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# Feasibility of salvaging genetic potential of post-mortem fawns: Production of sperm in testis tissue xenografts from immature donor white-tailed deer (*Odocoileus virginianus*) in recipient mice

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ABSTRACT

The purpose of this study was to evaluate the long-term outcome of testis tissue xenografting from immature deer. Testis tissue was collected post-mortem from a 2-mo-old white-tailed deer fawn (*Odocoileus virginianus*) and small fragments of the tissue were grafted under the back skin of immunodeficient recipient mice ($n = 7$ mice; 8 fragments/mouse). Single xenograft samples were removed from representative recipient mice every 2 mo from grafting for up to 14 mo post-grafting. The retrieved xenografts were evaluated for seminiferous tubular density (per $mm^2$) and tubular diameter, as well as for seminiferous tubular morphology and identification of the most advanced germ cell type present in each tubule cross section. Overall, 63% of the grafted testis fragments were recovered as xenografts. Testis tissue xenografts showed a gradual testicular development starting with tubular expansion by 2 mo, presence of spermatocytes by 6 mo post-grafting, round and elongated spermatids by 8 mo, followed by fully-formed sperm by 12 mo post-grafting. The timing of complete spermatogenesis generally corresponded to the reported timing of sexual maturation in white-tailed deer. This study demonstrated, for the first time, that testis tissue xenografting from immature deer donors into recipient mice can successfully result in testicular maturation and development of spermatogenesis in the grafts up to the stage of sperm production. These results may therefore provide a model for salvaging genetic material from immature male white-tailed deer that die before reaching sexual maturity.



## 1. Introduction

Species extinction rates seem to have accelerated in recent decades (Lenzen et al., 2009). The proportion of threatened species is much greater in mammals than that in other groups such as birds (Mace et al., 2008) and currently an alarming 23% of all mammalian species are considered threatened or vulnerable. This high ratio reflects species for which sufficient information is available, although the actual figures may be higher. Among mammals, ungulates are one of the groups with a greater percentage of threatened species. It is believed that ungulates are the only mammalian group in which hunting is a more frequent cause of threat than loss of habitat (IUCN 2011, www.iucnredlist.org).

To prevent the permanent loss of an individual's potential contribution to the genetic variability of a rare or endangered species, it is feasible to collect sperm before or even shortly after death by retrieval from the ejaculate, epididymis or testes, and cryopreserve the sperm for future use in assisted reproduction (Kishikawa et al., 1999; Martínez et al., 2008; Gañán et al., 2009). Preservation of

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sperm, however, is not an option when young offspring die prior to reaching sexual maturity. Cloning has been used for a number of species and especially where the goal has been to produce a genetically exact replica of an individual. However, development of cloning for a new species is technically demanding and costly, but more importantly cloning does not immediately provide the genetic diversity that would otherwise be offered by gametes.

Grafting of testis tissue fragments from donors of different species under the back skin of recipient mice resulted in the production of functional sperm (Honaramooz et al., 2002). Thereby, this system provided a novel tool for the study, manipulation, and propagation of the male germ line. Testis tissue xenografting has allowed the study of testis maturation and establishment of spermatogenesis in the grafted tissue from a number of species including laboratory and domestic animals (Honaramooz et al., 2002, 2004, 2008; Schlatt et al., 2002; Oatley et al., 2004; Snedaker et al., 2004; Rathi et al., 2006; Arregui et al., 2008). Using testis tissue xenografting, sperm have been produced from a variety of donor species, including mice, hamsters, cats, dogs, pigs, goats, sheep, cattle, bison, horses and primates (Honaramooz et al., 2002, 2004; Oatley et al., 2004; Snedaker et al., 2004; Rathi et al., 2005, 2006; Abrishami et al., 2010a; Abbasi and Honaramooz, 2011). The sperm recovered from such grafts are fertilization competent after intracytoplasmic sperm injection (ICSI) (Honaramooz et al., 2002, 2004, 2008), leading to the birth of healthy progeny (Schlatt et al., 2003; Nakai et al., 2010). Recently, we also developed methods for cool storage of immature testis tissue for up to 6 days and cryopreservation for long periods of time for such applications (Abrishami et al., 2010b; Yang et al., 2010). Therefore, testis tissue xenografting can be a unique solution for genetic conservation of immature males by producing sperm from these immature donors in xenografts, followed by extraction and cryopreservation of sperm for future use in ICSI.

