Urol Res (2001) 29: 178–181

ORIGINAL PAPER

Mehmet Dündar · Izzet Koçak · Nil Çulhaci

A new experimental model for cryptorchidism: inguinoscrotal approach

Received: 26 September 2000 / Accepted: 27 December 2000

Abstract We investigated the effect of inguinal canal closure as a new mechanically induced cryptorchid rat model. The effectiveness of this new model was evaluated by histopathological examination. Thirty-one 21-day-old Wistar rats were divided into four groups. In groups 1 ($n = 6$), 2 ($n = 6$) and 3 ($n = 7$), unilateral undescended testis was created by performing inguinal canal closure with inguinoscrotal approach. Sham-operated rats were used as controls in group 4 ($n = 12$). The rats were killed on day 30 after surgery in group 1, day 45 in group 2 and day 60 in group 3. The seminiferous tubular diameter, number of tubules with mature germ cell and Leydig cell clusters were evaluated. None of the rats were lost during the study period. Signs of infection were not detected in operation site although antibiotics were not used. Overall only three (16%) testes descended into scrotum in study groups. The operation time was 3–4 min for each rat. Histopathological examination revealed detrimental effects of cryptorchidism on testicular growth in study groups. In all groups, except the sham group, the mean tubular diameter and the number of tubules with mature germ cells in the left testicle were significantly decreased compared to the right ones. Our findings were in correlation with other experimental studies using different rat models of cryptorchidism. This new model of cryptorchidism is considered to provide a simple and effective technique for investigating the impaired development of the testes in cryptorchidism.

Key words Experimental model · Cryptorchidism · Inguinoscrotal approach

M. Dündar (✉) · I. Koçak
Department of Urology, Adnan Menderes University,
09100 Aydin, Turkey
e-mail: medundar@hotmail.com
Tel.: +90-256-2124078; Fax: +90-256-2120146

N. Çulhaci
Department of Pathology,
Adnan Menderes University, Aydin, Turkey

Introduction

Many experimental models have been used to constitute a cryptorchidism model. Undescended testis was usually induced by endocrinological, natural or mechanical methods [2, 3, 6, 7, 9, 10, 13, 14, 17–20]. Antiandrogen [2, 18], oestrodiol [7] or 5-α-reductase enzyme inhibitors [3, 15] for endocrinological model, congenital cryptorchid mutant rat for natural model [9] and intra-abdominal [6, 10, 19, 20] or extra-abdominal approach [13, 14] for mechanical model have been used to induce cryptorchidism. Of these models, the endocrinological model has partial success rate and probable side-effects on testis. The mechanical model has a risk for infection and mortality especially if abdominal approach is used. In addition, operation time may be longer and expensive material is used.

We investigated inguinal canal closure as a new experimental cryptorchid rat model. The effectiveness of this new model was evaluated by histopathological examination.

Materials and methods

Thirty-one 21-day-old Wistar rats were divided into four groups. At day21, the rats in all groups were weaned from their mothers and raised in a light-controlled (12 h light/12 h dark) environment, with free access to food and water. Anesthesia was achieved with intraperitoneal injection of ketamine hydrocholoride (Ketalar, 10 mg/kg) and xylazine hydrocholoride (Rompun, 2 mg/kg). In groups 1 ($n = 6$), 2 ($n = 6$) and 3 ($n = 7$), unilateral undescended testis was created by performing inguinal canal closure with inguinoscrotal approach. When they reached days 30, 45 and 60 after operation, the animals were sacrificed and the location of the testes was confirmed by manual examination. Sham-operated rats were used as controls in group 4 ($n = 12$). The experimental procedures were approved by the local animal ethics committee.

Surgical technique

After anesthesia, the scrotal region was shaved and cleaned by povidon iodine. Inguinoscrotal region was incised and the guber-

naculum was separated where it protruded from the abdominal wall, and then the external inguinal ring was revealed. After pushing the gubernaculum into the abdominal cavity, the external inguinal ring was closed by 6/0 nonabsorbable suture material (Prolen; Ethicon, UK). Inguinoscrotal wall was sutured by 5/0 absorbable suture (Vicryl; Ethicon, UK; Figs. 1–4).

