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Outcome of Fowler-Stephens Operation for Undescended Testes: An Experimental Study

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Abstract

The aim of this study was the outcome of Fowler-Stephens (FS) operations in prepubertal Wistar rats. Thirty-two 30-day-old rats underwent laparoscopic FS procedures on the right testicles (8 of them formed the control group). Nine, 30, 70, and 90 days later we assessed the testes histologically and determined inhibin β B serum concentrations; 1 day earlier, ultrasonography was also performed. Decreases in central testicular vascularity and heterogenous parenchymal echogenicity were the initial sonographic evidence of testicular damage, which either regressed in time or extended toward the periphery. Early degenerative changes either remained mild and restricted in the germinative epithelium or became more severe, affecting additional structures. Decreases in the seminiferous tubule area, thickening of the tunica albuginea, and increases in the number of mast cells were changes that reached significance. Significant decreases in the amount of serum inhibin β B were also found, and the decreases correlated significantly with both the thickening of the tunica albuginea and the increases in the number of mast cells but not with the decreases in the seminiferous tubule area. Division of the spermatic vessels caused severe testicular degeneration as evidenced by changes in ultrasonographic and histologic features combined with drops in the levels of serum inhibin β B.

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It is well known that an undescended testicle may present impaired development and potentially fertility problems. The situation can be surgically corrected simply by transposing the gonad in the scrotum. Fowler and Stephens (1959) proposed the division of spermatic vessels during orchiopexy if the testicular vascular pedicle was short. They relied on the formation of a collateral blood supply for the viability of the testicle. Despite the existing possibility of postoperative testicular atrophy, the Fowler-Stephens (FS) procedure remains a valid option for the correction of high undescended testes today (Docimo, 1995; Dhanani et al, 2004; Taran and Elder, 2006). Its effectiveness has been studied in various animal models, mostly rodents, with inconsistent results. Sprague-Dawley rats, at a prepubertal age in particular, seem to be able to develop sufficient collateral circulation (Pascual et al, 1989; Pascual et al, 1990; Levy et al, 1995), but this has not been confirmed by others (Kelly et al, 1992; Srinivas et al, 2003) or in Wistar rats (Salman and Fonkalsrud, 1990; Erçöçen et al, 2004). We aimed to investigate the temporal sequence of events that occur after laparoscopic FS operations and may contribute to testicular atrophy in Wistar rats. We selected this particular strain and intentionally divided the testicular vessels in a "low" level to find the middle ground between an increased chance of testicular degeneration and a successful simulation of the operation performed in humans. We assessed features of testicular development with ultrasonography and light microscopy together with inhibin β B expression in the testes and attempted to correlate these variables with inhibin β B serum levels.

Materials and Methods

Animals

This study was approved by the Ethics Committee of the University of Heidelberg and was in accordance with the German legislation on the welfare of laboratory animals. Thirty-two 30-day-old male Wistar rats, weighing approximately 200 g each, were used. At this prepubertal age, the testes of these animals were still intra-abdominal. The rats were kept in pairs in cages with controlled temperature, humidity, and light-dark cycles. Water and rat chow were permitted ad libitum. The animals were randomized into two groups: 24 rats underwent FS procedures (FS group) and 8, serving as controls, underwent sham operations. The FS group was further subdivided into 4 subgroups of 6 animals each. Nine, 30, 70, and 90 days after the operation, 1 group at a time together with 2 control animals were ultrasonographically, biochemically, and histologically assessed.

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Anesthesia and Operative and Postoperative Procedures

All examinations and operative procedures were performed under general anesthesia. This was achieved by intraperitoneal injection of 2.5% thiopental sodium diluted in 0.9% sodium chloride at a dose of 0.1 mL/100 g body weight after the animals were anesthetized by ether inhalation.

A 2-mm endoscope (0 degrees; 7210 AWA; Karl Storz, Tuttlingen, Germany) was inserted into the peritoneal cavity at the middistal of the xiphoid-pubic line. Carbon dioxide pneumoperitoneum was created by a venous catheter (22 G) inserted on the right side of the endoscope, and a 1.7-mm "working" cannula (Karl Storz) was inserted on its left side. The right spermatic vessels were identified, dissected, coagulated, and divided by means of a hook approximately 1 cm away from the testis avoiding any contact with the deferent duct. The instruments were removed, pneumoperitoneum deleted, and fascia and skin closed in layers. The animals were returned to their cages to recover.

