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A diagnostic model for histologic damage in undescended testes based on testis rigidity measurement: an experimental study with a novel device

Petros Mirilas, MD, MSurg, PhD,[a,b,*] *Dimitra Psalla, DVM, PhD,*[c] *and Anastasia Mentessidou, MD*[b]

[a] *Centers for Surgical Anatomy & Technique, Emory University Medical School, Atlanta, Georgia*
[b] *Unit of Experimental Pediatric Surgery-Microsurgery, 2nd Department of Pediatric Surgery, Aristotle University of Thessaloniki Medical School, Thessaloniki, Greece*
[c] *Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*

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ABSTRACT

Background: We aimed to test whether testis rigidity (hardness) measured using a newly-designed device we previously introduced would offer more reliable assessment of histologic damage in undescended testes than conventional methods (consistency feel at palpation, volume measurement).

Materials and methods: Forty-five 18-d-old Lewis rats underwent surgical inhibition of descent of left testes and were followed to 40 ($n = 16$), 63 ($n = 14$), or 90 days ($n = 15$). Another 45 18-d-old Lewis rats were sham operated (left side) and followed likewise ($n = 14$, $n = 15$, and $n = 16$). At the designated time points, testes were exposed bilaterally, rigidity was measured, and consistency at palpation was scored; testes were removed and subjected to length, width, weight measurements, volume calculation, and histomorphometry (mean Johnsen score [MJS], mean tubular diameter [MTD], and mean capsule width [MCW]). Testes of experimental group were compared with ipsilateral testes of sham-operated rats.

Results: At all time points, undescended testes had decreased rigidity, MJS, and MTD, increased MCW, decreased volume and weight; contralateral testes remained unaffected. Rigidity was associated only with MJS and MTD, and most strongly with MJS (multiple stepwise linear regression, $F = 694.44$, $P < 0.0005$). MJS could be precisely predicted from rigidity: MJS $= 0.699 \times$ testis rigidity ($F = 1358.82$, $P < 0.0005$). This model showed good fit between predicted and actual MJS values ($R^2 = 0.94$), low error, nonsignificant bias, sensitivity 75% and specificity 90%. Model validation showed low prediction error and nonsignificant bias, indicating generalizability. Testis volume and palpation proved imprecise MJS predictors.

Conclusions: Testis rigidity is an effective predictor of histologic damage in rat undescended testes, with diagnostic value superior to testis palpation scoring and volume measurement.

* *Corresponding author*. Center for Surgical Anatomy & Technique, Emory University School of Medicine, 1462 Clifton Road, Atlanta 30322, GA. Tel.: +1 404 727 1293, +30 6945 038083.
E-mail address: pmirilas@gmail.com (P. Mirilas).

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1. Introduction

Histologic lesions of cryptorchid testes may not be reversed by orchiopexy and result in infertility or cancer [1–3]. Despite some recent claims to the contrary [4], estimation of testis histologic damage present at orchiopexy has been previously proved useful in identifying patients at risk for long-term sequelae and has been suggested as potentially helpful in improvement of long-term management of the anomaly [5,6]. Testicular biopsy—the gold-standard [7]—is generally avoided in children, because of the evidence against its safety [8–10]. Thus, surgeons rely on the preoperative indirect estimation of histologic damage by measuring testis volume using ultrasound or by approximation of testis volume using orchidometers [11–13]. Surgeons also depend on the subjective intraoperative evaluation of testis consistency at palpation, that is, surface feel of testis hardness, which presumably reflects histologic damage [14].

To quantify testicular hardness, we previously developed a mechanical device—the Testis Rigidity Tester (TRT)—providing intraoperative measurements of testis "rigidity" [15]. Rigidity was defined as the force for a predetermined deformation of the testis. Measurement of testis rigidity in rats is shown in Figure 1. First experimental tests showed no significant intra- or inter-operator variation indicating good repeatability and reproducibility, respectively, of rigidity measurements [15]. Also, TRT revealed a rigidity reduction in undescended testes [15]; the relationship of rigidity alterations with underlying histologic lesions, however, was not examined.

