Function of Contralateral Testis after Artificial Unilateral Cryptorchidism in Dogs

Eiichi KAWAKAMI, Tatsuya Hori and Toshihiko TSUTSUI

Department of Reproduction, Nippon Veterinary and Animal Science University, 1–7–1 Kyonan-cho, Musashino-shi, Tokyo 180–8602, Japan

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ABSTRACT. The effects of a cryptorchid testis on the contralateral testis were investigated after artificially producing unilateral cryptorchidism in 8 beagle dogs. Bilateral testicular biopsy and collection of spermatic vein blood and peripheral vein blood were performed at the time of the operation to produce the cryptorchidism and 52 weeks later. The testicular tissue was used for histological examination by light microscopy and measurement of the testicular transferrin (Tf) concentration by enzyme immunoassay. Plasma testosterone (T), estradiol-17β (E2), and luteinizing hormone (LH) levels were measured by radioimmunoassay. Semen was collected weekly and its quality was examined. No spermatogenesis was observed in the cryptorchid testes at 52 weeks after the operation, and the number of germ cells in the contralateral testes had decreased but the number of Sertoli cells did not change. The Tf concentration in both testes had also decreased. The mean total number of sperm between 48 and 52 weeks after the operation (194 × 10⁶) was less than half the number before the operation (510 × 10⁶). Mean spermatic vein plasma T levels (51 ng/ml) in the cryptorchid testes 52 weeks after the cryptorchid operation were significantly lower than before the operation (91 ng/ml; P<0.05). By contrast, spermatic vein plasma E₂ levels (80 pg/ml) were significantly higher than the values before the operation (51 pg/ml P<0.05). The peripheral plasma LH levels decreased. These findings indicate that a large quantity of E₂ secreted by the cryptorchid testis inhibits the endocrine and spermatogenic functions of the contralateral testes in the dog. In particular, it is assumed that dysfunction of the contralateral testis is associated with Sertoli cell dysfunction suggested by the low Tf concentration.---KEY WORDS: artificial cryptorchidism, canine, estrogen.

Spermatogenesis is known not to occur in congenital [5, 12] and artificially produced [15, 28] cryptorchid testes in the dog or other species because of the higher temperature in the abdominal cavity or the inguinal subcutis. Spermatogenic function in the contralateral testis in the scrotum of unilaterally cryptorchid humans [16, 26] and dogs [13, 19, 28] has been reported to be inferior to function in normal males. Mengel and Moritz [19] have suggested that the function of the contralateral testa in the scrotum of the dog is inhibited by autoantibodies produced by the artificial cryptorchid testis in the dog. The authors have found that the endocrine and spermatogenic functions of contralateral testes of dogs with congenital unilateral cryptorchidism are improved by cryptorchidectomy [13]. Therefore appears that congenital and artificially produced cryptorchid testis inhibit the function of the contralateral testes in unilaterally cryptorchid dogs but there have been no reports on the mechanism of inhibition of spermatogenesis in the contralateral testes by the cryptorchid testis in the unilateral cryptorchid dog, except the paper by Mengel and Moritz [19]. To determine the mechanism of inhibition of spermatogenesis in this study, the authors investigated not only changes in bilateral testicular histology and semen quality after surgical production of unilateral cryptorchidism, but plasma sex hormone levels as well. It has been reported that transferrin (Tf), an iron-binding glycoprotein, is secreted by Sertoli cells [29], and that testicular Tf concentrations are an indication of Setroli cell function [21]. The Tf is associated with spermatogenesis by transporting Fe³⁺ to germ cells [31]. In the present study, testicular Tf concentrations were measured after artificial unilateral cryptorchidism.

MATERIALS AND METHODS

Animals and surgery: Eight normal male beagle dogs, all 2–4 years of age, selected from the beagle colony at our university, were used in this study. The dogs were housed in pens with runs under natural lighting, and water was available ad libitum. Under halothane inhalation anesthesia, the right testis in the scrotum of these dogs was passed through the subcutaneous tissue toward the inguinal ring, and after enlarging the inguinal canal by incising it, the testis was introduced into the abdominal cavity.

