Spermatogenic Function and Fertility in Unilateral Cryptorchid Dogs after Orchiopexy and Contralateral Castration

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ABSTRACT. Orchiopexy and contralateral castration were performed in a total of 5 adult and 2 young unilateral cryptorchid (CR) dogs. In every dog, biopsy was performed on the testes, and peripheral and spermatic venous blood samples were collected at intervals of 2, 4 or 8 weeks after the operation. Biopsy tissue specimens were observed after staining with PAS-hematoxylin. In the young CR dogs semen was collected by digital manipulation every week and the sperm fertility was examined by artificial insemination. Neither spermatid nor spermatozoon was noticed in any CR testis at the time of the operation. Spermatozoa were found in the seminiferous tubules of the young and adult CR dogs for the first time, 8 and 10 weeks after the operation, respectively. Thereafter, the number of sperms and germ cells in the seminiferous tubules increased. In the adult CR dogs plasma testosterone levels increased gradually in both peripheral blood and CR testicular venous blood after orchiopexy. In the young CR dogs spermatozoa first appeared in the ejaculate, 8 weeks after orchiopexy. Then, semen volume and sperm number increased gradually, but a high rate of sperm abnormality continued until 24 weeks after the orchiopexy. Semen quality, however, was relatively excellent when sperm fertility was studied 25 weeks after orchiopexy and later. Eight and three bitches were inseminated artificially with the semen of the 2 young CR dogs, respectively. As a result, two of the former and one of the latter became pregnant and gave birth to 2, 2 and 8 youngs, respectively. In conclusion, in the CR testes of the CR dogs the spermatogenic function was stimulated and spermatozoa with fertilizing capacity were produced by the orchiopexy, and puppies were born by artificial insemination of the spermatozoa. But the fertility of the CR dogs was lower than that of normal dogs.—KEY WORDS: cryptorchidism, dog, fertility, orchiopexy, spermatogenesis.


It is well known that androgen produced in the testis is closely related to the spermatogenesis [13, 18, 19]. In unilateral cryptorchid (CR) animals, it is considered that the androgen secretory function of the testis may be inferior to that of the contralateral one [2, 4]. The authors previously reported that spermatozoa appeared in the seminiferous tubules of the testes after orchiopexy in unilateral CR dogs [12]. But there have been no previous reports on fertility of spermatozoa produced in the CR testis after orchiopexy in any animal species. In unilateral CR dogs it is presumed that the occurrence of spermatogenesis in the CR testis after orchiopexy may be induced by androgen secreted from the contralateral testis and that spermatozoon produced in CR testis after orchiopexy has no fertilizing capacity. Therefore, in the present experiment young and adult unilateral CR dogs were subjected not only to orchiopexy but also to contralateral castration to remove any influence of androgen secreted from the contralateral testis. Thereafter, spermatogenic function of the testis descended in the scrotum was investigated histologically, endocrinologically, and from the point of quality. Moreover, fertility capacity of semen obtained from the CR dogs was investi-
gated by artificial insemination with estrous bitches.

MATERIALS AND METHODS

Five adult dogs (12 to 60 months of age) and two young dogs (24 weeks of age) with unilateral CR were used. All of them were subjected to orchiopexy and contralateral castration under anesthesia with sodium pentobarbital. After the contralateral castration, an incision was made on the skin over the external inguinal ring to expose the inguinal CR testis out of the tunica vaginalis. The testicular size (length, width, and thickness) was measured at the time of orchiopexy and 24 weeks after the operation. The testicular volume was represented by the product of the three dimensions.

Testicular biopsy was performed in 1-3 adult dogs at intervals of 2 or 4 weeks until 24 weeks after the orchiopexy and in 2 young dogs 8, 16, and 24 weeks after the operation. The excised tissues were fixed in Bouin’s fluid, cut into sections 3 μm thick and stained with PAS-hematoxylin. Cellular associations at the stage 9 of the cycle of the seminiferous epithelium were observed by the method previously described [11].

Peripheral and spermatic venous blood samples were collected in all the dogs at intervals of 4 or 8 weeks after the operation and the testosterone levels were measured by radioimmunoassay methods previously described [11].