In the sham-operated animals, we followed identical procedures, but instead of coagulating, we simply manipulated the vessels for approximately 6 minutes, time comparable to that needed for the FS procedure.

Both testes of all animals were ultrasonographically assessed 8, 29, 69, and 89 days after the operation. The day after (ie, the ninth, 30th, 70th, and 90th postoperative day [POD]), perfusion-fixation of the testes was performed on the animals by injecting 2% polyvinylpyrrolidone, 0.2% procaine hydrochloride, 1.5% glutaraldehyde, and 1.5% paraformaldehyde directly into the aorta. Then the gonads were removed and examined histologically and immunohistochemically. In addition, just before the perfusion of the fixative solution, blood was sampled to determine the levels of serum inhibin β B.

Ultrasonographic Examination

Ultrasonography was always performed by the same examiner in a standardized manner to minimize interobserver variability. We examined the borders and volume of each testis as well as the normality and echogenicity of testicular parenchyma with the Elegra apparatus (Siemens AG, Munich, Germany). Doppler sonography was also employed to determine testicular vascularity and blood supply. Unfortunately, the occasional development of massive calcification precluded accurate testicular volume assessment in some animals ([Figure 1](#)). Therefore, the volumetric data were considered unreliable and were disregarded.

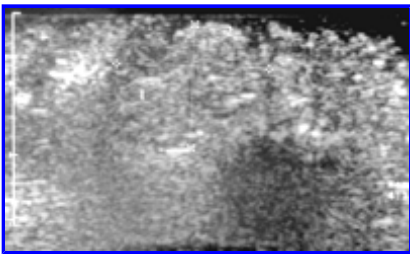


Figure 1. Ultrasonographic image 90 days after the Fowler-Stephens operation. Massive calcification precluded accurate testicular volume assessment in some animals. The testicular parenchyma showed severe heterogeneity (1), whereas the margins of the testis are not clearly detectable (2).

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Despite the standardization of the anesthetics, 2 animals of the ninth POD subgroup, 2 animals of the 30th POD subgroup, and 1 of the 70th POD subgroup died during the ultrasonographic scanning due to adverse drug reactions.

Histology and Immunohistochemistry

Pathology was assessed in paraffin- and epon-embedded sections stained with Richardson stain. Briefly, slices were incubated in 3,3'-diaminobenzidine, treated with 1% osmium tetroxide, and stained with 1% uranyl acetate. Then they were dehydrated, treated with propyleneoxide and epon, and embedded in epon. Richardson staining (azur II and methylene blue mixture) was selected because mast cells are easily identified due to metachromasia.

Immunohistochemistry was performed with a commercially available kit employing a monoclonal antibody against the inhibin β B subunit (Oxford Bio Innovation DSL Ltd, Oxfordshire, United Kingdom) and a biotinylated universal antibody (Vectastain biotinylated universal equine antibody; Vector Laboratories, Burlingame, Calif) according to the kit provider's instructions. In brief, thin

sections were deparaffinized, rehydrated in a xylene/ethanol sequence, pretreated in sodium citrate solution (pH 6.0), and incubated with 3% H₂O₂ in 100% methanol to inhibit endogenous peroxidase activity. Then the sections were incubated with normal goat serum with 0.1% azide to block nonspecific background. Subsequently, they were treated with the monoclonal antibody against inhibin BB and afterwards with the universal antibody. Following incubation with avidin-biotin complex, the slides were stained with 3-amino-9-ethylcarbazole and counter-stained with Mayer hematoxylin. With the same procedure, we prepared negative controls using the secondary antibody alone.

Morphometry

Morphometric studies were conducted with Leica Quantimet 500c software (Leica Microsystems GmbH, Wetzlar, Germany), a professional image analysis system commonly used in research and used as a standard for the evaluation of other systems ([Brockmann et al., 2004](#)) The mean seminiferous tubule area (STA) was determined semiquantitatively in the paraffin sections stained for inhibin BB. The area of 50 tubules in 4 cross sections of each gonad was determined, and the average of these 200 measurements was calculated for each testis. Tunica albuginea was determined as the mean value of 50 different measurements in each testis. The number of mast cells was calculated by counting the total number of mast cells in each section.

Determination of Serum Inhibin BB Levels

Prior to the perfusion-fixation procedure, we sampled blood from the inferior vena cava. Samples were centrifuged, and the supernatant serum was frozen and stored in liquid nitrogen at -130° C. Serum inhibin BB levels were determined by enzyme-linked immunosorbent assay.

