ANS 3319C Reproductive Physiology and Endocrinology Lab (25 pts)  

Name: ___________________________ Lab Date & Time __________

1) When evaluating bovine semen to determine if it is suitable for cryopreservation, factors such as motility, morphology, acrosome integrity, number of live/dead sperm cells, and concentration are evaluated. Semen evaluated for cryopreservation is more rigorously evaluated than semen in a breeding soundness exam.
   a) Please indicate what the minimum values are for the following categories to determine if it is suitable for cryopreservation in the bovine (3 pts)?
      1. Motility 60%
      2. Morphology 70% normal
      3. Concentration > 500 million/ml
   b) There are two types of sperm cell abnormalities, primary and secondary, that are typically evaluated in a morphology exam. Please provide a definition for each (2 pts).
      Primary: abnormalities associated with spermatogenesis or with the head of the sperm cell
      Secondary: abnormalities associated with epididymal maturation and (or) the mid-piece and tail
   c) Abnormally shaped sperm can have a negative effect on fertility in numerous species. Please describe three physiological processes where sperm abnormalities could have a negative effect on fertility in the female (3 pts)?
      1. Decreased ability of sperm to reach site of fertilization
      2. Inability to fertilize oocytes
      3. Inability to sustain embryonic development
   d) What is a typical volume and concentration of sperm in an ejaculate from an older mature bull (2 pts)?
      Volume: 6 to 15 mL  Concentration: 500 to 2,000 million sperm/mL.

2) As with semen collected in the stallion and boar, a bovine ejaculate that will be used for AI has extender added to it. In both the stallion and bull, glycerol is added to the extended semen to prepare it for freezing.
   a) What is the function of glycerol (1 pt)?
      Glycerol serves as a cryoprotectant to protect against the lethal effects of freezing by preventing crystallization of water within sperm cells.
   b) What is the volume and target sperm concentration of a single dose of extended bovine semen that will eventually be frozen (1 pt)?
      Semen is frozen in either 0.25 or 0.5 cc polyvinyl straws at a concentration of 20 x 10^6 live motile sperm/dose
   c) Why is it necessary that semen to be collected for freezing be of the highest quality and have more sperm cells packaged in a straw than needed (1 pt).
      Because all sperm cells do not survive the freezing and thawing process.
   d) What is the liquid and its temperature that packaged/frozen equine and (or) bovine semen is stored in (1 pt)?
      It is stored in liquid nitrogen at a temperature of -196°C
   e) It is important to accurately label a straw of extended bovine semen so we know what it contains. Please list several items that could be included on the straw to help identify its contents (1 pt).
      Sire name, breed code, breed association registration number, date frozen, and processing location.
3) Freezing semen in the equine industry is not as common as in the cattle industry even though the advantages to freezing equine are similar to many of those in cattle and pigs.

   a) Please list at least four disadvantages of frozen semen in horses (2 pts)?

   1. Takes considerable knowledge and technology to successfully freeze semen.
   2. Considerable cost and intensive management are needed to prepare for frozen semen insemination
   3. Genetic saturation of a particular stallion may occur if breedings are not limited.

4) The semen freezing process in the equine is streamlined and not as complicated or time consuming compared to the bovine. For either species, semen is packaged and frozen in 0.5 cc straws.

   a) Starting with semen collection/evaluation and ending with processing semen for insemination in the mare, please list the ten steps involved in freezing equine semen and fill in the blanks where required (8 pts).

   1. **Semen collection and evaluation**
   2. **Extension of semen**
      
      *Semen will be extended to a final concentration of 50 x 10⁶ sperm / mL*
   3. **Centrifugation to concentrate sperm**
   4. **Isolation of sperm pellet**
      
      *Process only allows for an 80% return on sperm after centrifugation & supernatant removal*
   5. **Addition of freezing extender**
   6. **Packaging semen for freezing**
      
      *Extended semen is loaded in 0.5 cc straws*
   7. **Freezing process**
      
      *When temperature in freezing container reaches -120°C, straws can be plunged into the liquid nitrogen*
   8. **Storage of frozen semen**
   9. **Post thaw motility test**
      
      *Each straw is thawed in a 37°C water bath for 30 sec and each straw is extended in 2.5 mL of extension media. Make sure the straws are free of water because water is toxic to sperm.*
   10. **Processing frozen semen for insemination**
      
      *Combine 4 straws of extended-packaged-frozen and eventually thawed to make one breeding dose that will contain approximately 800 x 10⁶ sperm. This preparation will have 10 mL of additional extender added to it to facilitate insemination of the mare.*