**MS Thesis**
**Department of Environmental Science and Policy**
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Title: The Influence of Retinoic Acid on Cell Proliferation and Differentiation in Lamb Testis Tissue

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## ABSTRACT

The majority of wild ungulate species are threatened or endangered by extinction. Populations managed in zoos and breeding centers serve as 'insurance' for species sustainability and future reintroductions. However, 10-15% of animals born in ex situ collections die before reaching puberty and fail to contribute to conservation breeding. Understanding the *in vitro* culture requirements for producing gametes from gonadal tissues could facilitate the rescue of germplasm from genetically valuable individuals. Most studies on this topic have focused on the laboratory mouse, with limited information on larger animal models. Retinoic acid (RA) is the biologically active form of vitamin A and is involved in the early steps of spermatogenesis by promoting meiosis to occur in progenitor cells. It was hypothesized that RA would promote Sertoli cell and gonocyte proliferation and/or differentiation in testicular explants that were cryopreserved and thawed before *in vitro* culture. Testicular pieces (1-2 $mm^3$) from 6-7 week-old lambs (n = 6) were cryopreserved using the slow-cool method. Tissues were thawed at room temperature (1 min), then in water (25°C; 1 min),

followed by three washes (5 min each) in MEM containing 20% FBS, 25 mM HEPES, and antibiotics). Thawed explants were cultured for 5 weeks in the absence (0 μM, control) or presence (1 μM, 2 μM, and 5 μM) of RA. Twenty tubules of uniform size per piece were evaluated for the number of gonocytes and Sertoli cells. Genes chosen for analyses were *PCNA* (proliferation), *c-Kit* (differentiation), *Stra8* (RA response), and *HSD3-β* (androgen synthesis). All analyses were performed with a logistic regression followed by a Tukey's post-hoc test. Gonocyte counts remained stable over time, and did not respond to RA1 or RA5 but declined with RA2 ($p < 0.05$). Sertoli cell counts were unaltered for the first three weeks of culture ($p > 0.05$) before declining by week 4 ($p < 0.05$), and decreased in the presence of RA2 and RA5 ($p < 0.05$). *PCNA* expression did not increase overall by week 5 and was not affected by RA ($p > 0.05$) though RA5 was less than RA1 and RA2 ($p < 0.05$). The expression of *c-Kit* increased overall by week 5 and increased with RA ($p < 0.05$). *Stra8* experienced oscillated over the five weeks but never returned to week 1 levels ($p < 0.05$), and responded positively to RA1 ($p <0.05$) compared to the other treatments. *HSD3-β* had the highest expression in weeks 4 and 5 ($p < 0.05$) but RA had a marginal impact as the three treatments were not distinct from each other ($p > 0.05$) with RA1 and RA5 being only slightly different from the control. This study demonstrates for the first time that lamb testicular explants can be 1) cultured *in vitro* for up to five weeks and 2) retinoic acid stimulates pathways involved in germ cell differentiation while promoting cell proliferation and steroidogenesis. Although continuation of spermatogenesis was not achieved in this study, results addressed critical knowledge gaps pertaining to long term culture of testicular tissue and the role of retinoic acid in lamb spermatogenesis *in vitro*.