Statistical Analysis

Nonparametric tests were used in this study. Mean values were compared with the Mann-Whitney *U* test and Kruskal-Wallis analysis of variance. Correlations were evaluated with Pearson's correlation coefficient. All analyses were performed with SPSS 10.0 for Windows software (SPSS Inc, Chicago, Ill).

▶ Results

Ultrasonography

The testes of the sham-operated animals had clearly visible margins, homogenous parenchyma, normal blood supply, and no calcification. The main vessels entering the gonads were always identified with a distinct flow signal. The same features characterized the left testes in all the rats that underwent the FS operation. On the contrary, the right testes of these animals displayed signs of atrophy (ie, less distinct margins and fewer calcifications, as early as the ninth POD [[Table 1](#)]). The central portion of the parenchyma appeared heterogenous with reduced blood supply; the peripheral part remained unaltered. The alterations became more prominent 30 days postoperatively as the right testes of all 4 animals were not echogenic due to marked calcifications. Blood supply was reduced peripherally and minimized centrally. Similar evidence of atrophy was seen in 3 out of the 5 animals of the 70th POD subgroup (subgroup 70B) and in 2 animals of the 90th POD subgroup (subgroup 90B). In contrast, 2 animals of the 70th POD subgroup (subgroup 70A) and 4 animals of the 90th POD subgroup (subgroups 90A and 90C) had normal right testes.

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following Fowler-Stephens procedure

Light Microscopy

The structures of both testes in the control animals and of the left testes in the FS group were normal. The seminiferous tubules had distinct, patent lumens and were densely packed in a hexagonal pattern. Numerous tubules were intimately associated with the tunica albuginea. Mean STA doubled ($P < .01$) at POD 30 and remained stable ($P > .05$) for the rest of the experiment ([Table 2](#)). Within the germinative epithelium, the spermatogonia and Sertoli cells formed a discontinuous layer adjacent to the basement membrane. Sertoli cells containing small lipid vacuoles surrounded the germ cells. The interstitial space contained a dense capillary network, a few macrophages, and small groups of Leydig cells clustered around the capillaries. They were spherical or elongated and contained small lipid vacuoles. A few mast cells were confined to the peripheral zone of the testis near the tunica albuginea. The number of mast cells remained constant throughout the experiment, whereas the thickness of tunica albuginea increased significantly ($P < .01$) between days 30 and 70.

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Table 2. *Changes in histologic parameters after ligation of the right testicular vessels**

The ischemic changes seen in the right testes after the division of their arteries could be graded into mild and severe forms. In the mild form, the apoptotic processes were restricted to the germ cells of the central area only. The severe form included progressive apoptosis of all germ cells, Sertoli cells, and Leydig cells; massive microlithiasis in the central zone; and complete obstruction of the seminiferous lumen by cellular debris. Of interest, those testes with severe degenerative alterations had reduced vascularity on ultrasound. In contrast, the testes with sonographically homogenous parenchyma and intact vascularity displayed either mild degeneration or no degeneration at all. Therefore, the 2 grades of injury probably reflect the sufficiency of the collateral blood supply.

Nine days after the procedure, the seminiferous tubules in all 4 right testes were shrunken with significantly reduced STAs ($P < .01$) compared with the left or control testes. The lumen contained numerous apoptotic cells in all stages of spermatogenesis and spermatid formation. The disorganized germinative epithelium consisted of disarranged spermatogonia, spermatocytes, and spermatids without stratification. The interstitial space was occupied by Leydig cells and macrophages. When compared with the left or sham-operated testes, the number of mast cells was significantly higher ($P < .01$) and the tunica albuginea was significantly thicker ($P < .01$) containing fibroblasts with numerous lipid droplets.

Thirty days after the procedure, all 4 right testes had significantly thicker ($P < .05$) tunica albuginea with newly formed vessels. Three characteristic and distinct zones were seen in the parenchyma ([Figure 2](#)). First, adjacent to the tunica albuginea, there was a discontinuous, peripheral zone ([Table 2](#), subgroup 30 per) in which the seminiferous tubules were large, atrophic,

