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Chemical castration by a single bilateral intra-testicular injection of chlorhexidine gluconate and cetrimide in bucks

A Mohammed & FO James

Department of Veterinary Surgery and Theriogenology, Faculty of Veterinary Medicine, University of Maiduguri, Borno-State Nigeria.

*Correspondence: Tel.: +2347035218214, E-mail: bdlhmohammed@yahoo.com

Abstract

Six apparently healthy Borno white bucks weighing 15± 1.6 kg and aged 1.3± 0.3 years were used for this study. Two and half (2.5) ml Purit® (chlorhexidine gluconate 0.3% B.P W/V and cetrimide 3.0% B.P W/V CAPL Lagos) were injected bilaterally into the caudae of each epididymis following sedation with xylazine hydrochloride. The pre-study scrotal circumference was 20.1±1.5cm; Significant decrease in scrotal circumference ($P<0.01$) 15.8±1.6cm occurred 35 days post-injection. There was no significant difference ($P>0.05$) between pre-injection semen volume 0.6±2.1ml and 24 hour post-injection semen volume 0.3±1.4cm. Subsequently, only few drops could be collected and from day 20 post- injection, no semen ejaculate could be collected from all the six bucks. Azoospermia was noted from day 16 post-injection with 0% motile cells, 95% dead cells and 25-60% abnormal sperm cells. From this study, Purit® (chlorhexidine gluconate B.P 0.3% W/V and cetrimide B.P 3.0% W/V) can be used as an effective chemical castration agent in small ruminants.

Keywords: Buck, Castration, Chemical, Intra-testicular injection, Purit.

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Introduction

Castration is any procedure that removes the testicles surgically, damaging them irreparably or causing them to atrophy by stricture of the blood supply (Currah, *et al* 2009). There are various castration methods used in animals, which include physical, chemical and hormonal methods (Rajkumar, 2013). The physical methods involve surgical removal of testicles, application of rubber ring at the base of the scrotum and bloodless castration by using burdizzo clamps (Stilwell *et al.,* 2008). While chemical castration involves injecting a sclerosing or toxic agents into testicular parenchyma to cause irreversible damage and functional loss (Fordyce *et al.,* 19897). In hormonal techniques an injection of immuno-contraceptive is given to induce antibody production against gonadotropin releasing factor leading to decreased production of endogenous hormone (Fisher *et al*., 1996). Various agents have been used for chemical castration in animals through intra-testicular injection. They include ferric chloride, BCG (Das *et al*., 1982), glycerol (Immegart & Threlfall, 2000), Chlorhexidine and cotton seed oil (Fadason 2001), formalin (Ijaz *et al*., 2000; Bakir *et al* ., 2002). All these have reported one complication or the other. The purpose of this work is to see whether Purit® (chlorhexidine gluconate B.P 0.3% W/V and cetrimide B.P 3.0%W/V) is a better agent for chemical castration in goats.

Materials and methods

Six apparently healthy Borno white bucks weighing 13-17kg and aged 1-1.5 years were used for this study. The bucks were purchased from Maiduguri metropolitan small ruminants market. Upon purchase they were de-wormed with albendazole at a dose rate of 7.5mg/kg body weight orally and stabilized for three weeks. They were housed at Large Animal Clinic pens Veterinary Teaching Hospital University of Maiduguri. They were fed with groundnut hay, bean husks and water provided ad-libitum. After stabilization semen samples were collected with the aid of an electric ejaculator from

each buck and analyzed for sperm count, sperm viability, motility and morphology before the injection of Purit® (chlorhexidine gluconate B.P 0.3% W/V and cetrimide B.P 3.0% W/V). The pre-injection scrotal circumference was taken for all the bucks.

The bucks were sedated with xylazine 0.2mg/kg body weight intramuscularly. Ten minutes after xylazine injection, the scrotum was scrubbed with methylated spirit and bilateral intra-testicular injection of 2.5ml Purit® (chlorhexidine gluconate BP 0.3% w/v and cetrimide BP 3.0 % w/v) into the tail of the epididymis was made.