Deer are a traditional ungulate game species and, as such, a primary focus of research in wildlife management. Farming of deer as a specialized hoofstock is also expanding into a viable industry in parts of North America, Europe and New Zealand. Farming of elk and deer is aimed at producing velvet antler for traditional medicine in domestic or Asian market, or at selecting and propagating genetically superior bucks for sports outfitting. Therefore, not only deer are subject of a small but increasing number of investigations into the reproductive physiology and management, but also provide excellent models for research in reproductive strategies for conservation purposes aiming at endangered or threatened ungulates. The objective of this study was to evaluate the long-term outcome of testis tissue xenografting using post-mortem white-tailed deer fawns.

## 2. Materials and methods

### 2.1. Donor testis tissue

Testes from a 2-mo-old white-tailed deer (*Odocoileus virginianus*) were collected immediately after euthanasia because of serious lesions (due to fighting with other deer) at a commercial deer farm. The testes were transferred to the laboratory in ice-cold Dulbecco's phosphate buffered saline (DPBS, Cat. No. 20-031-CV, Mediatech, Manassas, VA, USA). The testes were then washed three times with DPBS, containing 2% (w/v) antibiotic/antimycotic solution (Cat. No. 30-004-CI, Mediatech), and the tunica albuginea, rete testis and overt connective tissues were removed. The testis parenchyma was then divided into small fragments of ~5 mg, and maintained in Dulbecco's modified Eagle's medium (DMEM, Cat. No. 10-013-CM, Mediatech) on ice until grafting into recipient mice within 2 h. Prior to grafting, representative donor testis tissue fragments were fixed in Bouin's solution overnight, washed with and kept in 70% (v/v) ethanol and processed for histology as a reference for graft development.

### 2.2. Recipient mice and procedures for xenografting of testis tissue

Recipient mice were gonadectomised immunodeficient nude mice (NCr, *nu/nu*, Taconic, Germantown, NY, USA) that were ~10 wk old at the time of grafting and maintained aseptically in groups of 3 or 4 in plexiglass micro-insulators under controlled photoperiod environment (lights on from 06:00 through 18:00) with sterile water and mouse chow provided *ad libitum*.

In preparation for surgery, the mice were anaesthetised with intra-peritoneal injection of ketamine hydrochloride (100 mg/kg; Ketalene, Bimeda-MTC, Cambridge, ON, Canada) and xylazine hydrochloride (10 mg/kg; Vet-A-Mix, Shenandoah, IA, USA). Each mouse received eight transverse linear incisions (~5 mm in length) into the back skin, four on each side of the midline. A small subcutaneous pouch was made through each incision and a testis tissue fragment was inserted and the incision was closed using wound clips (Michel Clips 7.5 mm, Miltex, York, PA, USA). This work was approved by the University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

### 2.3. Gross and histological analysis

Representative recipient mice with deer testis tissue xenografts were anaesthetised and underwent single-graft removal or were killed at 2, 4, 6, 8, 10 and/or 12, and the remaining mice sacrificed at 14 mo after grafting and xenografts recovered. Visible testis tissue xenografts from each time point were dissected out and individually weighed. The retrieved grafts were fixed in Bouin's solution overnight, washed with and kept in 70% ethanol solution until processing for histology. The fixed tissues were then processed, paraffin blocked and sectioned (at 6 μm thickness) at the largest diameter of the graft. The sectioned tissues were stained with haematoxylin and eosin and analysed using light microscopy equipped with a digital camera.

For histological analysis of the xenografts, images were captured from all cross sections of each xenograft. The images were taken at 200× magnification using a calibrated microscope (calibrated using the eyepiece reticule