and partially calcified. In the noncalcified areas, the germinative epithelium consisted of apoptotic spermatogonia and a few Sertoli cells. Several layers of peritubular myofibroblasts, numerous intact blood or lymphatic capillaries, and a delicate network of thin, elongated Leydig cells and fibroblast-like cells surrounded the seminiferous tubules. This peripheral zone was characterized by an increased number of blood capillaries, abundance of mast cells, and focal proliferation of Leydig cells. Second, the intermediate zone had numerous small atrophic, noncalcified seminiferous tubules scattered in loose connective tissue and enclosed by a single layer of flat myofibroblasts. Large lipid-loaded macrophage-like cells marked the damaged tubules, and numerous typical macrophages were seen in the interstitial space. Accurate measurement of STA was impossible due to the degradation process and the irregularity of the tubule contours. Third, the central zone ([Table 2](#), subgroup 30 cen) contained relatively large, hexagonally, and tightly packed seminiferous tubules filled with cellular debris and lined with a completely atrophic epithelium. No spermatogenic cells or Sertoli cells could be recognized. A few blood capillaries; several calcified branches of the testicular artery, and widespread microlithiasis were also seen. Mean STA of the peripheral zone was significantly smaller ($P < .01$) than that of the central zone, and both differed significantly ($P < .01$) from those of the left testes. Also, the number of mast cells in the peripheral area was significantly higher ($P < .01$) compared with the left or sham-operated testes.

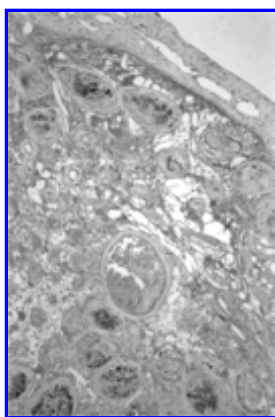


Figure 2. Thirty days after the procedure, all 4 right testes showed the characteristic zonation in their histologies. Three characteristic and distinct zones were seen in the parenchyma. Peripheral near the tunica albuginea (right, top of image), intermediate, and central zones (left, bottom of image) showing the distribution of specific types of cells. Magnification 100x.

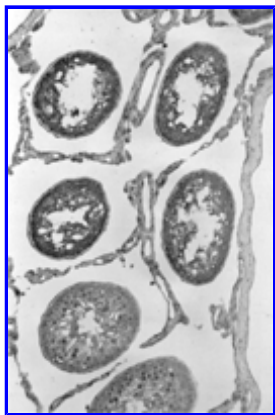
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Seventy days after the procedure, the 3 testes with decreased vascularity on sonography presented severe injury of seminiferous tubules, although the zone pattern was not prominent ([Figure 3](#)): all tubules were patent but markedly shrunk in cross sections, whereas the interstitial space was expanded. More than half of the seminiferous tubules ([Table 2](#), subgroup 70B s.at.) had a remarkably shortened and disintegrated germinative epithelium with almost no spermatogenic cells and were exclusively lined with large, highly vacuolated Sertoli cells. The degenerative changes in the remaining tubules were less pronounced ([Table 2](#), subgroup 70B m.at.) with identifiable spermatogenic cells in almost every tubule and a nearly typical, continuous, basal layer of Sertoli cells with smaller vacuoles. Analysis revealed no difference between the STAs of the severely and mildly degenerated areas, although both differed significantly ($P < .01$) from the left testes and the control animals. However, the number of mast cells differed significantly ($P < .01$) between these 2 areas, as well as from the left or sham-operated testes. Testicular damage was milder in the 2 rats with sonographically intact vascularity ([Table 2](#), subgroup 70A). Tunica albuginea was thickened in

all animals but not to a statistically significant level.



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Figure 3. Seventy days after the procedure, the 3 testes with decreased vascularities on sonography presented severe atrophy of the seminiferous tubules, although the zone patterns were not prominent. All tubules were patent but markedly shrunk in cross sections, whereas the interstitial space was expanded. More than half of the seminiferous tubules had a remarkably shortened and disintegrated germinative epithelium with almost no spermatogenic cells and were exclusively lined with large, highly vacuolated Sertoli cells. Magnification 200x.

Ninety days after the FS procedure, the testes of the 2 animals with decreased vascularity on ultrasonography were severely damaged ([Table 2](#), subgroup 90B). They possessed a thickened ($P < .01$) tunica albuginea with newly formed blood vessels. Again, the 3 distinct zone patterns of the parenchyma were prominent, but neither STA nor mast cell density changes reached statistical significance. Of the remaining 4 animals with normal testicular vascularities, the 3 that presented intermediate testicular damage ([Table 2](#), subgroup 90A; clusters of atrophic seminiferous tubules with significantly [$P < .01$] reduced diameters) were scattered among almost normal ones. The germinative epithelium was short, lacked the normal multilayered structure, and frequently consisted exclusively of highly vacuolated Sertoli cells. The last animal ([Table 2](#), subgroup 90C) had a right testis of normal morphology with well-developed seminiferous tubules, typical germinative epithelium and interstitial space.

