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Chemical sterilisation of *Bos indicus* bull calves following intratesticular injection of zinc acetate: Effects on semen quality and testicular changes

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ABSTRACT

The aim of this study was to determine the effects in *Bos indicus* bull calves of intratesticular administration of 1 mL of either saline ($n = 9$) or one of the two doses of zinc acetate (ZA1, 57.75 mg, $n = 10$ or ZA2, 71.75 mg, $n = 10$) on semen quality and testicular changes. Semen was collected by electroejaculation on Days 343, 524 and 783 and animals were slaughtered on Day 860. Treatment reduced median maximum number of progressively motile and morphologically normal sperm collected ($P = 0.001$) and the percentage of animals in which sperm were recovered (saline: 100%, 9/9; ZA1: 44.9%, 4/9 and ZA2: 40.0%, 4/10; $P = 0.013$). Compared to saline treated controls, treatment with ZA reduced the mean diameter of the testes after Day 34 of treatment (treatment × time, $P = 0.013$) and total testicular weight at slaughter (treatment: mean ± SEM; saline: 569.4 ± 59.0 g, ZA1: 249.3 ± 72.9 g, ZA2: 247.5 ± 68.1 g; $P = 0.004$). Histological changes in testes of bulls treated with ZA were characterized by germ cell depletion, vacuolation of Sertoli cells, interstitial fibrosis, epididymal duct atrophy with variable remnants of testicular tissue and degeneration. We conclude that intratesticular administration of two doses of ZA in *B. indicus* calves is able to severely impair spermatogenesis and cause varying degrees of testicular degeneration and a reduction in testicular diameter and mass. Further investigation is required to determine ways of obtaining more consistent results from treatment.

1. Introduction

There is increasing community concern about welfare implications of some routine husbandry procedures which are carried out on livestock. Castration of calves is known to cause acute and chronic pain although pain responses vary with both the age of calves and the method of castration (Robertson et al., 1994; Molony et al., 1995; Stafford and Mellor, 2005; Thuer et al., 2007; Lomax and Windsor, 2013). Castration of calves may come under closer public scrutiny and regulation in the future with the demand for the adoption of other sterilization techniques which have less of an adverse effect on animal welfare. Research into alternative means of sterilization could provide alternatives to surgical castration which may promote better welfare outcomes for animals and which could be utilized if restrictions are placed on the practice of surgical castration. In some environments non-surgical methods of castration may also be preferable where there is a significant risk of postoperative infections or myiasis.

Chemical sterilization by intra-testicular injection of chemical agents has been attempted as an alternative

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to surgical castration in a variety of domestic and laboratory animal species with variable responses being reported (Kutzler and Wood, 2006; Massei and Miller, 2013). Treatment is aimed at permanently disrupting the spermatogenic and endocrine function of the testes secondary to the destruction of testicular tissue, orchitis and fibrosis (Bowen, 2008) or destroying the patency of the epididymis or ductus deferens. Side effects such as pain, incomplete responses to treatment, persistence of male-like behaviour and scrotal ulcers have occurred with some treatments while some chemicals would not be acceptable for use in food producing animals.

Zinc gluconate has been used as an intratesticular sterilant in dogs (US FDA, 1993; Tepsumethanon et al., 2005; Oliveira et al., 2007; Soto et al., 2007, 2009; Levy et al., 2008) and cats (Fagundes et al., 2014) with side effects such as pain or injection site reactions being reported as uncommon. Testicular changes following treatment are characterized by inflammation, necrosis, testicular degeneration and fibrosis, germ cell depletion, and vacuolation of Sertoli cells. Effects on testicular size have been variable with both a decrease in testicular size (US FDA, 1993; Esquivel Lacroix, 2006) and no significant change in testicular size (US FDA, 1993; Soto et al., 2009) being reported. Sperm output following treatment in most species appear to be severely impaired with aspermia, azoospermia and oligospermia being reported although dose, species, age and the size of the testes may influence responses to treatment (US FDA, 1993; Soto et al., 2009; Brito et al., 2011; Oliveira et al., 2012, 2013).

