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Original Research

Effects of Intratesticular Injection of 70% Glycerin on Stallions

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ABSTRACT

The removal of endogenous germ cells of recipient stallions is a key step to produce donor germ cell-derived sperm using the germ cell transplantation technique. Six Thoroughbred stallions were divided into a treatment (n = 3) and a control group (n = 3), and 70% glycerin (1, 2, 3-trihydroxypropane, 40 mL per testis) or phosphate-buffered saline, respectively, was locally injected into testes. General semen evaluation, libido, and testicular volume were performed weekly from 3 weeks before to 10 weeks after treatment. The number of round germ cells in the ejaculate was counted using a hemocytometer. The hematoxylin and eosin staining was performed on the cross sections of testicular tissue obtained 11th week of treatment. Plasma testosterone levels in blood collected weekly were measured using a colorimetric competitive enzyme immunoassay kit. The sperm number was significantly lower than that of the control group at 5 and 10 weeks after glycerin injection. No differences in the status of spermatogenesis in the cross sections of seminiferous tubules and testicular volume were found between the two groups. The 70% glycerin-treated stallions had reduced total and progressively motile sperm and exhibited a significantly higher population of round germ cells in the ejaculate. Testosterone levels, testicular volumes, and libido of stallions were not significantly different between the groups. In conclusion, although intratesticular injection of 70% glycerin may have caused disassociation of some germ cells in the seminiferous tubules for several weeks, it did not significantly ablate germ cells in the tubules at 11 week in stallions.

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1. Introduction

Techniques for transplanting donor germ cells into the testes of recipient to produce donor-derived sperm are applicable in farm animals including bulls [1], boars [2], rams [3], and bucks [4]. However, the applicability of this technique in stallions remains to be elucidated. As one of key steps for the production of donor spermatogonial stem cell (SSC)–derived sperm, the techniques for endogenous germ cell removal in the seminiferous tubules of recipient models should be developed. The efficiency of SSC transplantation can be determined by the rate of endogenous germ cell removal [3]. While endogenous germ cells are removed in the recipient models, the place for donor germ cells and the libido should be maintained. The intraperitoneal injection of busulfan is the agent commonly used to deplete endogenous germ cells [5]. However, the administration of busulfan causes a side effect such as hematopoietic toxicity [6]. As an alternative, local irradiation treatment has been applied in recipient goats, rams, and bulls [1,7,8]. These results suggest that local testicular irradiation treatment may be applicable to produce recipient stallions, but it requires the irradiation (x- or gamma

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ray) machine. Recently, Pozor et al [9] reported that a single PO administration of 12.5 mg kg^{-1} indenopyride derivative RTI-4587-073 had potential for contraception in miniature stallions. The miniature stallions treated exhibited a significant reduction in sperm number and motility. The administration of indenopyride derivative RTI-4587-073 also resulted in degenerative changes in the cross section of seminiferous tubules in miniature stallions [10]. However, the compound is not publicly available.

A single intratesticular injection with 70% glycerol has been shown to be effective in long-term suppression of spermatogenesis in Sprague-Dawley rats, without altering serum levels of androgen, luteinizing hormone, or follicle stimulating hormone [11,12]. The suppression of spermatogenesis in response to the 70% glycerol treatment was also reported for squirrel monkeys [13]. The results of these studies suggest that an intratesticular injection of 70% glycerol might be applicable for removal of germ cells in recipient stallions. However, the effect of 70% glycerol appears to be species dependent. An intratesticular injection of glycerol failed to reduce sperm production in male dogs, although their libido was unchanged [14]. The effect of a single intratesticular injection of 70% glycerin in removal of endogenous germ cells should be tested for each species. Thus, the objectives of this study were to evaluate the effects of an intratesticular injection of 70% glycerin on (1) testicular volume, (2) endogenous germ cell removal in the seminiferous tubule, (3) sperm production and motility, (4) libido, and (5) testosterone level of stallions. We hypothesized that the injection of 70% glycerin in the testes would remove endogenous germ cells and reduce both sperm production and motility, without altering the libido.

