

ACT Certifying Examination Essay Questions and Answers

Question: Using the following headings, discuss the cloning of Dolly the sheep.

Principles of the procedure:

Preparation of the cloned nuclear material (3 pts):

The nucleus from a cell culture line of mammary gland was transferred into an enucleated oocyte (cytoplast). The culture cell line was passed between 3 and 6 times before being used as nuclear donors. The donor cells were fully differentiated. Recipient oocytes were flushed from the oviducts when arrested in metaphase II (MII). The DNA replication stage of the donor cell nucleus and the cytoplasmic stage of the recipient ooplasm were in synchrony. The recipient cytoplasts arrested at MII have high maturation promoting factor (MPF) activity. Since nuclei transferred into this environment undergo membrane breakdown and chromosome condensation it is important that transferred nuclei be diploid to continue their development. Donor cells were made quiescent by reduction of the serum concentration in culture media from 10% to 0.5% for 5 days, which caused cells to exit the growth cycle and arrest in the G₀ phase of the cell cycle, in a diploid state.

Transfer of nuclear material (3 pts):

Recipient oocytes were then transferred to calcium free media containing Cytochalasin B and a DNA specific fluorochrome; a glass pipette was used to remove the first polar body and the metaphase plate located under it. The Cytochalasin B allowed removal of the metaphase plate by disrupting myofilaments which increases cell membrane elasticity and allows membrane enclosed cytoplasm containing the metaphase plate to be drawn into the pipette and removed. Removal was confirmed by examining the (enucleated oocyte) under UV light (the fluorochrome stained DNA will be clearly visible if the metaphase plate was not removed). The donor cell was then aspirated into the enucleation pipette and introduced into the oocyte through the previously created hole in the zona pellucida. This cell sat within the zona but outside the oocyte plasma membrane.

Activation of the transferred nuclear material (2 pts):

The oocyte cytoplasm required activation into parthenogenetic development - this mimics the activation of fertilization where repeated transient increases in intracellular calcium occur. Electrical pulse application causes analogous changes in calcium concentration. A series of electrical pulses was used to

activate the oocyte and fuse the cells (oocyte and donor). An initial DC pulse was used to activate the oocyte cytoplasm then an AC pulse aligned the cells so that the fusion series was applied at 90 degrees to the plane of contact between the cells.

Transfer to a recipient animal (1 pt):

These reconstructed embryos were then cultured in vivo in the ligated oviduct of a recipient ewe till day 7 when they were reexamined and morula and blastocyst stages were transferred to synchronized recipients for development to term.

Implications for future genomic development (1 pt):

The real implications of the work involve the continuity of the genome during development. The results indicate that the genome of somatic cells does not undergo irreversible modification during the growth, development and differentiation of tissues.

Wilmot et al., were able to reset the clock for the genome of these cells and reactivate the entire genome of a differentiated cell so that the information within was able to develop a complete animal.

References:

Campbell KHS, McWhir WA, Ritchie WA, Wilmot I. Sheep cloned by nuclear transfer from a cultured cell line. Nature 1996;380:64-66.

Wilmot I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS. Viable offspring derived from fetal and adult mammalian cells. Nature 1997;385:810-813.

Paterson L, DeSousa P, Ritchie W, King T, Wilmot I. Application of reproductive biotechnology in animals: implications and potentials. Applications of reproductive cloning. Anim Reprod Sci 2005;793:137-143.

Question:

Using the following headings, describe the differences between the breeding soundness examination of the stallion and the bull. Assume semen collection in the bull is performed by electroejaculation and semen collection in the stallion is performed by collection with an artificial vagina.

Libido evaluation (1 pt)

Collection via electroejaculation reveals no information on copulatory ability. Alterations in the shape of the erect penis may occur with electroejaculation but

should not be considered significant. Copulatory ability needs to be assessed with a teaser cow/heifer if there is a concern about the ability of the bull to breed a cow. Libido and copulatory behavior can be assessed with semen collection with an artificial vagina, but one must consider behavior (previous experiences) and experience with the techniques in making judgments about copulatory behavior.

Testicular circumference (1 pt)

This is assessed based on age and breed in the bull. Bulls failing to meet or exceed their age or breed standards should not be passed. This is not considered a pass/fail category in the evaluation of the stallion. Total scrotal width is measured and should exceed 8 cm in a mature stallion, but age and breed will greatly affect this measurement and there is considerable individual variation. Failure to attain 8 cm TSW is not reason to fail an individual on BSE

Semen volume (1 pt)

Semen is generally quite concentrated with electroejaculate samples and total volume depends on how long the bull is stimulated. Volume is not evaluated in the bull BSE. Volume in the stallion is quite variable, some stallions have low volume and high concentration and others the opposite. Volume is also increased with extended teasing times. Volume is not considered significant in the stallion either.

Motility (2 pt)

Motility in the bull is evaluated as gross activity (wave like motion) and individual motility. Motility must be good and should be >50% progressive to pass (30-50% is questionable and should be rechecked). Motility in the stallion should be a minimum of 30% (50% progressive is preferable) to pass. The gel fraction of the ejaculate should be removed to facilitate motility evaluation.

Morphology (2 pt)

Morphologic evaluation separates primary from secondary or alternatively compensatory from non-compensatory from normal sperm. There should be a minimum of 50% normal sperm to pass the exam for either stallions or bulls and 30% is questionable.

Concentration (1 pt)

Concentration is not measured in the bull because of the great variability obtained in an electroejaculated sample. It is not representative of a normal ejaculate. In the stallion, concentration is measured and is then used to determine if the stallion can meet the needs of his intended book. As long as there is enough sperm to successfully impregnate one mare, the stallion may be allowed to pass the exam; however, if there is inadequate sperm for the intended book, the stallion may be classified as questionable or unsatisfactory depending on the needs of the stallion owner.

Number of ejaculates necessary to classify the individual: (1 pt)

A bull is generally electroejaculated once. If questionable, he may be re-evaluated in the future. Stallions are generally collected two times one hour apart for evaluation. This allows for evaluation of whether the ejaculates are representative.

How the satisfactory, questionable or unsatisfactory classification is determined: (1 pt)

In the bull, a minimum scrotal circumference, minimal % motile and minimum % morphologically normal sperm are required to be classified as satisfactory. Many times an individual that lacks one of these minimums will be re-evaluated later and is classified as questionable until he is able to be re-checked. In the stallion, passing is more subjective. The needs of the individual stallion (book) must be known and the semen parameters are evaluated in the face of the ability of the stallion to meet these needs. The examiner has significant leeway in the interpretation of the results of both the stallion and the bull (but more-so in the stallion).

References:

Love CC. Stallion semen evaluation and interpretation. In: Proceedings of the SFT Annual Conf. 2002;93-101.

Spitzer JC, Hopkins FM, Higdon HL III. Breeding soundness evaluation of yearling bulls: comparisons of outcomes with different classification systems. In: Proceedings of the SFT Annual Conf. 1997;108-115.

Chenoweth PJ. Genetic sperm defects. Proceeding of the SFT Annual Conf. Theriogenology. 2005;64:457-468.

Spitzer JC, Hopkins FM. Breeding soundness evaluation of yearling bulls. Vet Clin North Am Food Anim Pract 1997;13:295-304.