Testicular biopsy and blood collection: At the time of the cryptorchid operation, the size (length, L cm: width, W cm: and thickness, T cm) of both testes was measured, and testicular biopsy was performed for the purpose of histological examination and Tf assay. Testicular volume was calculated with the formula for an oval, 4/3π × L-W-T × 1/8. The spermatic venous blood and peripheral blood samples were collected at the time of the testicular biopsy.

Histological examination: Half of the testicular tissue was fixed in Bouin’s fluid, embedded in paraffin, sectioned at 3 μm and stained with PAS-hematoxylin. The seminiferous tubule diameter was measured with a micrometer, and germ cell counts were determined in 5 seminiferous tubules at stage 9 of the seminiferous epithelial cycle according to the classification of Ibach et al. [7] as well as by the method described previously [13]. The crude germ cell counts were corrected by the method of Abercrombie [1].

Hormone assays: Testosterone (T) and estradiol-17β (E₂)
levels in spermatic vein blood and peripheral vein blood samples were measured by radioimmunoassay (RIA) as described by Makino et al. [17] and Yoshida et al. [33], respectively. Rabbit antisera to T-11α-succinate-BSA and estradiol-17β-6-CMO-BSA were used. The intra-assay and inter-assay coefficients of variation were: for T, 3.0% and 9.6%, respectively, and for E₂, 7.3% and 12.6%, respectively.

Peripheral plasma luteinizing hormone (LH) was measured by a double-antibody RIA described by Nett et al. [22], except that radiolabeled porcine LH (LER-778) and anti-porcine LH serum were utilized, as report previously [14]. Purified canine LH (LER-1685) was used as the standard. The intra-assay and inter-assay coefficients of variation of LH were 3.2% and 16.0%, respectively.

Testicular Tf assay: Testicular tissue (20 mg) was homogenized in 2 ml of phosphate-buffered solution (40 mM Na₂HPO₄·12H₂O, 2 mM KH₂PO₄, 150 mM NaCl, and 3 mM KCl) with an ultra tissue homogenizer, and the suspension was centrifuged for 30 min at 600 g. The supernatants were preserved at -20°C until used as samples for enzyme immunoassay (EIA).

Testicular Tf concentrations were determined by our microtiter plate double-antibody EIA method [10]. Briefly, Tf was purified from male dog serum by DEAE-Affi-Gel Blue chromatography and gel filtration. Rabbits were immunized with the Tf, and anti-dog Tf antibody was purified from the rabbit serum as primary antibody by affinity chromatography. Peroxidase-conjugated goat anti-dog Tf antibody was used as the secondary antibody for EIA of Tf in canine testis. The optimum dilutions of the primary and secondary antibodies for the standard curves of Tf EIA were 1:4000 and 1:1000, respectively. The intra- and inter-assay coefficients of variation were 8.6% and 7.6%, respectively.

Semen collection and evaluation: Semen was collected from the 8 dogs by digital manipulation weekly between 8 weeks before the operation to produce unilateral cryptorchidism and 52 weeks after the operation. Semen samples were examined for total semen volume, total number of sperm, and percentages of actively motile sperm, viable sperm, and morphologically abnormal sperm by the methods described previously [11]. Briefly, the concentration of sperm in the semen was determined by hematocytometer counts. The percentages of motile sperm and viable sperm were determined by counting actively motile sperm on a warmed slide glass and by eosin-nigrosin staining, respectively. Sperm morphology was examined after rose-bengal staining.

Statistical analysis: Statistical significance was tested by the unpaired Student’s t test.

RESULTS

Testicular volume: The mean (± S.E.) volume of the cryptorchid testes at 52 weeks after the operation was markedly smaller (34 ± 4%) than at the time of the operation (Fig. 1). The volume of the contralateral testes had decreased slightly (96 ± 1%).

Testicular histology and Tf concentration: No spermatogenesis was observed and only Sertoli cells and spermatogonia were seen in the seminiferous tubules of all of the cryptorchid testes at 52 weeks after the operation (Table 1 and Fig. 2). The numbers of primary spermatocytes and round spermatids at 52 weeks after the operation were significantly lower than before the operation (P<0.05, 0.01) but there was no significant difference between the number of Sertoli cells in the contralateral and cryptorchid testes before and after the operation (Table 2).

