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Different approaches to establish infertile rooster

Fereshteh Ghadimi[a], Malak Shakeri[a,*], Mahdi Zhandi[a], Mojtaba Zaghari[a], Abbas Piryaei[b], Parham Moslehifar[a], Alireza Rajabinejad[a]

[a] Department of Animal Science, Faculty of Agricultural Science and Engineering, University of Tehran, Karaj, Iran
[b] Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences

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ABSTRACT

Several methods have been developed to suppress spermatogenesis in recipient males before spermatogonial stem cells (SSCs) transplantation. The aim of this study was to compare two different methods of depleting endogenous spermatogenesis in recipient ROSS 308 strain adult roosters. Gamma-radiation and alkylating agent busulfan were utilized to infertilize adult roosters (ROSS 308 strain). Two radiation therapy regimes (based on ^{60}co isotope) were conducted locally to testes using 40 Gy (5 × 8 Gy with three-day intervals) and 30 Gy (3 × 10 Gy with three-day intervals). And two different levels of busulfan 60mg(40 + 20) and 50mg(30 + 20) with 10-day intervals were injected intraperitoneally. The results showed that both radiation therapy regimes and both busulfan levels reduced sperm motility and sperm concentration significantly compared with control group. Moreover, there were no significant differences between gamma radiation and busulfan treatments in progressive and total motility of sperm reduction. Sperm concentration reached to zero at the end of the 4th week of experiment in all treatment groups. Also histological examinations revealed that both treatments could significantly reduce the diameter of seminiferous tubules and thickness of epithelium. None of the treatments had significant effect on body weight in comparison with control group and the health status of experimental roosters remained good throughout the study. Given that, the risk probability of high doses of radiation exposure and busulfan, it can be concluded that the 30 Gy (3 × 10 Gy) and 50 mg (30 + 20) are appropriate for suppression of endogenous spermatogenesis in mature roosters.

1. Introduction

Spermatogonial stem cells (SSCs) support spermatogenesis through an animal's life and are capable of treating some cases of male infertility (Hermann et al., 2012). In another hand, one of the ways to achieve superior genes in consecutive generations of certain species is using gene transfer technique to SSCs (Takehashi et al., 2010).

Birds have unique physiological characteristics and genetic properties that make them appropriate for developing transgenic bioreactors and experimental model (McGrew et al., 2004). Moreover the generation of germline chimeras in avian species has a valuable role in clarifying the fundamental mechanisms of germline development (Song et al., 2005). SSC transplantation also has the potential for producing interspecies germline chimeras in order to conserve endangered species (Kim et al., 2014). In relation to the production of transgenic animals, transplantation of spermatogonial stem cells is raised as a new reproductive and powerful technique that is also a suitable method for studying spermatogenesis and germinal stem cell biology and transgenic poultry production

* Corresponding author.
E-mail address: mshaker@ut.ac.ir (M. Shakeri).

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(Stern, 2005). In this method, donor SSCs are introduced into the seminiferous tubule lumen of testes of a sterile recipient to start donor-derived spermatogenesis (Brinster and Avarbock, 1994). One major obstacle to commercialization of testis germ cell transplantation (TGCT) in different species is to utilize suitable techniques to deplete endogenous stem cells in recipients that increase the colonization of transplanted donor cells (Herrid and McFarlane, 2013).

Several methods have been used to deplete endogenous spermatogenesis in recipient males before transplanting spermatogonial stem cells. Busulfan, an alkylating agent and used in chemotherapy, has been frequently used for depletion endogenous spermatogonial stem cells prior to germ cell transplantation (Bucci and Meistrich, 1987; Li et al., 2008). Busulfan is also known as a toxic chemical, for example, in order to avoid teratogenic effects of a direct application of Busulfan in domestic fowl embryo, busulfan is applied with sesame oil and injected directly into the yolk (Alg-Gil and Simkiss, 1991). Furthermore, the effectiveness of busulfan in depleting spermatogonial stem cells is dependent on method of application (oral, intravenously, intramuscularly, or intraperitoneally), the age of the animal, and the dissolvent of the drug (Bucci and Meistrich, 1987).

