Harvesting frog oocytes

PURPOSE: This document describes the ACUC approved methods for obtaining frog oocytes.

BACKGROUND: Frog oocytes are usually obtained by laparotomy of a suitably prepared female. Multiple major survival surgeries on a single animal may be justified considering the reduction in the total number of animals used over the long term. However, the pain or distress experienced by an individual animal following multiple major survival surgery must be considered vis-à-vis reduction in the total number of animals used.

PROCEDURE:

1. Personnel performing surgeries and other procedures must be properly trained.
2. Keep adequate records of all treatments and procedures performed.
3. All surgeries should be performed under general anesthesia.
4. Surgeries should be done as aseptically as practical. Use of sterilized instruments and gloves, and good surgical technique should be practiced.
5. Animals must be monitored until completely recovered from anesthesia.
6. Single housing or small group housing for several days after surgery is ideal for animal recovering from surgery.
7. Frogs should be monitored during this period for appetite, activity and complications such as wound dehiscence or infection.
8. Seek immediate veterinary assistance (5-3713) in case of problems.
9. Animals may have to be euthanized if the problems are serious.
10. The total number of laparotomies performed will depend on the condition of the animal, quality of the oocytes, duration of egg production and life span of the animal. A maximum of five recovery surgeries (the sixth would be terminal) per animal is acceptable. Additional surgeries must be approved by the institutional animal care and use committee (IACUC).
11. Females can also be induced to lay eggs by subcutaneous injection of 100 units of pregnant mare serum (gonadotropin) dissolved in sterile phosphate buffered saline, followed 4-9 days later by 800-900 units of human chorionic gonadotropin dissolved in phosphate buffered saline. Induced frogs should be kept in individual tanks containing 100 mM sodium chloride at room temperature. These frogs should be rested for at least 3 months between uses.

Adequate recovery time should be allowed between laparotomies, in any case no less than one month. A longer interval is preferred. The investigator can alternate oocyte collection between left and right ovaries and consider rotation of frogs so that the interval between surgeries in any individual is maximized.

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