Effect of testis nondescent or orchidopexy on antisperm antibodies and testis histology in rats

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Objective: To examine effects of nondescent of normal testis and of various orchidopexy techniques on antisperm antibody (ASA) production and histologic testicular lesions.
Design: Experimental cohort study.
Setting: Laboratories of surgical research and biology of reproduction, academic medical centers.
Patient(s): Lewis rats, immature and adult.
Intervention(s): Eighteen-day-old rats (6 groups): intra-abdominal stay of testis after closure of inguinal canal, classic dartos pouch orchidopexy, orchidopexy by testis fixation through tunica albuginea, orchidopexy by transparenchymal testicular fixation, sham operation, and bilateral vasectomy. Adult rats (1 group): transparenchymal testicular fixation.
Main Outcome Measure(s): The ASA—antiacrosome and antitail—were measured by indirect immunofluorescence in sera collected preoperatively, on 50th and 120th day in immature rats, and 90 days after surgery in adult rats. Testicular histology was also examined at the end of sera collection.
Result(s): Neither intra-abdominal testicular localization nor orchidopexies induced significant ASA. Testicular nondescent and fixation (transparenchymal or transtunical) caused hypospermatogenesis; dartos pouch was harmless. Bilateral vasectomy produced significantly increased ASA, but no significant testicular lesions. Contralateral testes were unaffected.
Conclusion(s): Intra-abdominal testicular stay and orchidopexy do not elicit autoimmune response to sperm; histologic testicular lesions might not be associated with ASA. In operated cryptorchids, ASA are probably due to other reason than testicular heat or orchidopexy trauma. (Fertil Steril® 2010;94:1504–9. ©2010 by American Society for Reproductive Medicine.)

Key Words: Antisperm antibodies, cryptorchidism, immunofluorescence assay, orchidopexy, testicular histology, testis nondescent

The surgeon treating cryptorchidism is often puzzled by postoperative serum antisperm antibodies (ASA) (1, 2). Despite early operation performed today (3rd-19th month), infertility is not ameliorated (3). If ASA develop in adulthood, avoiding infertility—one of the goals of surgery—may not be achieved.

Theoretically, there are many reasons for serum ASA production in cryptorchidism. The first question to be asked is whether the maldeveloped cryptorchid testis primarily has a defective blood-testis barrier permitting immunization toward sperm, or whether the barrier is affected by testis nondescent and hyperthermia (4, 5). Another question is whether there has been an adverse effect from orchidopexy. We have previously examined children after dartos pouch orchidopexy (6) and adolescents after dartos pouch and other fixation techniques performed in childhood (7). We did not find serum ASA; however, there has been no follow-up into adulthood. Another issue is whether ASA are etiologically associated with histologic lesions in cryptorchid testes after various orchidopexies. The humoral response mediated by ASA could also harm contralateral testes (8). In cryptorchid patients, all the above effects have been examined together previously and the cause of ASA has not been clearly defined. The final question is the etiology of increased serum ASA reported in infertile adults with a history of operated cryptorchidism [17% (6), 38% (1)]. It remains obscure whether ASA are due to primary developmental defect of cryptorchidism, intrabdominal stay and hyperthermia of testis, harmful orchidopexy technique, cross-reactions to other epitopes, or a combination of these.

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We designed the present prospective study to examine separately the effect of testis nondescent (i.e., exposure to increased temperature) or orchidopexy techniques on serum ASA, as tested by immunofluorescence assay. In separate groups of immature Lewis rats, we inhibited descent of left testes or applied various orchidopexy techniques. We added a group of bilaterally vasectomized rats expected to develop ASA (9) as well as a group with sham operation. Sera were taken before surgery and twice after surgery: in prepuberty, to assure that ASA do not develop against epitopes other than sperm-specific, and in adulthood to examine if ASA were evoked after sperm production. To investigate whether testis fixation could produce ASA in the presence of sperm, we performed it on adult rats and tested for ASA 3 months later, when immune response reaches a maximum (9). To investigate whether ASA were associated with testicular lesions, operated and contralateral testes were histologically examined at the end of follow-up.

MATERIALS AND METHODS

Experimental Groups and Sampling Schedule

This prospective study comprised a total of 84 male Lewis rats, including 74 immature and 10 adult rats. Rats were obtained from the Pasteur Institute, Athens, Greece, and were individually held in cages under a physical light/dark cycle and in a controlled atmosphere with a temperature range of 25 ± 3°C. Rats had access to food and water ad libitum. They were operated under anesthesia (ketamine-chloropromazine) with a surgical microscope (Carl Zeiss Co., Oberkochen, Germany) under aseptic conditions. All procedures with animals were conducted strictly in accordance with the Guiding Principles in the Care and Use of Animals (DHEW Publication, National Institutes of Health). Before the experiments, ethical approval was acquired from the National Ethical Committee for Animal Experimental Investigations (13/1667/February 4, 2008).