Subsequently, semen samples were collected from each buck on days 3, 10, 16, 20, and 30 post-injection. The samples collected were analyzed for volume, sperm motility, viability and other morphologic changes. In addition the scrotal circumferences were taken 24 hours post-injection and then weekly up to five weeks. The samples were analyzed using student's t-test (Chatfield, 1983).

Results

Following bilateral intra-testicular injections of 2.5ml Purit® (chlorhexidine gluconate B.P 0.3% W/V and cetrimide B.P 3.0% W/V), pyrexia 40±1.5^{o}C, reduced appetite and depression were observed in all the six goats for the first three days post-injection. Pre-injection scrotal circumference was 20.1±1.5cm whereas the circumference was 22.4±1.6cm 24hrs post –injection. This increase was a significant different ($P<0.01$). From the second week the scrotal circumference started decreasing though not significantly ($P>0.05$).However, there was a significant decrease ($P<0.01$) in the scrotal circumference 15.8±1.6cm at day 35 post-injection compared with the pre-injection value of 20.1±1.5 cm.

For the first week post-injection, all the animals were sensitive to touch and handling of the scrotum, which was also warm to touch. Varied testicular consistencies were also noted. They were initially firm and immovable within the scrotum but later on, two bucks showed soft fluctuating areas, whereas, the scrotum in the rest remained firm. Apart from the pyrexia, reduced appetite and depression earlier mentioned, no other clinically obvious systemic signs were observed.

Pre-study semen volume was 0.6±2.1 ml and that of the first day post-injection was 0.3±1.4 ml. Subsequently, only drops were obtained and from day 20 post-injection no semen or ejaculate could be collected from all the six bucks. Semen pH of 7.5±0 and 7.0±0 pre and post-injection were observed. Microscopic motility of sperm cells were initially good to very good (60-80%) pre-injection; but decreased to very poor (zero motility) from the third day post-injection. Also noticed were increasing sperm cell abnormalities. These were about 4% pre injection but rose to 25-60% post-injection. The abnormalities included head abnormality (loosed head), mid piece abnormalities (bent or coiled mid piece) and tail abnormalities (coiled or bent tails) and dead cells (95%).

Discussion

In attempts to achieve chemical sterilization, many chemical agents have been tried in several animals. With those agents that have showed various degrees of successes, it was obvious that single bilateral injections were much more efficient than single unilateral or multiple unilateral doses (Pineda *et al.,* 1977; Ahmad & Noakes, 1995). From these studies, the higher the volume (0.1ml, 0.5ml, 2.5ml, 5.0ml chlorhexidine gluconate and 5ml in 50% aqueous solutions of dimethyl sulphoxide (DMSO) or the higher the concentration (10, 20 and 40mg/kg body weight/testes calcium chloride) of such agents, the earlier or faster and better the sterilizing ability (Pineda *et al.,* 1977; Ahmad & Noakes, 1995; Jana *et al.,* 1991). From this study, 2.5ml Purit® 0.3% W/V chlorhexidine gluconate and 3.0% W/V cetrimide which is similar to the dose of chlorhexidine gluconate alone used in bulls (Pearson *et al.,* 1980) produced a more efficient result about the same time; the agent used, Purit®, being able to produce absence or lack of ejaculation in three weeks as against the azoospermia seen at the same time with chlorhexidine alone (Pearson *et al.,* 1980). Prepubertal animals (dogs) given bilateral injections of chemical sterilants began to ejaculate seminal fluid at approximately the same time as control animals but never had sperm in their ejaculates (Pineda *et al*., 1977). In this study however, matured animals were used but following the use of the chemical agent, they all could not ejaculate semen from the 20th day post-injection. Chlorhexidine gluconate had been used as a substitute for vasectomy to produce sterile males (Pineda, 1978). The time required to establish the obturating tissue reaction appears to be dependent on its concentration. In Beagle dogs, volumes of 0.5ml and 0.1ml of 3% chlorhexidine gluconate solutions were effective in inducing azoospermia by 42-77days, and 35days after treatment, respectively (Pineda, 1978). In bulls, 2.5ml or 5.0ml of 3.0% chlorhexidine

