

# TESTICULAR DEGENERATION IN STALLIONS

Regina M. Turner, V.M.D., Ph.D, Dipl. ACT

Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of  
Veterinary Medicine, Kennett Square, Pennsylvania

## Introduction

Testicular degeneration (TD), is a common cause of acquired, and often progressive infertility in stallions [1, 2]. In the stallion, TD often is loosely defined by a common set of clinical findings including decreasing fertility and testicular size and declining semen quality. In some cases, TD arises acutely secondarily to a known insult to the testis. For example, testicular trauma, exposure of the testis to heat, cold, radiation, specific toxins, or ischemia, certain nutritional deficiencies, administration of exogenous androgens, infection, autoimmune disease, sperm outflow obstructions and neoplasia all can lead to TD in the horse [3-6]. In these cases, the extent of TD is determined by the length and the severity of the causative insult. If the testis is only mildly affected, some areas may recover once the insult is removed. Even if the testis is severely affected, TD generally does not progress once the inciting cause is removed. However, separate from those cases of TD arising from known causes is another group of cases in which no underlying cause for the degeneration can be identified. This type of degeneration, also called idiopathic TD (ITD) [7, 8] is most often seen in middle aged or older stallions (senile or age-related testicular degeneration) but also can affect much younger animals [8]. Regardless of the age of onset, ITD is typically progressive and results in a steady decline in fertility, sometimes ending in sterility. It is possible that these different forms of TD actually comprise a group of heterogeneous disorders that all lead to a common endpoint. In support of the belief that ITD should be considered as a separate condition from TD arising from a known testicular insult, there have been reports of differences in plasma hormone concentrations and Leydig cell morphology in stallions with ITD compared to stallions with heat-induced or androgen induced TD [5, 6, 9].

## Pathogenesis

Testicular degeneration can be focal or diffuse and can affect one or both testes [2]. Generally, focal and/or unilateral TD is associated with some sort of traumatic insult or neoplasia, while systemic insults (e.g., toxins, exogenous androgens, nutritional deficiencies, etc.) and ITD more commonly uniformly affects both testes. Damage to the testis in the form of increases in scrotal temperature, trauma, exposure to toxins and neoplasia has been relatively well described in the stallion and in other species. The pathogenesis of each of these conditions varies widely and is beyond the scope of this report.

Senile (idiopathic) TD has been studied most extensively in rats. In this species, it has been shown that defects in the hypothalamic-pituitary axis are unlikely to be primarily responsible for age-related declines in testicular function [10]. Rather, the defect appears to reside within the testis itself. As rats age, testicular production of testosterone declines in the face of increasing FSH. What is the cause of the decline in testosterone? It has been shown that, although the

number of Leydig cells within the testis does not change, each individual Leydig cell's ability to make testosterone declines. In this regard, the number of LH-receptors on old Leydig cells is less than that found on young Leydig cells. Similarly, many steps in the steroidogenic pathway and in associated signaling pathways become impaired in ageing rat Leydig cells [11]. The cause of this ageing effect on the rat Leydig cell is currently being studied. One popular hypothesis is that reactive oxygen species that are produced during normal steroidogenesis cause damage to the Leydig cells over time. Additionally, it has been shown that differences in gene expression exist between young and old rat Leydig cells. However, the changes in expression were different depending on the stage of degeneration of the examined testis, suggesting that the consequences of aging were fundamentally different from the consequences of regression of the seminiferous tubules [12].

There is less information available on the pathogenesis of ITD in the stallion. Several investigators have studied endocrine changes in ageing, subfertile stallions [13-16], many of which were probably affected by ITD. These reports suggest that a stallion's endocrinologic status can vary based on the nature of the individual's subfertility and the severity of the problem. In general, subfertile animals with more mild testicular changes showed no consistent, statistically significant changes in plasma hormone concentrations compared to normal, fertile animals. More severely affected subfertile animals and infertile animals had elevated plasma FSH and LH concentrations, lower plasma estradiol concentrations and lower plasma and intratesticular inhibin concentrations. It has been suggested that low plasma estrogens in the presence of high plasma FSH is associated with low fertility and possibly testicular degeneration [14]. These and other studies designed to test hypothalamic, pituitary and testicular function in fertile, subfertile and infertile stallions all suggest that the testis, rather than the hypothalamus or pituitary, is the primary problem in cases of idiopathic stallion infertility [17].