The mean Tf concentration in the cryptorchid testes at 52 weeks after the operation was significantly lower than before the operation (P<0.01) (Table 2). The mean Tf concentration in the contralateral testes had also decreased but there was no significant difference in the Tf concentration of both the contralateral and cryptorchid testes before and after the operation.

Semen quality: Mean (± S.E.) semen quality from 48 to 52 weeks after the cryptorchid operation was compared with the mean values from 1 to 5 weeks before the operation (Table 3). The total number of ejaculated sperm after the operation was less than half the number before the operation. The percentages of actively motile sperm and viable sperm had decreased significantly (P<0.01), but the percentage of morphologically abnormal sperm had increased significantly (P<0.01).

Plasma hormone levels: The mean T level in the spermatic vein plasma of the cryptorchid testes at 52 weeks after the operation was significantly lower than the mean pretreatment value (P<0.05) (Table 4). By contrast, the mean E₂ level in the spermatic vein plasma had increased significantly (P<0.05) (Table 4). The changes in the peripheral plasma T and E₂ levels after the operation were similar to the changes in the values in the spermatic vein plasma of the cryptorchid testes. The peripheral plasma LH levels decreased (Table 4). After the cryptorchid operation,

![Fig. 1. Mean (± S.E.) testicular volume in 8 dogs 0 and 52 weeks after artificial unilateral cryptorchidism.](image)
both the T and E2 levels in the spermatic vein plasma of the contralateral testes had also decreased (Table 4) but there were no significant differences in the peripheral plasma LH and the spermatic vein plasma T and E2 levels of the contralateral testes before and after the operation.

**DISCUSSION**

The cryptorchid testis has been reported to inhibit spermatogenic function of the contralateral testis in the scrotum of dogs with congenital unilateral cryptorchidism [14, 19, 28]. In both the present study and the study reported by Mengel and Moritz [19], the spermatogenesis and endocrine function of the contralateral testes are suppressed by the effects of the cryptorchid testis in dogs with artificial unilateral cryptorchidism. The marked decrease in the volume of the cryptorchid testis is thought to be attributable to the loss of all types of germ cells except spermatogonia, and the slight reduction in volume and seminiferous tubule diameter in the contralateral testis appear to be attributable to the small number of germ cells. The authors have already certified that the testicular biopsy did not cause damage to spermatogenic function in canine testis [13].

It has been reported that the testes of azoospermic humans [4] and dogs [9] are capable of secreting a large volume of E2. Administration of E2 has been found to inhibit both the hypothalamic and pituitary of male animals [8], and the testicular function in the bull [24] and the dog [27] is suppressed by E2 injection. Mattheeuws and Comhaire [18] found that the plasma E2 levels in the spermatic vein of the abdominal cryptorchid testis of dogs with congenital cryptorchidism are higher than in normal dogs, and an increase in E2 production in the artificially cryptorchid testis has been observed in the rat [3]. In the present study, plasma E2 levels in the spermatic vein of the cryptorchid

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**Table 1. Mean (± S.E.) diameter of the seminiferous tubules (µm) and numbers of germ cells# per cross section in the testes of 8 dogs 0 and 52 weeks after artificial unilateral cryptorchidism**

<table>
<thead>
<tr>
<th>Type A spermatogonia</th>
<th>Preleptotene primary spermatocytes</th>
<th>Pachytene primary spermatocytes</th>
<th>Round spermatids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrotal Cryptorchid</td>
<td>Scrotal Cryptorchid</td>
<td>Scrotal Cryptorchid</td>
<td>Scrotal Cryptorchid</td>
</tr>
<tr>
<td>0</td>
<td>194.8 ± 8.2</td>
<td>196.6 ± 8.6</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>52</td>
<td>186.0 ± 4.0</td>
<td>133.4 ± 5.5</td>
<td>0.8 ± 0.1</td>
</tr>
</tbody>
</table>

# Germ cells were counted in stage 9 of the seminiferous epithelial cycle by the classification of Ibach et al. [7], and the crude germ cell counts were corrected by the method of Abercrombie [1]. *; P<0.05, **; P<0.01. a; compared with 0 week, b; compared with Scrotal.

