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Comparative studies on testicular and epididymal morphology, and serum hormone concentrations in foxes and the hybrids during the breeding season

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ABSTRACT

The silver fox and the blue fox belong to different genera, and the hybrid males are fully or partially sterile. In the present study, the objective was to evaluate the causes of hybrid male sterility, and therefore analyze the differences in testicular, and epididymal morphology and serum hormone concentrations among silver foxes, blue foxes, and the hybrids during the breeding season. Samples were collected from 20 male silver foxes, 20 male blue foxes, 15 male HSBs (silver fox female × blue fox male hybrids) and 14 male HBSs (blue fox male × silver fox female hybrids), respectively. Seminal evaluation showed large numbers of sperm present in the semen of blue foxes and silver foxes, but no sperm present in the hybrids. Mean testicular volume and the diameter of seminiferous tubules in silver foxes and blue foxes were greater than in the hybrids; and there were many Sertoli cells, spermatogenic cells, and sperm in silver foxes and blue foxes, while spermatogenic cells decreased with no sperm in the hybrids. Mean serum LH and prolactin concentrations in silver foxes and blue foxes were less and testosterone was greater than in the hybrids ($P < 0.05$). The results indicate that germ cell meioses in the hybrids were arrested at the prophase stage of meiosis, and that lesser concentrations of testosterone and greater concentrations of LH and prolactin can inhibit the completion of spermatogenesis.

1. Introduction

The silver fox (*Vulpes fulvus*) and the blue fox (*Alopex lagopus*) appear to be closely related despite belonging to different genera (Makinen and Gustavsson, 1982), while the hybrids of silver foxes and blue foxes belong to a distant hybridization event. The breeding seasons of silver foxes and blue foxes are different, although the seasons overlap partially, so it is possible to produce the hybrids by artificial insemination (Nyberg, 1980). The hybrids show obvious heterosis, with the advantages mainly displayed in the following aspects: larger body size, greater reproductive prolificacy, and a short and woolly pelt with less fur similar to the blue fox, but with greater fur economic value. However, empirically it is known that the fox hybrids are completely sterile, and the hybrids cannot produce normal spermatids and sperm (Wipf and Shackelford, 1949).

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Thus, the hybridization of silver and blue foxes holds true for Haldane's prediction (Haldane, 1922).

The diploid chromosomal number of the silver fox is 2n = 34–42, and the blue fox is 2n = 48–50, with the variations in chromosomal number due to B chromosomes in the silver fox and a centric fusion translocation in the blue fox. The diploid chromosomal number of the hybrids was 2n = 34–48. The differences in chromosomal number and morphology between the two purebred foxes must inevitably mean disturbance of the production of chromosomally balanced gametes in hybrid meiosis, and this would be a reason for the sterility of the hybrids (Makinen and Gustavsson, 1982). Synaptonemal complex analysis demonstrated that the sterility of HSB males was probably due to pairing problems of chromosomes caused by karyotypical variation between the two species, resulting in unpaired and broken chromosomes (Gustavsson et al., 1988). Investigations of male meiosis in HSBs have revealed meiotic arrest at the prophase stage of first meiosis (Nyberg, 1980), and analysis of the integrity of the blood-testis barrier indicated that spermatogenesis in HSBs was found to be arrested at the early pachytene stage of meiotic prophase; thus the hybrids lacked occluding junctions, and this may be another reason for the sterility of the hybrids (Andersen, 1984).

Although, there exist many studies on the hybrids of silver and blue foxes; to the best of our knowledge, there are no previous studies entirely focused on HSBs, and there is no similar study of HBSs.

The purpose of the present study was, therefore, to elucidate potential reasons as to why the fox hybrid males cannot reproduce. To accomplish this goal, two aspects of a study were conducted. First, a study was conducted to determine whether hybrid males were anatomically similar to pure-species with respect to testes and epididymides; and a second study was conducted to assess hormonal differences among the silver, blue and hybrid foxes. Furthermore, it proved to be helpful to assess fertility potential by initial hormonal examination during the breeding season.

2. Materials and methods

The study was conducted at the fur animal farm of the Institute of Special Animals and Plants, Chinese Academy of Agricultural Sciences, Jilin, China, from the period 10 January to 6 April 2015.

2.1. Animals and sample collection

The research with the animals used in the present study was approved by the Animal Welfare Committee of CAAS and was in accordance with The Code of Ethics of the World Medical Association.

There were 20 male silver foxes, 20 male blue foxes, 15 male hybrids of silver fox males × blue fox females (designated as HSBs), and 14 male hybrids of silver fox females × blue fox males (designated as HBSs) were used during the breeding season in this study. The blue foxes and silver foxes were produced by natural mating and the hybrids were produced by artificial insemination.

2.2. Sampling

Based on the different breeding seasons among silver foxes, blue foxes and the hybrids, the silver foxes were collected from 20 January to 4 February 2015, HSBs from 24 January to 8 February 2015, HBSs from 24 February to 11 March 2015, and the blue foxes from 10 March to 25 March 2015. Because most of the same fox species expressed signs of behavioral estrus, samples of peripheral blood and semen were collected at 9:00 to 11 am every 5 days, and there were four other collections from each fox. Testes and epididymides were collected on the same day when blood and semen were last collected.

Semen was collected by hand massaging, and directed into a clean semen cup that was immersed in a water bath at 37 °C. Semen was then smeared onto a glass slide for staining.

Blood samples were collected using a 5-mL injector into 5-mL coagulation promoting tubes with separation gel. The tubes were kept chilled until centrifugation at 3000*g* for 10 min. The serum was collected and stored at −20 °C.

Both sides of testes were gathered after weighing every fox, excess fat was removed, and weighing the bilateral testes singly occurred using a micro- electronic scale (500 g/0.01 g), and the major axis, minor axis and thickness of the testis was measured. The testicular volume was calculated using the formula for ellipsoid volume: $V = 4/3 \times \pi \times \text{major axis}/2 \times \text{minor axis}/2 \times \text{thickness}/2$ (Jallageas et al., 1994), and the relative weight of the bilateral testis was calculated by the formula: relative weight of bilateral testis = weight of bilateral testis/body weight. The testes and epididymides were kept in 10% formaldehyde after the measurement.

2.3. Semen evaluation

Immediately after collection, the semen volume was recorded as per graduated collection vial. To evaluate the gross and sperm progressive motility, semen diluted with fox artificial insemination specially dilution water (Xinyuan Co., LTD, Jinzhou, China) in a ratio of 1:2. Total and progressive sperm motility, VAP (average-path velocity, μm/s), VSL (straight-line velocity, μm/s), VCL (curvilinear velocity, μm/s), STR (straightness [VAP/VCL] 100; %), and LIN (linearity [VSL/VCL] 100; %) were determined by computerized analysis (CASA; IVOS Version 12.0, Hamilton Thorn Research, Beverly, MA, USA), as previously described (Waite et al., 2008). Moreover, semen samples were subjected to a staining procedure using Giemsa stain (Sigma, G4507, New York, USA) for simple morphologic assessment. For sperm production observations, an imaging system consisting of a Leica light microscope (Leica, DM2700, Heidelberger, Germany) was used.

2.4. Histological observations of testes and epididymides

The testis and epididymides were dehydrated in a series of ethanol, cleared in xylene and embedded in