In the 2 young unilateral CR dogs (Nos. 193 and 207), semen samples were collected weekly by digital manipulation. Each sample was examined on semen volume, total sperm number, sperm motility, sperm viability, immature sperm rate, seminal pH and osmotic pressure by the methods previously described [10].

Moreover, semen samples were collected from dog No. 193 at 25, 29, 30, 31, 36, 45, 50, and 52 weeks and from dog No. 207 at 39, 40, and 41 weeks after the operation. They were examined on semen quality, and the fertilizing capacity was examined by artificial insemination (AI).

Eleven bitches were inseminated once during an optimum period for fertile mating [16], between the third and the fifth days after the onset of estrus. Three semen fractions of the ejaculate were collected individually [6]. The second semen fraction (the sperm-bearing fraction) was added to the third semen fraction (prostatic fluid). The seminal volume used for AI was adjusted to about 3 ml. When the bitches were raised with the hind quarters, a rubber tube 4 mm in diameter was inserted deep into the vagina for insemination. In a bitch the oviducts were flushed for collection of embryo 6 days after AI. The ten other bitches were examined for pregnancy by external palpation 25 days after AI. Pregnant bitches were observed until parturition.

RESULTS

In the 5 adult unilateral inguinal CR dogs, the volume of the CR testes was smaller than that of the contralateral one. The ratio of the volume of the contralateral testis to that of the CR testis was 1.0 : 0.3±0.1 (M.±S.E.). All the CR testes increased in volume after the orchiopexy, reaching 1.0±0.2 at 24 weeks after the operation. Particularly, in 3 of the 5 adult dogs, the volume of the CR testes was 1.2–1.4 times as large as that of the contralateral one at 24 weeks after the orchiopexy.

In the 2 young unilateral inguinal CR dogs, Nos. 193 and 207, the volume of the CR testes was about the same as that of the contralateral testes (0.9 and 1.0, respectively) at 24 weeks of age, when the orchiopexy was performed. The CR testis descended in the scrotum increased in volume to 1.8 and 3.5 times as large as the contralateral ones in
Table 1. Mean diameter of seminiferous tubules, and number of germ cells per cross section in the testes of the 5 adult and 2 young unilateral cryptorchid dogs after contralateral castration and orchiopexy

<table>
<thead>
<tr>
<th>Weeks after operation</th>
<th>No. of dogs</th>
<th>Testis</th>
<th>Diameter of tubules (μm)</th>
<th>Number of cells</th>
<th>Spermatozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Type A spermatogonia</td>
<td>Pachytene spermatocytes</td>
</tr>
<tr>
<td>adult</td>
<td></td>
<td></td>
<td></td>
<td>5.6</td>
<td>15.9±0.5</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>S</td>
<td>205±4*</td>
<td>0.9±0.1</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>C</td>
<td>137±3</td>
<td>0.5±0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>C</td>
<td>162</td>
<td>0.8</td>
<td>2.3</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>C</td>
<td>151</td>
<td>0.6</td>
<td>3.9</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>C</td>
<td>165</td>
<td>0.9</td>
<td>4.0</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>C</td>
<td>177</td>
<td>0.9</td>
<td>3.7</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>C</td>
<td>230</td>
<td>1.0</td>
<td>6.8</td>
</tr>
<tr>
<td>24</td>
<td>3</td>
<td>C</td>
<td>226±4**</td>
<td>1.0±0.1</td>
<td>6.5±0.4**</td>
</tr>
<tr>
<td>young</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>S</td>
<td>146</td>
<td>0.7</td>
<td>4.2</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>C</td>
<td>125</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>C</td>
<td>165</td>
<td>0.6</td>
<td>4.8</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>C</td>
<td>212</td>
<td>0.9</td>
<td>6.6</td>
</tr>
</tbody>
</table>

a) S: Scrotal, C: Cryptorchid.
b) Mean from 10 seminiferous tubules of stage 9.
c) M±S.E.
d) Significantly different from scrotal testis at the time of orchiopexy at P<0.01.