Immature rats were operated on the 18th postnatal day. Testes were still undescended; descent occurs normally at 21–28 days in rats. We established six groups: 1) sham operation (SO; n = 12): negative control; 2) dartos pouch (DP; n = 11): left testis was placed in a pouch between skin and dartos muscle; 3) transtunical fixation (TF; n = 12): left testis was fixed into the scrotum with 9-0 nonabsorbable suture driven through the tunica albuginea without traumatizing testicular parenchyma; 4) fixation (F; n = 11): left testis was fixed into the scrotum (9-0 nonabsorbable suture driven through the parenchyma); 5) mechanically induced "cryptorchidism" (MC; n = 13): unilateral testicular nondescent after closure of the left inguinal ring (8-0 nonabsorbable suture) and anchoring the gubernaculum onto psoas muscle (9-0 nonabsorbable suture); and 6) bilateral vasectomy (BV; n = 15): positive control: the vas deferens was incised between two ligatures (8-0 nonabsorbable sutures). Sera were taken before surgery and twice after surgery: prepubertally (50th day) and in adulthood (120th day).

Adult rats, 120 days old, underwent transparenchymal fixation (AdF; n = 10), as described above. Sera were collected before surgery and 3 months after surgery.

Operated and contralateral testes were removed after last serum collection; then the animals were killed. Sera were stored at −30°C and later tested together.

Antisperm Antibody Assay

Sera were tested by indirect immunofluorescence assay on spermatozoa smears obtained from rat cauda epididymis (9). Smears were prepared with a cytocentrifuge at 800 rpm for 5 minutes, air dried, and fixed in methanol for 10 minutes at room temperature. Smears were incubated for 1 hour at room temperature with rat sera diluted at 1:10, washed with Earle bovine serum albumin (BSA) solution, then incubated for 30 minutes with the fluorescein-conjugated IgG fraction of antiserum to rat IgG, diluted 1:40, washed with Earle BSA, and mounted in Citifluor. Stained smears were observed under an epifluorescence microscope (Nikon E 600). Sera reactions were blindly rated by two independent readers according to an ordinal system scale of 5 categories (− or ±, +, ++, +++, and ++++), and expressed as 0–4 arbitrary units (AU).

Histology

Removed testes were fixed in Bouin solution and embedded in paraffin. Four μm sections were stained with hematoxylin and eosin. Microscopic evaluation was done blindly by a single observer. Seminiferous tubular diameter was measured using a micrometer eyepiece (×10); the mean was calculated by averaging the diameter of 50 randomly selected round seminiferous tubules in each section. Mean tubuli number and Leydig score (10) were also counted. Spermatogenesis was evaluated by the Johnsen scoring method on a scale of 1–10 (10). Morphology of tissue was studied to identify atrophy, vascular injury, calcification, edema or inflammation.

Statistics

The study had a mean sample size of 12 subjects per group; therefore, it had a power of 73% to detect a difference in means of 0.53 AU (SD = 0.93 AU) on Altman nomogram (11). Comparisons between groups within each measurement were performed using one-way analysis of variance (ANOVA) or Kruskal-Wallis test, depending on normal distribution of data (tested by Shapiro-Wilk test) (12); Bonferonni-Dunn tests followed. McNemar test assessed difference in ASA reactivity between consecutive time intervals (childhood–prepuberty, prepuberty–adulthood) in each group (11). Correlation between ASA reactivity and histologic variables was estimated by Spearman rank correlation coefficient (11, 12). Differences in histologic variables between groups were examined by one-way ANOVA or Kruskal-Wallis tests, followed by Bonferonni-Dunn tests. The SPSS program version 15.0 for Microsoft Windows was used for statistics.

RESULTS

Antisperm Antibodies

Antiacrosome and antitail reactivities in each group are depicted in Figure 1. Some reactivity was found preoperatively against sperm acrosome and tail (Fig. 1), i.e., reactivity >0 AU (1 or 2 AU) in 21 out of 74 rats (28%) and in 8 out of 74 (11%), respectively. Evidently, this reactivity in childhood cannot be attributed to sperm antigens but rather to cross-reactions with other epitopes.

Similarly, antiacrosome and antitail reactivity was found in the postoperative measurement in prepuberty (Fig. 1). We found reactivity of 1 or 2 AU in 40 out of 74 (54%) against acrosome and in 23 out of 74 (31%) against tail. The increase of these percentages compared with the first measurement may be the effect of aging; sperm had not been produced yet and these reactivities cannot be attributed to sperm epitopes. However, these reactivities were not affected by testicular nondescent, various orchidopexies (F, TF, DP), or vasectomy (no difference from SO group; Shapiro Wilk tests: $P < .05$; Kruskal-Wallis tests: antiacrosome: $H = 6.245$; $df = 5$; $P = .283$; antitail: $H = 10.903$; $df = 5$; $P = .053$).

In the postoperative adulthood measurement, i.e., after sperm production, sperm-specific reactivity due to inhibition of testis descent, orchidopexy, or vasectomy may be added to the nonspecific reactivity acquired with aging. Only the effect of aging was present in the SO group; comparison with it allows for separate examination of the effects of testicular nondecent or orchidopexy on ASA reactivity. Neither minimally invasive orchidopexy techniques in childhood (DP, TF) nor testicular fixation in childhood or adulthood (F, AdF) induced a significant antiacrosome or antitail antibody response compared with the SO group (Shapiro-Wilk test: $P < .05$; Kruskal-Wallis tests: antiacrosome: $H = 28.277$; $df = 6$; $P < .0001$; antitail: $H = 39.43$; $df = 6$; $P < .0001$; Bonferonni-Dunn tests: $P = 1.0$). Similarly, testicular nondescent (MC) did not influence antiacrosome or antitail reactivity (Bonferonni-Dunn tests: $P = 1.0$). Only BV significantly increased antiacrosome