**Table 2. Mean (± S.E.) number of Sertoli cells a) per cross section in seminiferous tubules and testicular transferrin (TF) concentrations (ng/ml) in 8 dogs 0 and 52 weeks after artificial unilateral cryptorchidism**

<table>
<thead>
<tr>
<th>Number of Sertoli cells</th>
<th>TF concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrotal Cryptorchid</td>
<td>Scrotal Cryptorchid</td>
</tr>
<tr>
<td>0</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>52</td>
<td>3.7 ± 0.3</td>
</tr>
</tbody>
</table>

a) The crude Sertoli cell count was corrected by the method of Abercrombie [1]. ** P<0.01, in comparison with 0 week.
testis were rose after the operation to produce abdominal cryptorchidism. It therefore appears that a large volume of 
E₂ secreted by the cryptorchid testis of the unilateral cryptorchidism. It therefore appears that a large volume of testis were rose after the operation to produce abdominal cryptorchidism. It therefore appears that a large volume of E₂ secreted by the cryptorchid testis presumably caused of the cryptorchid testis [2, 25]. In this study, a large volume temperature in the abdominal cavity or the inguinal position 
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of the cryptorchid testis [2, 25]. In this study, a large volume 
of testis becomes a clinical index of Sertoli cell function and 
of the cryptorchid testis induced by the high plasma E₂ levels caused the 
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of dogs with artificial unilateral cryptorchidism.

The results indicate that the reduced function of the contralateral testis in dogs with unilateral cryptorchidism may be caused by high E₂ production by the cryptorchid testis. It is considered that Sertoli cell dysfunction suggested by the low Tf concentration in the contralateral testis after the cryptorchid operation was caused by both low FSH secretion by the anterior pituitary and low T secretion by Leydig cells in the contralateral testis induced by the high plasma E₂ levels. The reduced spermatogenic dysfunction of the contralateral testis is assumed to be associated with Sertoli cell dysfunction. In the future the findings may be useful for investigating the cause of spermatogenic dysfunction and treating it in the dog. It will be necessary to investigate which cells in the cryptorchid testis are capable of secreting a large volume of E₂.

REFERENCES

3. Damber, J.-E. and Bergh, A. 1980. Decreased testicular re-
duction by the anterior pituitary and T secretion by the contralateral testis induce the poor semen quality of the 
of dogs with artificial unilateral cryptorchidism.

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Table 3. Mean (± S.E.) semen quality in 8 dogs from 1 to 5 week before (Pre) and from 48 to 52 weeks after (Post) artificial unilateral cryptorchidism

<table>
<thead>
<tr>
<th></th>
<th>Total volume of semen (ml)</th>
<th>Total number of sperm (× 10⁶)</th>
<th>Sperm motility (%)</th>
<th>Sperm viability (%)</th>
<th>Sperm abnormality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>14 ± 2</td>
<td>510 ± 45</td>
<td>90 ± 2</td>
<td>95 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Post</td>
<td>11 ± 1</td>
<td>194 ± 19**</td>
<td>75 ± 3**</td>
<td>82 ± 2**</td>
<td>14 ± 2**</td>
</tr>
</tbody>
</table>

a) Semen was collected weekly for 8 weeks before and 52 weeks after the production of artificial unilateral cryptorchidism. ** P<0.01, in comparison with Pre.

Table 4. Mean (± S.E.) testosterone (T), estradiol-17β (E₂) and luteinizing hormone (LH) levels in the spermatoc vein and peripheral vein plasma of 8 dogs 0 and 52 weeks after artificial unilateral cryptorchidism

<table>
<thead>
<tr>
<th></th>
<th>Scrotal testes</th>
<th>Cryptorchid testes</th>
<th>Peripheral venous plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks</td>
<td>T (ng/ml)</td>
<td>E₂ (pg/ml)</td>
<td>T (ng/ml)</td>
</tr>
<tr>
<td>0</td>
<td>95.2 ± 10.4</td>
<td>50.8 ± 11.3</td>
<td>91.4 ± 6.3</td>
</tr>
<tr>
<td>52</td>
<td>80.6 ± 6.8</td>
<td>44.8 ± 7.1</td>
<td>51.0 ± 8.1*</td>
</tr>
</tbody>
</table>

* P<0.05, in comparison with 0 week.

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