centrifugation, remove the pellet from the microcentrifuge tube and place into the WASH tube.
4. Centrifuge the WASH tube for 5 min at 300 x g.
5. After washing, remove the supernatant from the tube, add about 100  $\mu$ L of IVF-TALP.
6. Calculate the concentration of sperm with a hemacytometer and estimate the volume of the sperm pellet with a pipettor.

### Fertilization

1. Once the concentration of sperm has been determined, dilute to 4 x 10<sup>6</sup> cells/ml. The following equation can be used to calculate the dilution required volume of IVF-TALP needed to dilute the sperm suspension to obtain a final concentration of sperm in the fertilization drop of 1 x 10<sup>6</sup>/mL.

$$\text{Milliliters of IVF-TALP to add to sperm pellet} = (\text{sperm concentration} \times \text{sperm pellet volume in mL} / 4 \times 10^6) - \text{sperm pellet volume in mL}$$

2. Add 20  $\mu$ L of the diluted sperm suspension to each fertilization drop (approximate volume=60  $\mu$ L consisting of 50  $\mu$ L of IVF-TALP and 10  $\mu$ L of oocytes). Note that the final concentration of sperm is 1 x 10<sup>6</sup>/mL.
3. Place 3  $\mu$ L of PHE in each fertilization drop.
4. Return fertilization plates to the incubator and coincubate sperm and COCs for 8 hrs.

### Note on Contamination of Semen

Some straws of sexed semen contain a bacterium that is resistant to the antibiotics commonly used in IVF media. Often, a brown cloud of microorganisms is seen surrounding COCs after fertilization. Such contamination has severe deleterious effects on the outcome of IVF. Personal communication from Fuliang Du (Evergen) indicates that the antibiotic Amikacin can sometimes resolve the problem. Amikacin can be obtained from Med-Shop Total Care Pharmacy (Longview TX 75605; 1-888-769-4710) at a concentration of 50 mg/ml. The working solution is 20  $\mu$ g/ml (40  $\mu$ l into 100 ml solution). All solutions used for IVF and culture should receive Amikacin.

