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Original Research

Effects of Repeated Testicular Biopsies in Adult Warmblood Stallions and Their Diagnostic Potential

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ABSTRACT

Testicular biopsy is still uncommonly used in equine andrological diagnostics although several studies supported that it is a relatively safe procedure. So far, no data exist regarding repeated testicular biopsies in stallions. In the present study, repeated testicular biopsy samples were obtained from four stallions at 4-week intervals alternatingly from both testes over the period of 1 year. The objectives were to assess (1) effects of repeated testicular biopsies by clinical, morphologic, histologic, ultrasonographic, and infrared thermographic examinations and (2) the utility of the biopsy samples for diagnostic purposes, namely hematoxylin–eosin staining, immunohistochemistry, Western blot analysis, and polymerase chain reaction. No significant side effects could be determined on clinical healthiness, testis size, libido, testicular blood flow, testicular histology, and scrotal surface temperature. The biopsy samples provided sufficient tissue for assessing spermatogenesis, detecting and localizing proteins (immunohistochemistry), and for protein/messenger RNA extraction for Western blot analyses and polymerase chain reaction. The authors conclude that taking even repeated testicular biopsies is a practicable and safe technique for equine practice having only minimal side effects. The biopsy specimens are suitable for various diagnostic cell and molecular biology applications for identifying testicular causes of male equine infertility. Prospectively, also therapeutic applications might be possible to extend the prospects of already established assisted reproductive techniques (e.g., testicular sperm extraction followed by intracytoplasmic sperm injection) in stallions suffering from obstructive azoospermia or oligospermia. Repeated testicular biopsies might also provide benefits for scientific work, for example, to monitor effects of pharmaceuticals or seasonal variations on testis function and spermatogenesis in the same animal.

1. Introduction

Gaining tissue samples for histologic evaluation of testes and/or assessment of testicular disorders (e.g., neoplasia, azoospermia, oligospermia) without removing the whole organ can be achieved by testicular biopsy.

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Different methods have been described for the use in stallions including the open method (most invasive), needle puncture with different needle types (e.g., Tru-Cut [1], Biopty instrument [2], Monopty instrument [3]), and fine needle aspiration (least invasive; summarized by Roser and Faber [4] and Threlfall [5]).

Testicular biopsy is not commonly used in the diagnosis of equine cases of subfertility or infertility, probably due to deleterious side effects suspected by the owner or clinician. These side effects include hemorrhage, adhesions, tubule degeneration, coagulation necrosis, interstitial fibrosis, orchitis or local inflammation, hypospermatogenesis, and increased morphologic aberrations of spermatozoa [5–7]. Thus, several studies have been performed regarding side effects of a single testicular biopsy by different methods in stallions [1,2,8,9]. There, it was reported that testis function and spermatogenesis of nine stallions was not greatly altered during 27 days after testicular biopsy using the open method with the horses in dorsal recumbency [8]. Assessed parameters before and after biopsy included measurements of width of each testis as well as total scrotal width and evaluation of ultrasonographic echogenicity of the testicular parenchyma [8]. Bartmann et al. [9] performed testicular biopsies under general anesthesia in dorsal recumbency on six fertile and healthy stallions, three stallions with a unilateral increase in testicular size, and three stallions exhibiting azoospermia. After biopsy, no animal showed clinical or ultrasonographically detectable signs of hemorrhage or inflammation. In five of the six stallions with testicular anomalies, a definite diagnosis could be made by testicular biopsy. The tissue specimen of one stallion could not be assessed because of low biopsy quality. Thus, testicular biopsy seemed to be a useful additional method for defining testicular pathology in stallions if less invasive methods did not provide a definite diagnosis [9]. Furthermore, testicular biopsy samples may be useful in analysis of abnormalities in testicular restricted gene expression [10] and complementary DNA (cDNA) mutation analyses of testicular genes in subfertile and infertile stallions [11]. In a case report by Richterich and Wehrend [12], testicular biopsy also led to a particular diagnosis in two infertile stallions. In humans, needle biopsy specimens were compared to specimens gained by open biopsy in ten infertile men. Both techniques revealed no postoperative complications, such as infection, hematoma, orchialgia, or testicular atrophy. The authors concluded that although needle biopsy samples contained fewer tubules than open method samples, the needle specimens provided adequate material to make an accurate diagnosis anyway [13]. In another study, testicular biopsies were performed as a standing procedure under sedation and local anesthesia on seven stallions using a sterile 14-gauge (G) split needle coupled to a spring-loaded Biopty instrument. To evaluate long-term effects of the biopsy, the fertility of the stallions was assessed before and after testicular biopsy by semen evaluation, changes in total scrotal width, pregnancy rates per estrus cycle, serum and seminal plasma antisperm antibody concentrations, and determination of plasma luteinizing hormone, follicle-stimulating hormone, estradiol, testosterone, and inhibin concentrations. It was concluded that obtaining biopsy samples from equine testes by this method does not have apparent negative effects on prospective stallion fertility [2]. Carluccio et al. [1] investigated testicular biopsies on stallions aiming to determine their usefulness, adequacy of the specimens regarding the needle size and mechanism (manual vs. automatic), and short- and long-term side effects. They ascertained minimal side effects caused by testicular biopsy and concluded that the least testicular damage with concurrent most reliable histology specimens is achieved by using the automatic 18-G needle [1].

To our knowledge, effects of repeated testicular biopsies in stallions have not been reported so far. However, there are studies concerning rams and dogs, on which repetitive testicular biopsies using the open method have been performed [14,15]. In rams, unilateral testicular biopsy was accomplished at 4-week intervals during puberty and did not influence the development of either the biopsied or nonbiopsied testis [14]. In dogs, influence of repeated testicular biopsies on spermatogenesis, semen quality, and sperm output was assessed. It was shown that repeated testicular biopsies do not have significant influence on canine spermatogenesis [15].

The objectives of the present study were to determine (1) effects of repetitive testicular biopsies using a technique previously described by Richterich and Wehrend [12] on stallions and (2) the usefulness and diagnostic potential of the obtained specimens for histology, cell biology, and molecular biology.

2. Materials and Methods

2.1. Testicular Biopsies

2.1.1. Animals

Biopsy samples were taken from four healthy Hanoverian stallions (stallion 1–4) with expected normal spermatogenesis (nsp) aged 4 up to 19 years (Table 1). The stallions were housed individually in box stalls with daily discharge on sand paddocks. They were fed with hay and oats twice a day and had free access to water.

To demonstrate the use of testicular biopsies to identify pathologic findings, testicular biopsy samples from a Haflinger stallion (3 years old) were also collected (clinical case, Table 1). The stallion was presented at the Unit for Reproductive Medicine, Clinic for Horses, University of Veterinary Medicine Hannover, for breeding soundness evaluation because of his inability to impregnate mares.

Table 1
Overview of the animals of the study.

Animal	Breed	Age (y)	History
Stallion 1	Hanoverian horse	8	Expected normal spermatogenesis
Stallion 2	Hanoverian horse	10	Expected normal spermatogenesis
Stallion 3	Hanoverian horse	19	Expected normal spermatogenesis
Stallion 4	Hanoverian horse	4	Expected normal spermatogenesis
Haflinger stallion	Haflinger horse	3	Clinical case