This experimental study aims to investigate the relationship between testicular rigidity—measured with TRT—and histology of the undescended testis and examine whether this relationship permits prediction of the histologic damage based on testis rigidity. We also aimed to compare testis rigidity with scoring of testicular consistency at palpation and with testis volume measurement, in terms of prediction of histologic damage in the undescended testis.

2. Materials and methods

This randomized controlled experimental study included 90 Lewis rats obtained at the age of 14 d from Pasteur Institute (Athens, Greece) and housed—along with dams until 18 days—in a temperature-controlled atmosphere under a physical light–dark cycle with free access to food and water. Procedures were conducted under general anesthesia (ketamine 100 mg/kg, intramuscular; chlorpromazine 2 mg/kg, intramuscular) with a surgical microscope (Carl Zeiss Co, Germany).

Operations were performed on the 18th postnatal day, when testes were still intra-abdominal (rat testes descend between 21–28 d). We established two parallel groups of rats: (1) rats with left testicular undescent (UD, $n = 45$), in which the internal inguinal ring was obstructed (9-0 nonabsorbable sutures) and the gubernaculum anchored onto the psoas muscle (10-0 nonabsorbable suture); (2) rats with sham operation (SO, $n = 45$), in which left testes were delivered through an abdominal incision (as in 1) and then repositioned into the abdomen and left free to descend into the scrotum. Rats of each group were followed up until three different ages, after random selection: 40 d, that is, prepuberty (UD, $n = 16$; SO, $n = 14$), 63 d, that is, puberty (UD, $n = 14$; SO, $n = 15$), 90 d, that is, adulthood (UD, $n = 15$; SO, $n = 16$).

At these time points, the rats were anesthetized and both testes of each rat were exposed. The surgical approach was transabdominal for undescended testes and transscrotal for descended testes. Rigidity of all testes was measured using the device TRT, as shown in Figure 1. Testicular consistency was evaluated in all testes by palpation and scored on an ordinal scale from 1 (softest) to 4 (normal) arbitrary units (AU) by a single investigator (A.M.). Then, testes were removed; length, width, and weight were measured. Testicular volumes were calculated using Hansen's formula [16]. Rats were euthanized by cardiac exsanguination while still under anesthesia.

2.1. Ethical considerations

International Guiding Principles in the Care and Use of Animals (DHEW Publications) were followed. Ethical approval was acquired from the National Animal Investigation Committee (13/14346/29.10.2007).

2.2. Histologic examination

Excised testes were fixed in Bouin solution and embedded in paraffin. Four micrometer sections from each testis were stained with hematoxylin–eosin. Examination was done blindly by a single investigator (D.P.). Germ cell maturation and count was evaluated by Johnsen histologic grading system (Johnsen score) on a scale of 1-10 AU (Table 1) [17,18]; per testis, 50 seminiferous tubules were scored and the mean Johnsen score (MJS) was recorded. Mean tubular diameter (MTD) was calculated by averaging the diameters of 20 consecutive cross-sectioned tubules; per cross-section, two perpendicular diameters were measured using an ocular micrometer [18]. Testicular capsule thickness was measured in 10 randomly selected areas; the mean capsule width (MCW) was recorded per testis.

2.3. Study design

Four groups of testes per evaluation time point were obtained, bearing the followings effects: (1) left testes of UD rats (LT-UD): intra-abdominal stay, surgery, and aging; (2) left testes of SO rats (LT-SO): surgery and aging; (3) right testes of UD rats (RT-UD): effect of undescended testis on its counterpart, and aging; (4) right testes of SO rats (RT-SO): aging. LT-SO served as controls for LT-UD and RT-SO for RT-UD.

The study design avoided direct comparisons between LT-UD and RT-UD to avoid the bias from a possible effect of testis undescent on histomorphometric parameters of the counterpart testis, for which there were previous claims [19]. Instead, both these groups were compared with the ipsilateral testes of sham-operated rats, which permitted us to examine separately the effect of testis undescent from the effects of