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# Sensitivity of spermatogonia to irradiation varies with age in prepubertal ram lambs

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ABSTRACT

Although germ cells from donor rams transplanted into irradiated recipient testes have produced donor derived offspring, efficiency is low. Further optimization of recipient irradiation protocols will add precision to the depletion of recipient spermatogonia prior to germ cell transplant. Three irradiation doses (9,12,15 Gy) were administered to ram lambs aged 14 weeks (Group 1) and 20 weeks (Group 2), then testicular biopsies were collected 1, 2 and 3 months after irradiation. At 1 month after irradiation of Group 1, only the largest dose (15 Gy) reduced spermatogonia numbers below 10% of non-irradiated controls, whereas in Group 2 lambs, each irradiation dose reduced spermatogonia below 10% of controls. In both Groups, fewer differentiated germ cells were present in seminiferous tubules compared to controls. At 2 months after irradiation, spermatogonia numbers in both Groups increased more than sixfold to be similar to controls, whereas fewer differentiated germ cells were present in the tubules of both Groups. At 3 months in Group 1, each irradiation dose reduced spermatogonia numbers to < 30% of controls and fewer tubules contained differentiated germ cells. Lesser expression of spermatogonial genes, VASA and UCHL-1, was observed in the 15 Gy group. In Group 2, only 12 Gy treated tubules contained fewer spermatogonia. Knowledge of these subtle differences between age groups in the effect of irradiation doses on spermatogonia or differentiated germ cell numbers and the duration of recovery of spermatogonia numbers after irradiation will aid the timing of germ cell transplants into prepubertal recipient lambs.

## 1. Introduction

The germ cell transplant technique in rodents has proved to be a valuable technique to study spermatogonia and the stem cell niche (Oatley and Brinster, 2006; Brinster, 2007). Its translation into domestic species has introduced a novel option for breeding improvement programs and an alternate method to produce transgenic animals (Hill and Dobrinski, 2006). Successful germ cell transplantation with donor derived spermatogenesis and offspring was first described in mice followed a decade later by the adaptation of the technique for agricultural animals (Brinster and Avarbock, 1994; Brinster and Zimmermann, 1994; Honaramooz

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et al., 2002; Honaramooz et al., 2003). Although colonization of unprepared recipient testes by germ cell transplantation occurs, prior removal of endogenous germ cells should allow for increased colonization of the donor spermatogonial stem cells (Shinohara et al., 2001; Brinster et al., 2003; Honaramooz et al., 2003).

Key to adapting the germ cell transplant from rodents to domestic animals is solving issues related to scaling up the procedure to physically much larger animals with much greater testis volumes. Younger animals of smaller size have, therefore, been preferred as recipients for germ cell transplant particularly when testis irradiation is used to deplete endogenous germ cell populations. Several studies have used irradiation in rodents and various domestic species to estimate the optimal time for germ cell transplantation (Izadyar et al., 2000; Creemers et al., 2002; Schlatt et al., 2002; Izadyar et al., 2003; Honaramooz et al., 2005; Oatley et al., 2005; Kim et al., 2006; Trefil et al., 2006; Zhang et al., 2006). Most studies have proposed, based on the timing of endogenous recovery, that the optimal time to perform germ cell transplants in livestock animals is between 1 and 2 months post irradiation (Honaramooz et al., 2005; Herrid et al., 2006, 2011). The majority of studies have used large irradiation doses (> 5 Gy) which are generally needed to deplete spermatogonia stem cells. Large irradiation doses must be balanced against the potential to cause long-term sterility. Doses of 9 Gy in rats and 12 Gy in mice (Meistrich et al., 1978; Pinon-Lataillade et al., 1991) have resulted in permanent sterility.

The current study in prepubertal rams expands upon earlier transplant studies using pubertal rams irradiated with 9 Gy or 15 Gy followed by germ cell transplant 6 weeks later (Herrid et al., 2009). The effect of irradiation on seminiferous tubules was not studied specifically although reductions in testicular size (scrotal circumference) in the 3 months following irradiation were indicative of reduced cellularity within seminiferous tubules and intertubular compartments. To quantify the impact of irradiation on seminiferous tubules the current study analysed testicular biopsies taken from pubertal ram lambs for 3 months following irradiation.

## 2. Materials and methods

### 2.1. Study design

Two age groups of animals were randomly selected ($n$ = 12 per group). Group1 contained animals aged 14 weeks ( ± 2 weeks) with mean body weights of 22.6 kg and mean scrotal circumference of 13.4 cm. Group 2 were older and aged 20 weeks ( ± 2 weeks) with mean body weights of 27.5 kg and mean scrotal circumference of 21.5 cm. Animals from each group were randomly allocated to receive irradiation doses of 0, 9, 12 or 15 Gy. Animals were handled and treated according to the guidelines of the CSIRO animal ethics committee and located at CSIRO, Armidale, NSW, Australia.

### 2.2. Selection of animals

The two age groups were selected to compare the effects of irradiation in young Merino rams during early and late puberty. During early puberty (Group 1 animals aged 14 weeks with a scrotal circumference range of 13–15 cm) the most advanced germ cell types within the seminiferous tubules are spermatogonia and/or spermatocytes (Skinner et al., 1968). This early puberty group was compared to a late puberty group, (Group 2 aged 20 weeks with a scrotal circumference range of 18–25 cm) which corresponds to seminiferous tubules containing active spermatogenesis with spermatozoa at the most advanced germ cell stage.

### 2.3. Testicular irradiation

The irradiation doses of 9, 12 or 15 Gy were delivered by a 6 MV photon beam produced by a linear accelerator at a dose rate of 2.5–3 Gy/min. Each lamb was anesthetized using a 0.1 ml/kg i.v. of a combination of tiletamine and zolazepam (Zoletil 100 mg/ml; Virbac Corporation). The diameter of the testes was measured, and delivery of irradiation calculated accordingly to deliver a consistent dosage throughout the full depth of the testis to within ± 6.5%, at a dose rate of 2.5–3 Gy/min. Control animals were not placed inside the linear accelerator and did not receive an irradiation dose.

### 2.4. Records and sample collection

At 1, 2 and 3 months after irradiation, the left testis was biopsied. The biopsies were performed under general anaesthesia (2%–3% Isoflurane vapour in oxygen + $NO_2$). The biopsies were fixed in Bouin's solution for 3–6 h, followed by transfer into 70% alcohol.

At 3 months after irradiation both testes were removed under general anaesthesia. Testis and epididymis weights were recorded. Testis fluid was collected by an impression smear taken from the freshly cut surface of the testis, while fluid from the head and tail of the epididymis was collected and transferred to a glass slide. All slides were examined using a light microscope for the presence of spermatozoa. The presence/absence, motility and morphology of spermatozoa in the testis and epididymal fluids were recorded.

Testis tissue was collected and fixed in Bouin's solution overnight and transferred into 70% Ethanol prior to paraffin embedding and processing. Sections were cut at 5 µm then left unstained for immunohistochemistry or stained with haematoxylin and eosin (H& E).

### 2.5. Histology and immunohistochemistry

Immunohistochemistry was conducted essentially as described previously using antibody dilutions of 1:400 for PGP 9.5/UCHL-1