In an attempt to determine the testicular defect that is responsible for ITD in the horse, researchers have compared the number of LH receptors (LH-R) in the testes of normal and subfertile stallions. Unlike the rat, the number of LH receptors on membrane preparations from subfertile stallion testicles does not appear to decline compared to normal, fertile controls [18]. The defect in the testis therefore may reside downstream of the LH-R, possibly in the steroidogenic pathway itself [17]. Additionally, it has been shown that a decline in testicular inhibin concentrations is the first observed change in testicular steroid levels in subfertile stallions. Thus suggesting that, in cases of idiopathic infertility, the primary defect resides in the Sertoli cell rather than in the Leydig cell [16]. Regardless of the primary cell type that is involved, these studies supply further evidence to support the hypothesis that the primary cause of ITD resides at the level of the testis, rather than in the hypothalamus or pituitary.

Based on the likelihood that the primary problem in stallions with ITD resides in the testis, our lab took a different approach to the study of ITD in the stallion. Using the technique of differential display, we searched for differences in gene expression in the testes of a fertile stallion compared to the testes of one young (7 yr old) stallion and one old (23 year old) stallion diagnosed with severe ITD. We found no repeatable differences in gene expression between both of the ITD stallions and the fertile stallion. This finding adds further weight to the argument that what we currently call ITD comprises a heterogeneous group of problems. When the data was reexamined to look at differences between the fertile stallion and either one (but not

both) of the two ITD stallions, several genes were found to be differentially expressed (either up or down regulated) in one of the two ITD stallions when compared to the control [19]. The roles that each of these genes might play in the pathogenesis of ITD is unclear. Surprisingly, to date we have not identified differences in expression for any genes involved in the steroidogenic pathway.

Blanchard and Johnson [20] reported increased germ cell degeneration rates in stallions producing low sperm numbers. The germ cell loss was especially evident during early meiosis and spermiogenesis. Additionally, a lower germ cell:Sertoli cell ratio was reported in these stallions. In general, earlier stage germ cells (e.g. spermatogonial stem cells) and testicular somatic cells (Leydig and Sertoli cells) appear to be more resistant to degenerative changes. Thus, if the inciting cause of TD can be identified and removed, these remaining germ cells may have the ability to repopulate the testis with normal spermatogenesis. Thus, for example, in cases of heat-induced TD, once the testes are returned to a physiologic temperature, normal spermatogenesis often resumes. However, in cases of ITD in which no inciting cause can be identified, the disease is typically progressive.

### History

An accurate breeding history is critical to making an accurate diagnosis of TD. Clinically and histologically, TD may be indistinguishable from testicular hypoplasia. Since testicular degeneration is an acquired condition, while testicular hypoplasia is congenital, a firm diagnosis of TD can be made only if the stallion has a history of declining reproductive efficiency. This often is coupled with declining testicular size. As such, information on the stallion's past book sizes, seasonal pregnancy rates, and average numbers of heat cycles per pregnancy is very important. It should be kept in mind that many animals with testicular hypoplasia often are affected by degeneration as well [2].

Information on a possible cause for TD also may be found in the history. For example, the history may include an incident of trauma to the testicle, a history of recent illness associated with fever, administration of anabolic steroids or administration of other potentially damaging substances. In these cases, the onset of infertility is generally sudden and closely associated with the cause. If an inciting cause can be identified and removed, the prognosis for future fertility is generally better than for cases of true ITD.

If a stallion presents with a history declining fertility over time, ITD should be suspected. This is particularly true in older animals. Although classic ITD is considered to be a slowly progressive problem, it is surprising how many stallions present for what is perceived to be an acute onset of infertility or subfertility.

### Clinical Signs and Diagnosis

For cases in which TD results from a known, finite cause (such as an increase in scrotal temperature secondary to fever or trauma to the scrotum), azoospermia may be seen within the

first 2 weeks after the insult. If the inciting factor is removed, semen quality should improve gradually over the next 2 months. In severe cases, it may take up to 5 months for complete recovery and return to normal sperm production [1]. However, the resistant nature of spermatogonial stem cells and testicular somatic cells (Leydig cells and Sertoli cells) to injury usually provides for a population of cells that are capable of repopulating the testis.

