

ABSTRACTS

**Abstracts: UC Davis Transgenic Animal Research
Conference VI**

Isolation and selective culture of quail primordial germ cells and the development of a cross culture system to harbor development of manipulated embryos

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Primordial germ cells (PGCs) are the embryonic precursors of adult egg and sperm. Isolation, propagation, genetic manipulation and reimplantation of modified PGCs that retain their commitment to the germline have been recently described for the successful generation of transgenic chickens [1]. Here, we report the adaptation and application of these techniques to Japanese quail for the purpose of future applications involving the introduction of a phytase transgene as previously described [2]. Quail PGCs are harvested by aspiration of whole embryonic blood from embryos that have been incubated for 48–52 h. Blood is aspirated using a mouth pipette fitted with a 37 μm i.d. embryo biopsy needle and then cultured in media that has been conditioned on buffalo rat liver feeder cells to selectively propagate PGCs. We are continuing to refine methods for the selective culture and propagation of PGCs for genetic manipulation and reimplantation into developing embryos. Modified PGCs must be reinjected into an embryo of the same developmental stage, and recipient embryos must then be allowed to develop to hatch. This requires the use of a cross culture system involving transfer of the manipulated quail embryo to foster chicken egg shell beds. We have explored various conditions to optimize this system including egg shell bed size, use of antibiotic and fungicidal compounds, incubation environment, and supplementation with thick and thin chicken albumen. For unmanipulated embryos entering the cross culture system, we have experienced hatchability rates ranging between about 10% and 30%. We have also observed that embryos entering this cross culture system require an additional 12–48 h to complete development.

1. Marie-Cecile van de Lavoie et al (2006) Germline transmission of genetically modified primordial germ cells. *Nature* 441:766–769

2. Garret Guenther et al (2005) Development of methods for the production of transgenic quail expressing an *E. coli* phytase gene. *Transgenic Res* 15:115–130 (abstr.)