Testis and Antler Dysgenesis in Sitka Black-Tailed Deer on Kodiak Island, Alaska: Sequela of Environmental Endocrine Disruption?

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It had been observed that many male Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) on Kodiak Island, Alaska, had abnormal antlers, were cryptorchid, and presented no evidence of hypospadias. We sought to better understand the problem and investigated 171 male deer for pheno-
typic aberrations and 12 for detailed testicular histopathology. For the low-lying Aliulik Peninsula (AP), 61 of 94 deer were bilateral cryptorchids (BCOs); 70% of these had abnormal antlers. Elsewhere on the Kodiak Archipelago, only 5 of 65 deer were BCOs. All 11 abdominal testes examined had no spermatogenesis but contained abnormalities including carcinoma in situ-like cells, possible precursors of seminoma; Sertoli cell, Leydig cell, and stromal cell tumors; carcinoma and adenoma of rete testis; and microlithiasis or calcifications. Cysts also were evident within the excruciant ducts. Two of 10 scrotal testes contained similar abnormalities, although spermatogenesis was ongoing. We cannot rule out that these abnormalities are linked sequelae of a mutation(s) in a founder animal, followed by transmission over many years and caus-
ing high prevalence only on the AP. However, based on lesions observed, we hypothesize that it is more likely that this testis–antler dysgenesis resulted from continuing exposure of pregnant females to an estrogenic environmental agent(s), thereby transforming testicular cells, affecting development of primordial antler pedicles, and blocking transabdominal descent of fetal testes. A browse (e.g., kelp) favored by deer in this locale might carry the putative estrogenic agent(s).

Key words: antler dysgenesis, CIS, cryptorchidism, Leydig cell tumor, microlithiasis, rete carci-
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Sitka black-tailed deer (SBTD; *Odocoileus hemionus sitkensis*) were introduced into the Kodiak Archipelago before 1935 [Supplemental Material Figure 1 and “Background” ([http://www.ehponline.org/members/2005/8052/supplemental.pdf](http://www.ehponline.org/members/2005/8052/supplemental.pdf)]. There were occasional reports of cryptorchidism in SBTD on Kodiak Island, often with abnormal antlers (i.e., sharp tips, atypical/bizarre shape, retention of velvet) or an atypical (enlarged, convex) bony antler pedicle after antlers were shed. Apparently, incidence of such abnormal deer on the Aliulik and Hepburn Peninsulas increased after 1990 (Van Daele and McDonald 2001). Without scrutiny of the problem, wildlife personnel attributed the cryptorchidism to inbreeding (Lewis 1991).

Elsewhere, deer with abnormal antlers and atrophic scrotal testes have been reported (Chapman et al. 1984; Tiller et al. 1997). Occasionally, males of other deer species lacking scrotal testes have been reported (Hofmann 1968; Leader-Williams 1979; Marburger et al. 1967), but they had normal antlers. Hoy et al. (2002) found high propor-
tion of males with malpositioned testes among road-killed deer in an area of Montana, but they apparently had normal antlers. Reported abnormalities are different from those reported herein for SBTD. Cryptorchidism also occurs in domesticated animals or humans, with prevalence ranging from 3 to 20% (Klonisch et al. 2004; Nielen et al. 2001).

A lay publication (Jacobson 2003) and earlier reports (Bubenik et al. 2001; Bubenik and Jacobson 2002) of unusually high inci-
cidence of cryptorchidism in SBTD led to the current study. To determine if there really is a problem of cryptorchid SBTD with abnormal antlers in the Kodiak Archipelago, we gathered additional data and compiled and scrutinized all data. We were intrigued concerning the possible cause(s). Because we suspected a prob-
dlem during fetal development and because analyses of maternal blood serum or fat for an array of potential endocrine disruptors was expensive, we focused on collection of properly fixed testicular tissue and critical evaluation thereof (Higuchi et al. 2003; Veeramachaneni 2000; Veeramachaneni et al. 1986, 2001). We anticipated that these evaluations would:

- determine if there were many unusual deer on a portion of Kodiak Island;
- point to the cause of the unexplained phenomenon (genetic basis vs chemical agent); and
- provide a database, critical for any future study.