Cases of ITD generally present with a spectrum of clinical signs. Mild cases of ITD may not be associated with any noticeable change in testicular character. Specifically, studies on germ cell loss rates in stallions indicate that ITD can be present before any clinically significant decrease in testicular size can be appreciated [20]. As such, early signs of ITD may only be noticed if semen quality is being frequently and carefully monitored. A gradual decline in overall semen quality (including a decline in total sperm numbers and/or declines in the percentages of motile and morphologically normal sperm) may be the only clinical signs early in the disease. As the disease progresses, clinical signs become more apparent and include decreasing testicular size, palpable softening of the testicular parenchyma, decreasing sperm numbers, low DSO per ml of testis, the appearance of increasing numbers of immature round spermatogenic cells and/or multinucleate giant cells in the ejaculate and an overall decline in semen quality [2, 3, 7, 21]. In advanced cases, stallions may become azoospermic. Because the size of the epididymis usually does not change in cases of ITD, the epididymis may seem to be disproportionately large with respect to testicular size [1]. If a stallion's fertility is not regularly monitored, some cases of ITD may present for what is perceived to be an acute onset of subfertility or infertility. In fact, in many of these cases, the problem was more likely progressive over time but went unnoticed until it had become a severe problem. In severe, end-stage ITD, the testicles may become overly firm [22].

In stallions that can be followed over time, it is a good idea to measure total scrotal width, DSO, and testicular volume at least annually. Any trends suggestive of ITD then can be identified early (e.g., decreasing testicular size/volume, declining semen quality, declining sperm numbers). ITD might be suspected if a stallion at DSO is producing low sperm numbers for his testicular volume. The volume of a single testis can be calculated using the following formula:

$$\frac{4}{3} \pi \times (\text{length of testis (cm)})/2 \times \text{width of testis (cm))/2 \times \text{height of testis (cm))/2}$$

And total testicular volume equals:

volume of the left testis + volume of the right testis [23].

Additionally, Daily Sperm Output (DSO) per ml of testis can be calculated by dividing the total number of sperm in the ejaculate at DSO by the total testicular volume [21]. Low DSO/ml of testis, together with a low percentage of morphologically normal sperm in the ejaculate have been recommended as good indicators of the possible presence of TD [21].

Another hallmark of TD (whether it results from a known cause or is idiopathic) is the appearance of immature spermatogenic cells (round cells) in the ejaculate. In an unstained semen sample, these cells can sometimes be confused with white blood cells. However, because different stages of spermatogenic cells typically appear in a single ejaculate, spermatogenic cells

usually vary in size. White blood cells are typically much more homogeneous. A simple Diff-Quik stain can quickly identify neutrophils and lymphocytes and so aid in the identification of spermatogenic cells. Multinucleated giant cells also may be present [2, 3]. It should be kept in mind that low numbers of immature spermatogenic cells may be found in the ejaculates of normal stallions [24]. However, in normal stallions, other signs of abnormal spermatogenesis (small testicular size, poor semen quality, low sperm numbers, etc.) should not be found.

Because of the variation in plasma hormone levels seen in normal and subfertile stallions, circulating hormone levels may not be a good predictor of mild to moderate TD [20]. In severe cases, elevated FSH and LH as well as low plasma estradiol are consistent with a diagnosis of TD.

Histopathology of affected testes reveals a common group of spermatogenic abnormalities including cytoplasmic vacuolization and a loss of the normal architecture of the seminiferous epithelium [3]. The diameter of the seminiferous tubules may be decreased and immature spermatogenic cells may be shed into the lumen of the seminiferous tubule. In more severe cases, these immature (or 'round') spermatogenic cells may appear in the ejaculate in increasing numbers, as described above. As TD progresses, there is an increased loss of germ cells from the seminiferous tubule. In the most extreme cases, fibrous tissue may be present and tubules can become almost devoid of spermatogenic cells and may be left with only Sertoli cells and few spermatogonia. Fibrosis and calcification of the testicular parenchyma also may be seen [25]. It should be kept in mind that even normal testes have some focal areas of abnormal spermatogenesis. Thus, the percentage of the testicular parenchyma that is affected as well as the severity of the histological lesions should be taken into account before a diagnosis of TD is made.

Because histopathologic findings can help to define TD (and testicular hypoplasia), evaluation of a testicular biopsy sample does provide definitive evidence of these conditions. However, in practice, testicular biopsy is rarely indicated. Once the clinician has obtained an adequate history and has performed a complete physical and reproductive examination, a diagnosis of TD can usually be made with some confidence and a biopsy sample is not necessary. Additionally, there is some concern that a single biopsy sample may not be representative of the condition of the entire testis and thus may not be of significant prognostic value. If a biopsy sample is to be taken, the testes should be examined ultrasonographically prior to obtaining the biopsy [26]. The ultrasonographic appearance of the parenchyma can help the clinician to choose a representative site for sampling. Several reports have indicated that obtaining testicular biopsy samples in the stallion can be done safely and with minimal permanent damage to the remaining testicular parenchyma [27, 28]. However, many of these studies were performed on normal stallions and thus the risk to an already compromised testicle (e.g. a degenerating testicle) is more difficult to ascertain. Clinicians must carefully weigh the diagnostic benefits of obtaining a biopsy sample against the risk of damaging some portion of an already marginally functional testicular parenchyma.