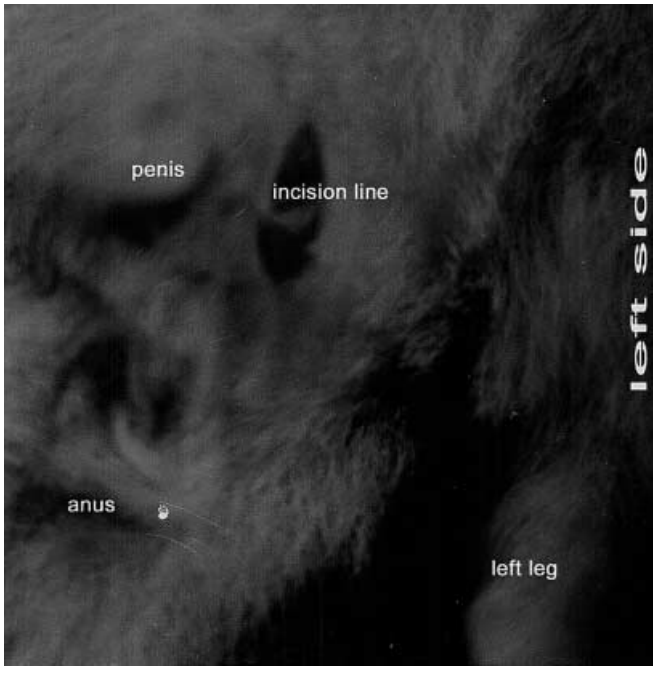

Fig. 1 Incision line on inguinoscrotal region

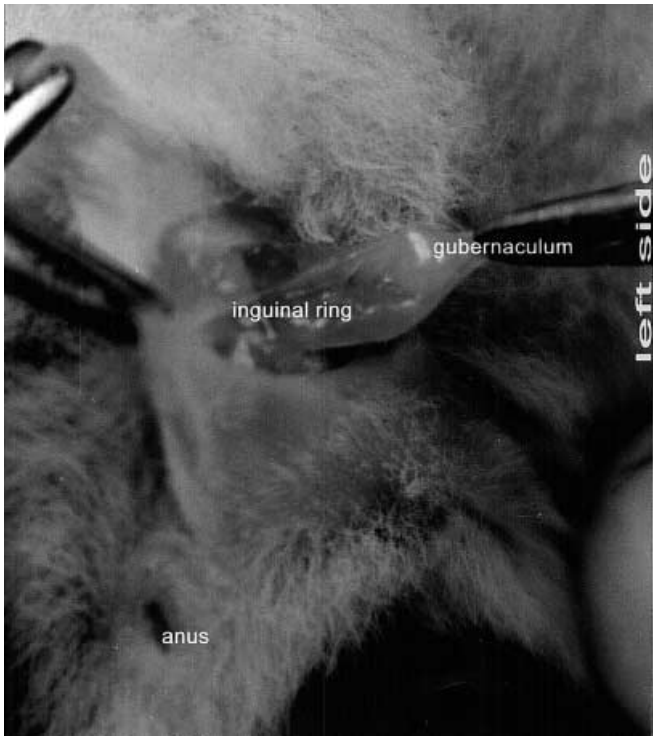

Fig. 2 Gubernaculum was separated where it protruded from the abdominal wall

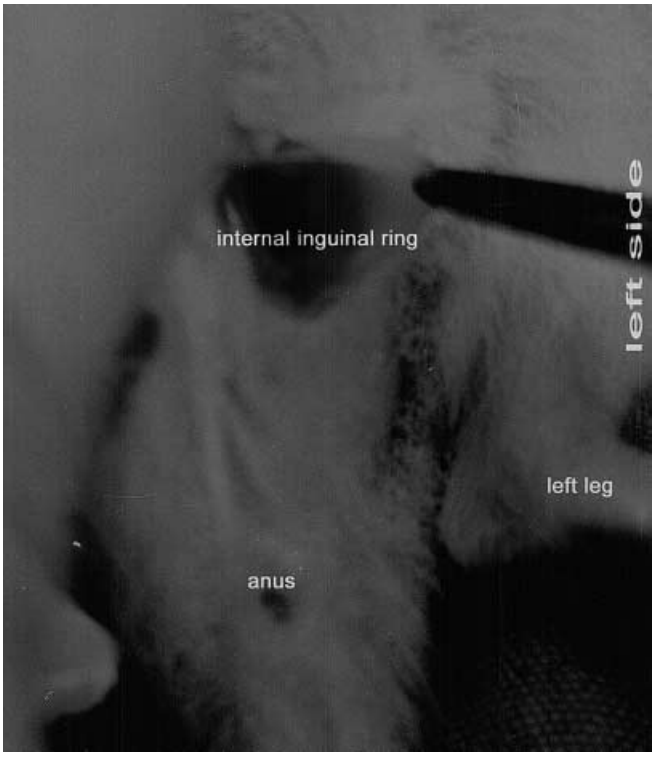

Fig. 3 Appearance of inguinal canal

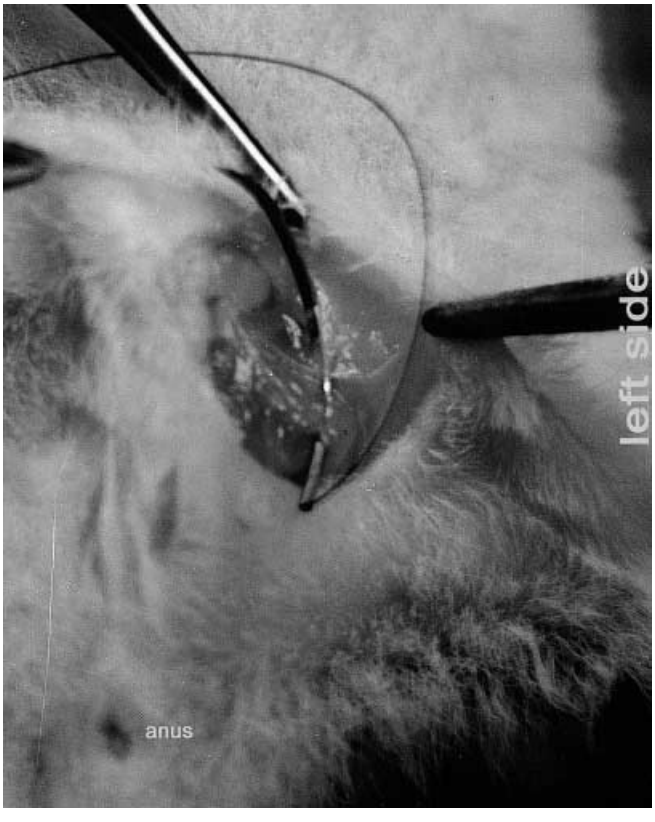

Fig. 4 Closure of inguinal canal

Histopathological evaluation

Rats were killed on day 30 after surgery in group 1, day 45 in group 2 and day 60 in group 3, respectively. Their testes were removed for histopathological examination. A total of 62 orchiectomy specimens were obtained; 10% formalin-fixed, paraffin-embedded tissue was sectioned at 5 μm and stained with haematoxylin-eosin (H&E) for histological examination. Testes were all examined on light microscopy for seminiferous tubular diameter, number of tubules with mature germ cell, and number of Leydig cell clusters were evaluated. Tubular diameter was measured in microns. Fifty tubules were randomly chosen and evaluated. Kruskal-Wallis one-way analysis of variance test, Mann-Whitney *U*-tests and Wilcoxon test were used for statistical analyses.