Using gamma radiation is another approach to depleting spermatogenesis in recipient males before SSCs Transplantation (Trefil et al., 2003). The efficiency of it, is dependent on the dose of radiation and the age of the animal at the time of exposure. Additionally, the need for specialized facilities and equipment is an obstacle when applying this method on large animals (Herrid and McFarlane, 2013). There are also large differences between animals in responding to irradiation. For example, in cattle 10–14 Gy of irradiation is required to delete endogenous spermatogenesis in prepubertal bull calves (Izadyar et al., 2003), while achieving this result in adult roosters requires 40Gy(5 × 8 Gy) of irradiation (Trefil et al., 2003).

The objective of our work was to compare two different approaches in preparing ROSS 308 strain roosters testis as recipient for exogenous germ-line cells.

2. Materials and methods

Fifteen 38-week-old ROSS 308 strain roosters were obtained for this experiment. This study was approved by the Animal Welfare Committee of Department of Animal Science, University of Tehran. The roosters were housed in individual cages (45 × 35 × 55 cm) and were kept under standard husbandry condition:14L:10D, 21 °C,55 to 60% RH. The rooster's health, feed and water intake were monitored daily. There were five treatment groups and each group consisted of three birds. Treatment groups constituted of 40-R and 30-R related to gamma radiation and 60-B and 50-B related to busulfan. Both testes of roosters in groups 40-R 30-R were irradiated locally with gamma radiation. The 40-R group received 40 Gy gamma radiation in repeated doses of 8 Gy in 5 times over 13 days. And the 30-R received 30 Gy of gamma radiation in repeated doses of 10 Gy in 3 times over 6 days. The radiation treatment unit Theratron T780 (Theratronics, Kanata, Canada) was used to irradiate roosters testes. As a source T780 uses isotope ^{60}C0, and an isocentric irradiation technique with a source subject distance (SSD) 80 cm was used. In order to keep roosters in a fixed position during exposure a proprietary fixation was prepared and roosters were kept in a lying position with their wings aside the body so that the testes could be easily exposed.

Intraperitoneal injection of busulfan (Lot. No. BCBK7370 V, Sigma chemical Co., Product of USA) was performed as describe by Gardner et al. (2004). Briefly, Busulfan was dissolved in dimethyl sulfoxide (DMSO; Cas-No: 67-68-5. Merck, Germany) and just before the injection, an equal volume of heated (38–40 °C) sterile distilled water (Because of poor solubility of busulfan, and to prevent precipitation) was added to reach a final concentration of 4 mg/ml. Roosters in 60-B were weighted and given the first dose of busulfan (40 mg) and the second dose (20 mg) was given 10 days later. Similarly roosters of 50-B were given the first injection (30 mg) after being weighed and then 10 days later received the second one(20 mg). A control group was treated with neither treatments.

Roosters were trained and semen was collected from each bird using abdominal massage (Burrows and Quinn, 1937) a week prior to the start of treatment. Semen collection continued for four weeks after treatments were completed. Total and progressive motility and concentration of sperm was determined from each sample by a visual estimate. Concentration of spermatozoa was estimated using hemocytometer.

The roosters were killed at the end of the experiment and testes were isolated and fixed in Bouin's fixator, dehydrated and embedded in paraffin. The 5 μm microscopic sections were prepared and at least 2 slides from each testis were stained with hematoxylin and eosin for histological assessment. Also an average of at least 30 round or nearly round tubules of each birds' testis were measured for diameter of seminiferous tubules and epithelium thickness with DinoCapture 2.0 camera and light microscopy.

The results were analyzed by SAS 9.1. Data related to body weight, total and progressive motility with GLM procedure and T test, spermatozoa concentration with Mix procedure and histological data with GLM procedure were analyzed.

3. Results

Examination of body weight change in treatment groups showed that there were no significant differences in body weight after and before treatment (Fig. 1). Moreover, comparison of body weight change in treatment groups with control group were not significant. Since none of the birds died during the experiment, so it seems that the levels of busulfan and gamma radiation were well-tolerated by birds.

Sperm motility that is the net movement of sperm population and quantitative trait of domestic fowl (Forman et al., 1999) was measured in two parts, progressive and total motility. It was observed, as expected, that total and progressive motility significantly decreased in the first week after treatments in all treated groups in comparison with control (Table 1).

However decreasing in total and progressive motility were not significantly different between treatments.