## Treatment

There is no known, proven successful treatment for TD. If the cause of the degeneration is known (e.g., fever, toxin), successful treatment of or removal of the inciting cause should at least prevent further progression of the disease. If the degeneration is not severe and if the inciting cause is removed, the testicle may at least partially, and sometime fully, recover depending on the degree of damage sustained.

In cases of unilateral TD, some have recommended removal of the affected testis. The reasoning behind this recommendation is that the damaged testicular tissue could result in the production of anti-sperm antibodies [29] that might adversely affect sperm produced by the normal testis. Additionally, removal of one testis often results in hypertrophy of the remaining testis and a resultant increase in sperm numbers. The practice of unilateral castration is debatable, however, as there are reports of acceptable fertility in stallions with unilateral TD in which the affected testis was not removed [1].

There are some reports of the successful use of GnRH therapy as a treatment for infertility in stallions [30, 31]. However, these successes have not been duplicated in controlled studies [14, 32, 33]. GnRH therapy has been highly successful in treating men with hypogonadotropic-hypogonadism, however this condition has not been clearly documented in stallions. As such, the use of GnRH implants or pulsatile administration of GnRH as a treatment for stallion infertility in general or ITD specifically is questionable. If this therapy is to be attempted, it has been suggested that treatment must start before the testis has reached a severe state of degeneration [34].

Because there is no proven treatment for TD *per se*, the basis of dealing with this problem centers around stallion management. The veterinarian first should determine the number of progressively motile, morphologically normal sperm that the stallion is capable of producing on a regular basis. The stallion's mare book then should be adjusted accordingly to insure that the stallion is not overused. If a stallion with low or marginal sperm numbers is required to breed daily, it is not uncommon for the animal's sperm numbers to drop below what would be required for a minimum insemination dose. Limiting the animal's book so that he has one or more days of sexual rest between each ejaculate often can help boost sperm numbers and improve pregnancy rates in mares. If possible, the semen quality of each ejaculate should be monitored to be certain that each mare is receiving a minimum insemination dose. Addition of an extender to the ejaculate may help improve longevity of sperm motility in some cases.

Semen from stallions with severe TD should be handled with particular care. Mares should be inseminated as quickly as possible after semen collection. Semen should be carefully evaluated as to its suitability for cooled transport. However, in many cases of moderate to severe TD, sperm longevity of motility is poorly maintained and pregnancy rates may be significantly reduced in mares bred with cooled semen. If this is the case, it may be prudent to discontinue the use of shipped semen and only breed mares on site with fresh, extended semen or by natural cover.

More intensive mare management also can be used to improve pregnancy rates. By breeding mares very close to the time of ovulation, and in extreme cases within 6 hours post ovulation, the veterinarian can minimize the requirement for sperm longevity.

## Summary

If a cause for TD can be identified, it should be treated or eliminated. In these cases, depending on the degree of testicular damage, testicular function should improve and possibly return to normal over a period of months.

If a cause for the TD is not known then, by definition, the case should be classified as ITD. It should be kept in mind that this is a catch-all phrase and that it is possible and even likely that stallions diagnosed with ITD probably represent a very heterogeneous group. Changes in plasma hormone levels, particularly during the early stages of ITD, can be variable. Additionally, no consistent differences in gene expression have been identified in normal vs. ITD testes. As such, some of the most reliable signs of ITD can be identified as part of the routine breeding soundness examination. These include small, soft testes, poor semen quality, low numbers of sperm for testicular size and the presence of immature germ cells in the ejaculate. Unfortunately, by the time changes in testicular size are noticed, the damage to the testes is already significant. As such, valuable animals should be monitored carefully with regular semen evaluations and testicular measurements to try to identify subtle changes in semen quality and sperm numbers over time. Currently, there is no known successful treatment for ITD. If caught early, and if breed registries permit, semen can be frozen in anticipation of a gradual decline in the stallion's fertility over time. Stallions diagnosed with ITD should be managed intensely to maximize fertility in the face of progressively declining semen quality.

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