Results

None of the rats were lost during the study period. There was no infection in operation site although antibiotics were not used. Overall only three (16%) testes descended into scrotum in study groups. In these groups rudimentary scrotum was developed. The operation time was 3–4 min for each rat. Histopathological examination revealed detrimental effects of cryptorchidism on testicular growth in study groups. In all groups, except the sham group, the mean tubular diameter and the number of tubules with mature germ cells in the left testicle were significantly decreased compared to the right ones ($P < 0.05$; Tables 1, 2). The difference in the number of the Leydig cell clusters was not significant ($P > 0.05$; Table 3). No significant differences in tubular diameter of either the left or right testis were noted between the three groups at days 30, 45 and 60 after surgery.

Discussion

The aetiology of cryptorchidism and its effect on testicular function is still controversial despite a large body of clinical and experimental research. In prior studies with rats cryptorchidism was usually induced by endocrinological, natural or mechanical methods [9].

Antiandrogen (flutamide), oestrodiol or 5-α-reductase enzyme inhibitors (finasteride) have been used for endocrinogically induced undescended testis models [3–5, 15, 18]. This method may not be successful in all male rats to induce cryptorchidism and the effects of the hormone may enhance the consequences of undescended testis.

Besides, there are cryptorchid rat models to obtain undescended testis naturally, without any intervention. In this model, testicular maldescent occurs in roughly 68% of animals due to aberrant implantation of the gubernaculum into the inguinal canal [2, 9]. This model seems to be very convenient for studies on cryptorchidism since it is natural. However, it is not possible for every study group to achieve rat lineages with naturally formed undescended testis.

The most frequently used mechanical method is the modified abdominal approach [6, 10, 14, 17, 19, 20]. This method is easy to practice but mortality is higher since the abdominal cavity is opened. Risk of infection is high and abdominal organs are exposed to manipulation, which may lead to intra-abdominal adhesions and unwanted effects on testis. Also, more time and suture material is required for this method.

The method most similar to ours is the broad scrotal skin excision of Shono [13]. Since the inguinal canal is not closed in this model and the scrotal wall develops partially in some rats, testis may descend to different localizations.

Rats were operated in the prepubertal period on day 21 in our study [11, 20]. In the post-operative period, they were sacrificed on days 30, 45 and 60 and testes were extracted. Our histopathological results were compared with data of other experimental studies to evaluate their validity. The time period used for eval-

Table 1 Average seminiferous tubule diameter (μm) of both testes

Localization	Group1 (means ± SEM)	Group2 (means ± SEM)	Group 3 (means ± SEM)	Group 4 (means ± SEM)
Left	306.62 ± 40.25	266.58 ± 15.79	281.00 ± 21.55	345.66 ± 19.98
Right	359.83 ± 12.77	339.83 ± 27.49	334.35 ± 21.55	347.66 ± 16.03

Table 2 Average number of tubules with mature germ cell of both testes

Localization	Group 1 (means ± SEM)	Group 2 (means ± SEM)	Group 3 (means ± SEM)	Group 4 (means ± SEM)
Left	1.00 ± 0.51	2.33 ± 0.76	2.20 ± 1.02	4.50 ± 0.54
Right	4.83 ± 0.87	5.00 ± 0.89	4.85 ± 0.59	4.75 ± 0.55

Table 3 Average number of Leydig cell clusters of both testes

Localization	Group 1 (means ± SEM)	Group 2 (means ± SEM)	Group 3 (means ± SEM)	Group 4 (means ± SEM)
Left	0.83 ± 0.40	1.66 ± 0.71	2.42 ± 0.61	0.75 ± 0.36
Right	1.16 ± 0.30	2.00 ± 0.68	1.57 ± 0.36	1.00 ± 0.50

uation was the same [12] or prolongs up to 150 days [1, 16, 19] in previous studies. The testes were evaluated for the diameter of seminiferous tubules, number of tubules containing mature germ cells and Leydig cell clusters. The histopathological changes obtained in our study were found to be compatible with results of previous studies. The histopathological changes began on post-operative day 30 and effected the opposite testis, too [7–9, 11, 17]. These data support the validity of our model.

The operation time is quite short in our experimental study model. The abdomen was not opened and no sign of infection was detected although we did not use prophylactic antibiotic therapy. Any rat death related to the study was not observed. The development of the scrotum wall was less than the opposite side in rats with undescended testis. The only disadvantage of this model was about 16% of testis descended to scrotum, which is an acceptable rate for experimental studies.