There are currently no reports on the effects of zinc containing compounds when used as a potential chemical sterilant on semen quality and testicular changes in bulls. A companion report to this study has reported on effects of intratesticular administration of zinc acetate (ZA) on growth, hormone and carcass characteristics in *Bos indicus* bulls (Cavalieri and Wang, 2015). The main findings included a decrease in mean serum concentrations of testosterone and concentrations 48 h following administration of hCG without significantly affect body or carcass weights. The aim of this study was to investigate the effects of administering two doses of the potential intratesticular sterilant, ZA to bull calves on testicular size, histology and semen quality in *B. indicus* bulls following treatment.

2. Materials and methods

The experimental protocol and procedures used in this study were approved by the James Cook University Animal Ethics Committee (approval number: A1304).

2.1. Location, treatment and nutrition

Animals were located at the James Cook University, Tropical Veterinary Research Station, Fletcherview (latitude 19° 53′4″ S; longitude 146° 10′43″ E) located in the dry tropics, of Northern Queensland. Brahman calves ($n = 38$), 5–6 months of age were first weighed and assigned to treatments on Day -7 of the study. Groups were balanced for live weight and treatments were administered on Day 0. Calves ($n = 9$) were surgically castrated as part of a separate but related study (Cavalieri and Wang, 2015). Calves in the control group (saline, $n = 9$) had each testis injected with 1 mL of saline (0.9% NaCl) solution. Other calves had each testis injected with 1 mL of a solution containing ZA at a concentration of 57.75 mg/mL (ZA1; $n = 10$, 17.2 mg Zn; Fahim Technology™, Inc. Columbia, Missouri) or 71.75 mg/mL (ZA2; $n = 10$, 21.4 mg Zn).

Intratesticular injections were administered with a 25-gauge needle, 25 mm in length. The scrotal skin was first swabbed with a povidone iodine antiseptic scrub. The needle was inserted into the dorsal half of the testis. Small quantities of the solution (approximately 0.2 mL) were injected in one location and then the needle was repositioned adjacent to that location and another similar quantity was injected. This procedure was repeated with the intent of avoiding the deposition of product in one location only but instead fanning the substance across the dorsal pole of the testis to inject in the region of the ductuli efferentes which is similar to the technique that has been used (Oliveira et al., 2007) and is recommended when administering zinc gluconate to sterilize dogs (Arksciences, 2014).

Between Days 545 and 775 due to local flooding bulls could not be recovered for data collection until Day 775. Two bulls (ZA2 treatment) could not be found when remaining stock were recovered and by the time of slaughter. From Days 787 to 859 bulls were maintained in a feedlot (72 days) and then slaughtered (Day 860).

2.2. Measurements and blood samples

Testicular measurements and blood samples were collected on days 7, 34, 62, 132, 201, 263, 343, 448, 524 and 783, where Day 0 was the day when treatments were applied. Details relating to blood sampling, hCG stimulation testing and assay for testosterone have been described (Cavalieri and Wang, 2015).

Testicular diameters were monitored using transcutaneous ultrasound throughout the study using a 6.0 MHz (Days 0–524, Aquila Pro Vet, Medical Plus Australia, Crows Nest, NSW) or 5.0 MHz linear probe (Days 783, My Lab™ 30 Vet, Medical Plus Australia, Crows Nest, NSW). Diameter was recorded at the point of maximum width of the testis with the probe oriented perpendicular to the long axis of the testis and the region corresponding to the mediastinum located centrally when it was visible. Electronic callipers were used to make testicular measurements. During the course of the study some testes moved from a scrotal to an inguinal location. Testes located outside of the scrotum were identified by their location between the scrotal neck and external inguinal ring, and palpation of structures which resembled the regions corresponding to the head, body and tail of the epididymis associated with the organ. Testes from some bulls ($n = 5$) that could not be definitively located in the latter stages of the study were assigned a diameter corresponding to the 25th percentile of the diameter of testes located out of the scrotum on the day in question rather than a zero value.