Fig. 1. Histological findings of testes in adult and young unilateral cryptorchid dogs after contralateral castration and orchiopexy. PAS-hematoxylin, ×100. A: Contralateral testis in a adult cryptorchid dog at the time of operation. B: Cryptorchid testis in the same dog at the time of operation. C: Cryptorchid testis in the adult dog 10 weeks after the operation. D: Cryptorchid testis in the same dog of 24 weeks after the operation. E: Contralateral testis in a young cryptorchid dog at the time of operation, 24 weeks of age. F: Cryptorchid testis in the same dog at the time of operation. G: Cryptorchid testis in the young dog of 8 weeks after the operation. H: Cryptorchid testis in the same dog of 24 weeks after the operation.
the 2 dogs, Nos. 193 and 207 at 24 weeks after the orchiopexy.

Histological findings of the testes in the unilateral inguinal CR dogs are shown in Table 1 and Fig. 1.

In the adult CR dogs, the mean diameter of the seminiferous tubules in the CR testes was about two-thirds of that in the contralateral ones at the time of the orchiopexy. After the orchiopexy, it increased gradually to 1.6 times as long as the latter 24 weeks after the operation. In all the CR dogs the seminiferous tubules of the CR testes contained type A spermatogonia. A few pachytene primary spermatocytes, however, were seen only in some seminiferous tubules of the CR testis in one of the 5 adult CR dogs. Increased pachytene primary spermatocytes were observed in many seminiferous tubules of the testis in the 2 dogs 4 weeks after the orchiopexy.

Neither round spermatid nor spermatogonia was seen in any CR testis until 8 weeks after the orchiopexy. A few round spermatids and spermatogonia appeared first in the seminiferous tubules of the CR testis in an adult CR dog, which were subjected to testicular biopsy 10 weeks after the orchiopexy, and in the two other adult CR dogs at 12 weeks after the operation. Thereafter, both round spermatids and spermatogonia began to increase markedly in number in the CR testis. The number of germ cells was significantly more (P<0.01) in the cryptorchid testis 24 weeks after orchiopexy than in the contralateral testes at the time of the operation.

Sertoli and Leydig cells were slightly atrophic in the CR testes at the time of the orchiopexy. They became normal in morphology 8 weeks after the operation.

In the 2 young CR dogs, the mean diameter of seminiferous tubules in the CR testes was six-sevenths of that in the contralateral one at 24 weeks of age, when the orchiopexy was performed. These tubules increased in diameter in both testes of the 2 dogs with growth after the operation. Therewith, the difference in tubular diameter became small between the CR and contralateral testes. In the CR testes of the 2 young dogs at 24 weeks of age, a few spermatogonia were found in the seminiferous tubules. No primary spermatocytes were seen. Sertoli cells were slightly atrophic, while Leydig cells seemed to be normal in morphology at that time. At eight weeks after the orchiopexy, Sertoli cells showed normal morphological feature.

Not only spermatocytes and spermatids but also spermatogonia first appeared in many seminiferous tubules of the CR testes. Type A spermatogonia, pachytene spermatocytes, round spermatids, and spermatogonia began to increase gradually in number in both testes at 48 weeks of age, or 24 weeks after the operation. There was little difference in number of germ cells of each type in the CR testis between the young and the adult CR dogs.

Fig. 2 shows the plasma testosterone levels of peripheral and spermatic venous blood in the 5 adult and the 2 young unilateral inguinal CR dogs after the orchiopexy.

In the adult CR dogs the mean peripheral plasma testosterone level was 1.7±0.6 ng/ml at the time of the orchiopexy and was more than 2.6 ng/ml 12 weeks after the operation and later.

In the young CR dogs it was 0.4 ng/ml at 24 weeks of age, when the orchiopexy was performed. Peripheral plasma testosterone level in the young CR dogs began to increase from 8 weeks after the orchiopexy, reaching about 2.0 ng/ml at 24 weeks after the operation.

In all the CR dogs, except one adult, the spermatic venous plasma testosterone level was lower in the CR testis than in the contralateral one at the time of the orchiopexy. At that time, adult CR dogs the
mean plasma T levels of spermatic venous blood in the CR and contralateral testes were 43±8 and 59±9 ng/ml, respectively, and in the young CR dogs 22 and 36 ng/ml, respectively. When spermatogenesis was first recognized in the seminiferous tubules of the CR testes (12 and 8 weeks after the orchiopexy in the adult and the young CR dogs, respectively), these levels increased to attain 87±8 ng/ml in the adult CR dogs and 50 ng/ml in the young ones. Finally, the testosterone levels reached 97±5 ng/ml in the former and 76 ng/ml in the latter 24 weeks after the orchiopexy.