Analysis revealed that the increase in the number of mast cells correlated positively with the thickening of tunica albuginea (Spearman's $r_s = .9$; $P < .01$) and negatively with the decrease in STA in the severely atrophic tubules (Spearman's $r_s = .7$; $P < .01$).

Immunohistochemistry

In the control animals only, the Sertoli cells presented intense immunohistochemical staining for inhibin β B within the germinative epithelium. Leydig cells, macrophages, spermatogonia, spermatocytes, and spermatids exhibited very weak or no reactions at all. Peritubular myoid cells, blood, and lymphatic endothelial cells were completely negative. The intertubular space occasionally displayed homogeneous weak to moderate staining for inhibin β B. Immunohistochemistry of all the left testes yielded comparable results.

Nine days after the FS procedure, distinct inhibin β B staining was easily identified in the rarely seen, flattened, alive Sertoli cells lining the atrophic tubules of the peripheral zone of the right testes. The remaining seminiferous tubules lacked inhibin β B staining. In the later groups, the flattened and vacuolated Sertoli cells maintained intense staining for inhibin β B in the mildly damaged testes, although this was slightly weaker compared with the sham-operated animals. In

contrast, the severely damaged testes presented inhibin β B staining patterns similar to the zones seen in light microscopy. Staining was positive only in the few atrophic tubules of the peripheral zone and specifically in the clusters of 2 to 4 intact Sertoli cells in the basement membrane. On the contrary, the intermediate zone was completely negative. Moderate to intense nonspecific staining was observed in the cellular debris inside the calcified atrophic tubules of the central zone. This could represent either cell remnants or viable Sertoli cells scattered in the cellular debris.

Serum Inhibin β B Levels

Serum inhibin β B levels in the sham-operated animals showed normal age-dependent decreases ([Table 1](#)). In the FS-operated animals, serum inhibin β B levels were lower from those of the control animals after 9 and 30 days. Later the serum levels varied according to the degree of testicular degeneration seen in pathology or sonography. The animals with normal sonography and mild or no degradation on histology presented normal levels. On the contrary, the animals with severely degenerated testes exhibited less than half of the inhibin β B levels compared with controls ($P < .05$). This decrease in the levels of inhibin β B correlated negatively and significantly to both the increase of mast cell density and the thickening of tunica albuginea (Spearman's $r_s = .8$ and $r_s = .5$, respectively, $P < .01$ for both), but it did not correlate to the decrease in STA.

Discussion

We attempted to investigate the sequelae of FS operations in Wistar rats. Division of the spermatic vessels caused severe testicular degeneration in 68% of the animals. This was evidenced by changes on ultrasonographic and histologic features, by a decrease in the number of Sertoli cells positively stained for inhibin β B, and by a drop in serum inhibin β B levels.

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Ligation of testicular vessels decreases testicular capillary blood flow from the first hour ([Erçöçen et al, 2004](#)). Reduction of testicular blood flow initially impairs spermatogenesis through apoptosis ([Bergh et al, 2001](#)), perhaps through a nitric oxide-mediated mechanism ([Taneli et al, 2005](#)). After a period of time, parenchymal perfusion seems to be more severely affected than capsular ([Huang et al, 1992](#)), and additional testicular structures are probably injured. Ischemia due to the FS maneuver results in central coagulative necrosis and peripheral interstitial fibrosis ([Erçöçen et al, 2004](#)). The seminiferous tubule borders become indistinct and spermatogenesis impaired ([Erçöçen et al, 2004](#)). Spermatozoa and spermatid cells are affected as early as 1 week and tubular morphology becomes disrupted in 2 weeks after the operation ([Huang et al, 1992](#)). In the long run, atrophy and fibrosis become apparent with reduction of testicular weight, sperm density, seminiferous tubule diameter, and various scores assessing the different cell populations ([Salman and Fonkalsrud, 1990](#); [Huang et al, 1992](#); [Kelly et al 1992](#); [Sperling et al, 2000](#); [Guler et al, 2004](#)).