As a result, we propose that the inguinoscrotal approach is a convenient and safe method to obtain undescended testis model.

References

1. Diamond DA, Flores C, Kumar S, Malhotra R, Seethalakshmi L (1992) The effects of an LHRH agonist on testicular function in the cryptorchid rat. J Urol 147: 264
2. Goh DW, Momose Y, Middlesworth W, Hutson JM (1993) The relationship among calcitonin gene-related peptide, androgens and gubernacular development in three animal models of cryptorchidism. J Urol 150: 574
3. Husmann DA, McPhaul MJ (1992) Reversal of flutamide-induced cryptorchidism by prenatal time-specific androgens. Endocrinology 131: 1711
4. Juenemann KP, Kogan BA, Abozeid MH (1986) Fertility in cryptorchidism: an experimental model. J Urol 136: 214
5. Kogan BA, Gupta R, Juenemann KP (1987) Fertility in cryptorchidism: further development of an experimental model. J Urol 137: 128
6. Kort WJ, Hekking-Weijma I, Vermeij M (1990) Artificial intra-abdominal cryptorchidism in young adult rats leads to irreversible azoospermia. Eur Urol 18: 302
7. Lein M, Fahlenkamp D, Schonberger B, Prollius S, Loening S (1996) The pharmacological effect of the gonadotrophin-releasing hormone on experimental cryptorchidism in rats. Scand J Urol Nephrol 30: 185
8. Patkowski D, Czernik J, Jelen M (1992) The natural course of cryptorchidism in rats and the efficacy of orchidopexy or orchidectomy in its treatment before and after puberty. J Pediatr Surg 27: 870
9. Penson DF, Lugg JA, Coyne C, Sadeghi F, Freedman AL, Gonzalez-Cadavid NF, Rajfer J (1997) Effect of cryptorchidism on testicular histology in a naturally cryptorchid animal model. J Urol 158: 1978
10. Quinlan DM, Gearhart JP, Jeffs RD (1988) Abdominal wall defects and cryptorchidism: an animal model. J Urol 140: 1141
11. Rajfer J, Coffey DS (1978) Sex steroid imprinting of the immature prostate. Long term effects. Invest Urol 16: 186
12. Salman T, Adkins ES, Fonkalsrud EW (1988) Morphologic effects of unilateral cryptorchidism on the contralateral descended testis. J Pediatr Surg 23: 439
13. Shono T, Suita S (1995) The effect of the excision of future scrotal skin on testicular descent in neonatal rats: a new experimental model of cryptorchidism. J Pediatr Surg 30: 734
14. Shono T, Zakaria O, Imajima T, Suita S (1996) Extra-abdominal fixation of the gubernaculum inhibits testicular descent in newborn rats. J Pediatr Surg 31: 503
15. Spencer JR, Torrado T, Sanchez RS, Vaughan ED Jr, Imperato-McGinley J (1991) Effects of flutamide and finasteride on rat testicular descent. Endocrinology 129: 741
16. Stewart RJ, Brown S (1990) Fertility in experimental unilateral cryptorchidism. J Pediatr Surg 25: 672
17. Stewart RJ, Brown S (1990) Unilateral cryptorchidism: an animal model. Aust NZ J Surg 60: 905
18. Van der Schoot P (1992) Disturbed testicular descent in the rat after prenatal exposure to the antiandrogen flutamide. J Reprod Fertil 96: 483
19. Zakaria O, Shono T, Imajima T, Suita S (1998) Fertility and histological studies of the contralateral testes in two different intra- and extra-abdominal rat models of unilateral cryptorchidism. Br J Urol 82: 574
20. Zini A, Abitbol J, Schulsinger D, Goldstein M, Schlegel PN (1999) Restoration of spermatogenesis after scrotal replacement of experimentally cryptorchid rat testis: assessment of germ cell apoptosis and eNOS expression. Urology 53: 223