2. Materials and Methods

2.1. Animals

The experiment was performed at the research facility for domestic animals at Kyungpook National University and approved by the Institutional Animal Care and Use Committee of Kyungpook National University (2014-0135). Six Thoroughbred stallions were used in this study. The average age of these stallions was 4.5 ± 0.56, ranging from 3 to 7 years. All stallions were individually housed in a stable ($3 m \times 4.5 m$) and rotationally turned out to the paddock ($20 m \times 30 m$) for a half day. Generally, horses were exercised for 30 minutes per day. Stallions were fed 1.5% of their body weight of timothy hay supplemented with 0.5% body weight of commercial feed per day. Stallions had ad libitum access to water.

2.2. Experimental Design

Six Thoroughbred stallions were randomly divided into a treatment ($n = 3$) and a control group ($n = 3$). Local injection of 70% glycerin (40 mL per testis) or phosphate-buffered saline (PBS) was performed on the bilateral testes of stallions in treatment or control group, respectively. Prior to the experiment, stallions were trained to mount and ejaculate on the phantom by collecting semen once a week for 3 to 6 weeks. To obtain baseline data, semen and blood were collected weekly for 4 weeks (weeks −3, −2, −1, and 0) prior to treatment (Fig. 1). The libido of stallions was also monitored during the experimental period. At the fourth week of experiment (week 0), stallions in each group were treated with 40 mL of 70% glycerin (DUKSAN pure chemicals, Ansan-si, Gyeonggi-do, Republic of Korea; treatment) or PBS (WELGENE, Gyeongsan-si, Gyeongsangbuk-do, Republic of Korea; control) per testis. After treatment, semen and blood were collected weekly, and libido was monitored for an additional 10 weeks. Testicular volume of stallions was measured prior to injection and at 10 weeks following treatment. Stallions were hemicastrated 11 week after treatment.

2.3. Intratesticular Injection of Treatment

The 70% glycerin diluted with PBS was filtered through a 0.2-μm syringe filter (Chromdisc, Daegu, Gyeongsangbuk-do, Republic of Korea), and 20 mL of solution was loaded in each of two 50-mL syringes (Buguang medical, Yangju-si, Gyeonggi-do, Republic of Korea). For intratesticular injection, stallions were sedated by IV injection of 1.5 mL of Dormosedan (Zoetis, Florham Park, NJ). Two 26-gauge needles (Sungshim medical, Bucheon-si, Gyeonggi-do, Republic of Korea) connected to an infusion set (Korea vaccine, Ansan-si, Gyeonggi-do, Republic of Korea) were inserted into the core of each testes through midtestis simultaneously. Each solution was infused at 1 mL min^{-1} using a fusion touch impregnator (Model Fusion 720, Chemyx Inc, Stafford, TX). During the injection, a researcher slowly moved the tip of needles back and forth to spread the solution throughout the testes.

2.4. Testicular Volume Measurement

The length, width, and height of each testis were measured using a caliper. The volume of each testis was calculated using the following formula [15].

$$[(0.5233) \times (length - cm) \times (width - cm) \times (height - cm)] = volume\ of\ the\ testis\ in\ cm^3$$

2.5. Analysis for Testicular Tissue

For the preparation of testicular tissue section, five 1-cm^3 sections of testicular parenchyma were obtained from randomly selected sites of testes and fixed in 4% paraformaldehyde overnight. Tissues were then dehydrated using increasing concentrations of ethanol (70, 80, 95, 100% for 1 hour each) and incubated in the xylene for 1 hour. Tissues were then embedded in paraffin. Testicular tissue was sliced using RM2255 Fully Automated Rotary Microtome (Leica, Wetzlar, Germany), and 5-μm sections of tissue were placed on microscope slides to air dry. For hematoxylin and eosin (H&E) staining, sliced tissue on the slide were deparaffinized in xylene (SAMCHUN chemical, Seoul, Korea) for 10 minutes and rehydrated using decreasing concentrations of ethanol (100, 95, 80, 70% for 1 hour each). After washing the slide with running water for 5 minutes, slides were incubated in hematoxylin (BBC Biochemical, Mt

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