Fig. 3 shows semen volume, total sperm number, sperm motility, sperm viability, sperm abnormality, immature sperm rate, pH of seminal plasma and seminal osmotic pressure in the 2 young unilateral inguinal CR dogs after the orchiopexy.

At the time of the orchiopexy (24 weeks of age), ejaculation was noticed in dog No. 193. But in dog No. 207 the first ejaculation was found at 26 weeks of age. The seminal volume in dog No.' 193 and 207 was 1.5 and 0.4 ml, respectively. In the 2 dogs, Nos. 193 and 207, semen volume began to increase at 27 and 28 weeks of age and reached to 2.7–6.8 ml and 10.2–16.9 ml after 34 weeks of age, respectively.

In these 2 dogs the spermatozoa first appeared in the ejaculate at 32 weeks of age (8 weeks after orchiopexy). The mean values determined in dogs No. 193 and No. 207 at 32 weeks of age were as follows: 0.04×10^6 and 0.46×10^6 for total sperm number, 64% and 70% for sperm abnormality, and 44% and 7% for immature sperm rate, respectively. Thereafter, semen quality in the 2 dogs, except sperm abnormality, changed for the better. Sperm abnormality continued to be at a high level (38–58%) in dog No. 207 after 37 weeks of age. In dogs Nos. 193 and 207 at 48 weeks of age (24 weeks after the orchiopexy), the total sperm number was 170.5×10^6 and 136.0×10^6,
sperm motility 4 and 3, sperm viability 94% and 79%, sperm abnormality 16% and 38%, and the immature sperm rate 9% and 7%, respectively. Their seminal pH was 7.7 and 7.4, respectively, at 26 weeks of age. Then it began to decrease gradually, and reached to 7.0 and 6.7, at 48 weeks of age, respectively. In the 2 young CR dogs seminal osmotic pressure showed remarkable fluctuation (270–320 mOs/kg) throughout the experiment period.

Table 2 shows fertilizing capacity of sperm in the 2 young unilateral CR dogs, Nos. 193 and 207, on which the orchiopexy and the contralateral castration were performed at 24 weeks of age.
Table 2. Characteristics of semen from 2 young cryptorchid dogs after contralateral castration and orchiopexy and results of artificial insemination

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weeks of age</th>
<th>Weeks after operation</th>
<th>Total sperm count (×10⁶)</th>
<th>Sperm motility</th>
<th>Spermi viability (%)</th>
<th>Sperm abnormality (%)</th>
<th>Immature sperm rate (%)</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>25</td>
<td>80</td>
<td>3</td>
<td>78</td>
<td>15</td>
<td>13</td>
<td>2 cell stage embryo found in oviduct</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>53</td>
<td>29</td>
<td>59</td>
<td>3</td>
<td>85</td>
<td>10</td>
<td>18</td>
<td>Not pregnant</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>54</td>
<td>30</td>
<td>24</td>
<td>3</td>
<td>86</td>
<td>12</td>
<td>10</td>
<td>Not pregnant</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>55</td>
<td>31</td>
<td>43</td>
<td>3</td>
<td>88</td>
<td>15</td>
<td>11</td>
<td>Not pregnant</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>193</td>
<td>36</td>
<td>135</td>
<td>3</td>
<td>88</td>
<td>11</td>
<td>13</td>
<td>2 puppies resulted in stillbirth</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>69</td>
<td>45</td>
<td>347</td>
<td>4</td>
<td>92</td>
<td>13</td>
<td>12</td>
<td>1 healthy puppy delivered and 1 puppy stillbirth</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>74</td>
<td>50</td>
<td>250</td>
<td>3</td>
<td>94</td>
<td>12</td>
<td>8</td>
<td>8 healthy puppies delivered</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>76</td>
<td>52</td>
<td>324</td>
<td>4</td>
<td>91</td>
<td>12</td>
<td>10</td>
<td>8 healthy puppies delivered</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>63</td>
<td>39</td>
<td>130</td>
<td>4</td>
<td>95</td>
<td>20</td>
<td>4</td>
<td>Not pregnant</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>64</td>
<td>40</td>
<td>205</td>
<td>4</td>
<td>91</td>
<td>25</td>
<td>6</td>
<td>8 healthy puppies delivered</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>65</td>
<td>41</td>
<td>137</td>
<td>4</td>
<td>92</td>
<td>24</td>
<td>7</td>
<td>Not pregnant</td>
<td>Not pregnant</td>
</tr>
</tbody>
</table>

