

# RSPCA Australia Scholarship for Animal Welfare

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**Project title:** Hormonal induction of sperm release in the Roseate Frog (*Geocrinia rosea*)

## Progress report 2007-08

### Project summary

Amphibian decline is recognized as a serious phenomenon affecting amphibian populations worldwide (Stuart *et al.* 2004). Australia is among the countries most adversely affected, with 47 of 214 described frog species threatened with extinction (IUCN 2006). To reduce further loss of amphibian biological and genetic diversity, captive breeding and assisted reproductive technologies (ART) have been identified as a mechanism which will allow development of captive populations to be used in future reintroduction programs when threatening processes have been identified and ameliorated (Clulow *et al.* 1999, Mahony *et al.* 1999).

*In-vitro* Fertilization (IVF) is an integral component of assisted reproduction and first requires the collection of fresh gametes (eggs and sperm). Traditionally, sperm collection techniques for frogs have either involved euthanasia of males and post-mortem removal of testes or injection protocols. The former is of no value for breeding of threatened species where the loss of reproductive individuals is unacceptable, and the latter may be traumatic for small frog species such as *G.rosea* (mass < 1 gm, body length 20- 25 mm).

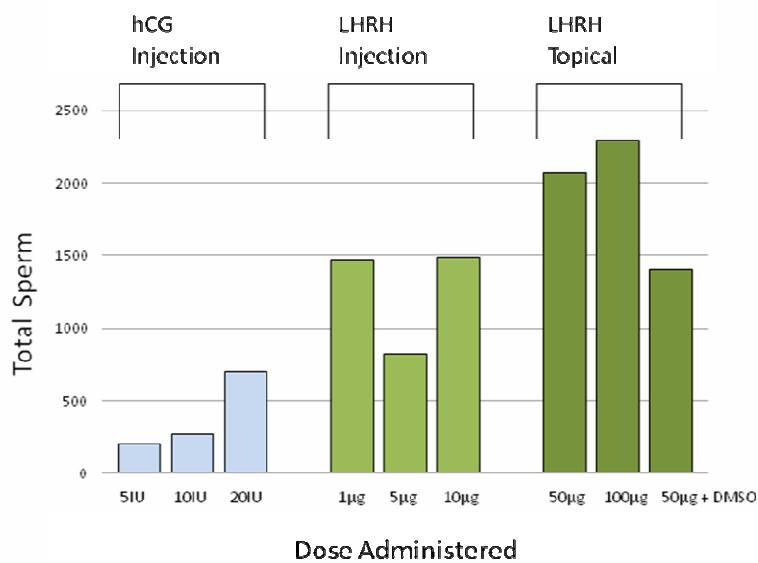
Frogs possess highly permeable ventral abdominal surfaces (Bentley & Main 1972), providing the opportunity to investigate the effectiveness of placing reproductive hormones on the abdominal skin as an alternative, non-invasive means of initiating sperm release. Such a non-invasive method of administering luteinizing hormone-releasing hormone (LHRH) has been shown to be effective in inducing sperm release in the American and Gulf Coast Toads (Rowson *et al.* 2001). If topical administration is successful in Australian frog species, it would allow Zoo keepers and Research Assistants who are not trained in administering animal injections to safely and effectively administer reproduction hormones and avoid trauma that may be associated with injection of very small frogs.

### Project Objectives:

- 1) Investigate the sperm-release response to subcutaneous injection of varying concentrations of human Chorionic Gonadotrophin (hCG)
- 2) Investigate the sperm-release response to topical administration of varying concentrations of human Chorionic Gonadotrophin (hCG) with or without the aid of penetration enhancer Dimethylsulfoxide (DMSO)
- 3) Investigate the sperm-release response to subcutaneous injection of varying concentrations of luteinizing hormone-releasing hormone (LHRH)
- 4) Investigate the sperm-release response to topical administration of varying concentrations of luteinizing hormone-releasing hormone (LHRH) with or without the aid of penetration enhancer Dimethylsulfoxide (DMSO)

*Geocrinia rosea* exhibited a sperm-release response following the injection of hCG and both the injection or topical administration of LHRH (Figure 1). Males did not respond to the topical administration of hCG on its own or when administered in combination with the penetration enhancer DMSO. *Geocrinia rosea* responded by producing higher sperm yields when injected or topically administered the reproductive hormone LHRH compared to the injection of hCG (Figure 1).

Results from this study have positive implications for captive breeding programs, as a non-invasive method of hormone administration has been proven effective at inducing the release of viable sperm of comparable numbers to those obtained via hormone injection. It is hoped that these findings may aid the conservation of two closely related species *Geocrinia alba* and *G.vitellina* that are currently threatened with extinction.



**Figure 1:** The average total sperm yield over a 12 hour period post administration of hCG (human Chorionic Gonadotrophin) or LHRH (Luteinizing hormone-releasing hormone) via subcutaneous injection or topical administration onto the ventral abdominal surface. n=8 per dose administered