Our findings are consistent with the literature because we observed early decreases in the central testicular vascularity. This in time became more severe and extended toward the periphery in those testes that eventually degenerated. In contrast, in the animals in which testicular blood flow had been restored, milder or no testicular degeneration was present. On histology, the early effects of ischemia included decrease in STA, accumulation of mast cells, and thickening of tunica albuginea, all at statistically significant levels. The inhibin β B-positive, alive Sertoli cells became rarer and were located in the periphery of the testes, whereas serum inhibin β B dropped to approximately half of the control animal levels. Later the animals in which blood perfusion had been restored

maintained homogenous testicular parenchyma on ultrasonography, whereas the mild ischemic changes were restricted to the germ cells on histology. Inhibin β B-producing Sertoli cells seemed unaffected, and inhibin β B serum levels were normal. In contrast, the animals in which perfusion derangement persisted were additionally characterized by sonographically heterogenous parenchyma, probably reflecting the more severe changes caused by ischemia. The histologic parameters remained significantly different than the contralateral testes and control animals. The 3-zone pattern we noticed, with the more severely apoptotic processes located centrally, perhaps mirrors the distribution of blood flow impairment. Furthermore, the few alive, inhibin β B-positive Sertoli cells were mostly located in the peripheral, least degenerated zone. Serum levels of inhibin β B in these animals were less than half of the corresponding controls, a decrease that correlated significantly to mast cell accumulation and tunica albuginea thickening but not to seminiferous tubule narrowing.

Serum inhibin B levels in the rats have been related to the number and activity of Sertoli cells ([Buzzard et al, 2004](#)). Inhibin B production and secretion is stimulated by the presence of specific germ cells ([Pineau et al, 1990](#); [Allenby et al, 1991](#)); therefore, its levels may be regarded as markers of spermatogenesis. The levels decrease with testicular torsion ([Ozkan et al, 2001](#)) or by hemicastration ([Klajj et al, 1994](#)), although these procedures affect the whole testis and not the germinative epithelium selectively. In our study, we observed both damage of the germinative epithelium and restriction in the number and location of Sertoli cells, although the latter was not quantified. Inhibin B subunit expression and localization to interstitial and Sertoli cells in the adult rat are similar to human prepubertal testicles ([Roberts et al, 1989](#); [Majdic et al, 1997](#)). However, the extension of these conclusions from rodents to children and vice versa requires caution. Inhibin B secretion requires functional interaction of germ cells with Sertoli cells in the adult human testis ([Andersson et al, 1998](#); [Andersson, 2000](#); [Frydelund-Larsen et al, 2002](#)) but not in the prepubertal one ([Andersson et al, 1998](#); [Petersen et al, 1999](#)), which is why inhibin B is readily detectable in prepubertal boys with Sertoli cell only syndrome but not in men with the syndrome ([Andersson et al, 1998](#)). A similar germ cell-Sertoli cell interaction seems to be required in mature rats ([Allenby et al, 1991](#)) but it may be more complicated in prepubertal rats ([Klajj et al, 1994](#)). This discrepancy may account for the lack of correlation between the decrease in levels of serum inhibin B and the reduction in STA in our study, although we cannot ignore the limited power due to the anesthesia-related mortality.

Of interest is the significant negative correlation of the reduction in serum inhibin β B levels with both the thickening of tunica albuginea and increase in the number of mast cells. Changes in tunica albuginea due to new vessel formation following the FS procedure in rats has been reported ([Guler et al, 2004](#)), but the increase in the number of mast cells represents new information to the best of our knowledge. Although this increase could be related to angiogenesis, it is also likely that it may be related to the process of testicular degeneration and fibrosis. In humans, the chymase-containing subtype of mast cells has been implicated in testicular fibrosis and infertility ([Meincke et al, 2000](#); [Yamanaka et al, 2000](#)), and mast cell blockers have already been used as treatment ([Yamamoto et al, 1995](#)). Furthermore, the mast cell accumulation in human pathologic testicular tissue correlates significantly with inducible nitric oxide synthase scores ([Sezer et al, 2005](#)). In rodents, mast cells probably participate in the regulation of steroidogenesis ([Mayerhofer et al, 1989](#); [Aguilar et al, 1995](#)), and their number increases with testicular injury ([Gaytan et al, 1989](#); [Gaytan et al, 1990](#)). In rats, inhibin B secretion by Sertoli cells is inhibited by inflammatory mediators ([Okuma et al, 2005](#)), which may be derived from mast cells. This could, at least partially, explain the correlation between the decrease in inhibin B levels and the increase in the number of mast cells, and the latter could be related to the testicular tissue damage through a nitric oxide-dependent mechanism ([Sezer et al, 2005](#); [Taneli et al, 2005](#)). Whether this hypothesis is valid and whether mast cells contribute to testicular degeneration following the FS operation remains to be

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