a) Contralateral castration and orchiopexy was performed at 24 weeks of age.
b) Whole ejaculate was inseminated.

Eight bitches were inseminated with the semen of dog No. 193. The oviducts of one of the eight were flushed 6 days after AI and a two-cell embryo was collected. Morphology of this embryo was normal. Two of the other 7 bitches were diagnosed as pregnant by abdominal palpation 25 days after AI. Two puppies were born from each of the 2 bitches 61 and 63 days after AI, respectively. However, 3 of the 4 puppies were stillborn. Three bitches were inseminated with the semen of dog No. 207. One of them became pregnant and gave birth to 8 normal puppies 63 days after AI. Comparing the fertile semen quality with the sterile semen quality, there were no significant differences in the mean values between the two.

DISCUSSION

The authors previously described that orchiopexy in the young and adult unilateral CR dogs gave rise to appearance of the spermatozoa in the CR testes 8 and 10 weeks after the operation, respectively [12]. The results of the present histological and endocrinological studies on spermatogenesis after the orchiopexy and the contralateral castration agreed almost completely with those reported in the authors' previous paper [12]. Therefore, it is now clarified that appearance of spermatozoa in the CR testes after the orchiopexy was not caused by androgen secreted from the contralateral testis in the unilateral CR dogs. It appears that when the CR testes were descended from the high thermal abdominal cavity or the inguinal region into the low thermal scrotum, its androgen secretory function became active and gave full play to a potential spermatogenic function.

Both Takahashi [14] and Yamauchi et al. [20] reported that histological findings of the testes in normal young dogs about 40 weeks of age were almost the same as those in adult normal dogs.

There was no remarkable difference in histological findings of the testes between the 2 young CR dogs (48 weeks old) 24 weeks after the orchiopexy and adult nor-
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Some reaction of sperm. It seems, therefore, necessary to study the fertilizing capacity and ultrastructure of spermatozoa originated from the CR testis in the future.

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要約

片側性陰莖術における死抜卵卵摘出と陰囊内固定手術後の造精機能および受精能について：河上栄一，筒井敏彦・山田昌一・小笠光・山内亮（日本薬剤研究大学薬学臨床薬理学教室）——片側性陰茎の成犬5頭および未成熟犬2頭について，死抜卵卵摘出と陰囊内固定手術を実施し，配偶4頭または8週間隔で精巣バイオプシーおよび末梢血・精巣静脈血を採取した。精巣組織については，PAS-ヘマトキシリン染色を施して観察し，血液中テストステロン値はRIA法により測定した。未成熟犬については，1週間隔で精液性状検査を行い，人工授精による受胎試験を実施した。手術時における陰睾側精巣の精細管内には，精子細胞および精子は認められなかったが，未成熟犬，成犬それぞれ陰囊内固定手術後8，10週で少数の精子が発見し，その後精細管内の精細胞数，精子数は著しく増加して，精細管径も増大した。未成熟犬，成犬ともに末梢血および陰睾側精巣静脈血中テストステロン値は，陰囊内固定手術後数週に増加した。未成熟犬では，術後8週で初めて射精精液中に精子が出現した後，精液量，精子数ともに増加し，精子密度率はやや高値を示したが，術後25週以後の精液性状は良好であった。これら陰睾犬2頭の精子をそれぞれ人工授精した雌犬8頭中2頭および3頭中1頭が妊娠し，産子数はそれぞれ2，2および8頭であった。以上の成績から，犬では陰囊内固定手術により，陰睾側精巣自身の能力で造精機能が発現し，その精子は受精能を保有しているが，その受胎率は正常より低いことが判明した。