gluconate solution resulted in azoospermia within 3 weeks after treatment (Pearson *et al.,* 1980). Unilateral intratesticular injections of 5ml sterile chlorhexidine solution in 50% aqueous solutions of DMSO in goats and ram was considered probably too low to produce a sufficiently severe reaction necessary to result in azoospermic ejaculates (Ahmad & Noakes, 1995). From this study it has been found out that 2.5% 0.3 W/V chlorhexidine gluconate and 3.0% cetrimide W/V have been able to result in azoospermia by day 16 post-injection. This may not have resulted from 0.3% W/V chlorhexidine gluconate, which is not too high a concentration when compared to other works but may result from the 3% cetrimide.

The onset of sterility as typified by azoospermia, poor sperm motility, high percentage or proportion of dead sperm cells, increased sperm abnormalities, decreased volume of ejaculate, varying semen colour, atrophy of the testes, and abnormal individual sperm cell motility (Zemjanis, 1970). Azoospermia is referred to when not more than five sperm cells are observed in ten microscopic fields (Pineda *et al.,* 1970). From this study azoospermia was observed from day 16 post-injection as well as the other indexes of sterility mentioned above. Sperm abnormalities can be either primary or secondary. Primary abnormalities are considered to reflect disturbances of spermatogenesis and the different forms include head abnormalities (giant heads, small heads, pyriform heads, tapering and narrow heads, other deviations in size and form and loose abnormal heads), mid piece abnormalities (abaxially attached mid piece, double mid piece, coiled, frayed, granular or swollen mid piece) and tail abnormalities (tightly coiled and double tails). Secondary abnormalities, on the other hand, are believed to arise after the spermatozoa have left the seminiferous tubules (Zemjanis, 1970). Both types of abnormalities have been observed in this study with the predominance of loosed head; this is in agreement with studies in goats (Ahmad & Noakes, 1995). The incidence of each abnormality is determined by a differential count of the abnormal forms. Each abnormal form is recorded and all abnormal and normal cells in a field are counted (Zemjanis, 1970). Less than 10% motile cells, 81.91% dead and 70.81% abnormal cells with commonest abnormalities being degenerated detached heads or abnormal tails including looped tails or bent tails have been observed (Ahmed and Noakes, 1995). In this study, 0% motile cells, 95% dead cells and 25-60% abnormalities were also observed.

The onset of azoospermia or sterility following treatment with other agents apart from chlorhexidine gluconate was 26 days in dogs using balanced zinc gluconate (Tepsumethanon *et al.,* 2005), 30 days using calcium chloride (Jana *et al.,* 1991), 35 days in ram using formaldehyde in ethanol (Plant *et al.,* 1979) but 16 days in this study. From this study gross testicular changes noticed were in conformity with previous findings; the increased testicular changes observed were as a result of inflammatory reactions because of the sclerosing properties of the agent used (Pineda *et al.,* 1977). This inflammatory reaction, expectedly, was responsible, also, for the pain-associated depression in the first three days. Palpation of the testes performed daily after the treatment revealed hard, adherent, non-freely moving testes which reflected past localized inflammatory process whereas abscesses are recognized as localized areas of softer and more fluctuating consistency (Zemjanis, 1970). Increased sensitivity to the touch or handling of the testes noticed in the first week was a manifestation of acute orchitis. The absence of undesirable clinical side effects other than the transient swelling of the scrotum is an advantage noted with the use of the agent (Pineda *et al.,* 1977). It could therefore be said that the agent, Purit® (chlorhexidine gluconate B.P 0.3% W/V and cetrimide B.P 3.0% W/V) was capable of causing sterility in Borno white bucks characterized by increased dead cells and sperm cell abnormalities, zero sperm cell motility and finally inability to ejaculate. There were no noticeable systemic effects apart from initial three-day pyrexia, reduced appetite and depression. Based on the findings, Purit® may be recommended for use as an agent for chemical castration in small ruminants.

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