Materials and Methods

Population sampling. This study involved observations on 171 male SBTD legally hunted in mid-October through December during 1999–2004. Hunting occurred in the vicinity of 36 general sites [Supplemental Material Figure 2 ([http://www.ehponline.org/members/2005/8052/supplemental.pdf](http://www.ehponline.org/members/2005/8052/supplemental.pdf)], 8 of which were on the Aliulik Peninsula (AP) of Kodiak Island and 28 elsewhere on Kodiak Island or nearby islands of the Kodiak Archipelago. Hunters decided what deer to shoot, and we must assume that they repre-
sent all in that geographic area. SBTD in this study include those in earlier reports (Bubenik et al. 2001; Bubenik and Jacobson 2002), but we did not rely on histological observations in the latter report.

Tissue sampling, processing, and evaluation. For 2003 and 2004 our goal was to obtain blood serum and tissue from at least six cryptorchid and six noncryptorchid (NCO) males annually. Soon after a male was shot, an ID number was assigned, normalcy of penis and scrotum was recorded, and blood was taken and allowed to clot. Serum was separated and stored at −20°C.

Testes and epididymides were photo-
graphed alongside a ruler. Slices of testes at two loci were fixed in Bouin’s fluid or 4% glu-
taraldehyde. A cross-section through the cauda epididymis was fixed in Bouin’s fluid. Tissues were fixed in the field, usually < 3 hr after death. Tissues were processed for light and transmission electron microscopy and evaluated based on criteria described by Markwald (1968) and Veeramachaneni et al. (1986). Briefly, 50- to 100-tubule cross-
sections per testis were classified as one of eight grades denoting progressive loss of germ cells and degenerative changes in seminiferous epithelium, and the relative degree of germinal epithelial loss (DGEL) for each section of testis was calculated [Supplemental Material ([http://www.ehponline.org/members/2005/8052/supplemental.pdf](http://www.ehponline.org/members/2005/8052/supplemental.pdf))].

Detailed evaluations of morphology of the four cell types (germ cells, Sertoli cells, Leydig cells, and stromal cells) within each

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Quantification of serum testosterone. We measured concentration of testosterone using a validated radioimmunoassay (Berendtson et al. 1974) in all available serum samples \(n = 86\) of male SBTD shot during 2000–2004. Sensitivity of the assay was 0.024 ng testosterone/mL serum. Based on duplicate aliquots of each sample, intraassay variation was 4.4%. The data summary also included values \(n = 19\) from Bubenik et al. (2001) for males shot in 1999.

Statistics. We summarized descriptive data within and across sites for males with both, one, or no testes within the scrotum and for normalcy of antlers. Statistics were not applied to these descriptive data or to semiquantitative classifications of testicular histology and DGEL. Values for estimated age for males shot at sites on or not on the AP were compared using a \(t\)-test. Testosterone concentrations were subjected to a log transformation, and geometric means are presented. Differences were compared with a \(t\)-test or analysis of variance or, alternatively, a Mann-Whitney or Kruskal-Wallis test. Differences between certain ratios [unilateral cryptorchid (UCO) + bilateral cryptorchid (BCO) vs. NCO deer] were substantial, but the nonsystematic harvest of SBTD made statistical testing illogical.

Results

Biometric data. Estimated age was available for 112 of the 171 SBTD and averaged 5.3 years (range 1.5–12.5 years). On average, deer shot on the AP were older than those shot elsewhere (5.8 vs 4.6 years; \(p = 0.01\); \(n = 64, 48\)).

Numbers of NCO, UCO, and BCO deer at each site [Figure 1; details in Supplemental Material Table 1 (http://www.ehponline.org/members/2005/8052/supplemental.pdf)] revealed that cryptorchid deer represented 72% of the population on AP but only 12% averaged across all other sites. Across all sites the ratio of UCO to BCO was 1:6.6. Occasional cryptorchid deer were far distant from the AP and likely were present at low incidence throughout the archipelago. Prevalence of cryptorchidism on the AP might have increased since 1999. Numbers of NCO, UCO, and BCO were respectively 9, 3, and 10 in 1999 compared with 10, 2, and 25 in 2003–2004. Respective ratios of NCO:UCO + BCO deer were 1:1.4 versus 1:2.7.

Two of 4 abdominal testes in UCO deer and 40 of 58 in BCO deer were recovered between the kidney and inner inguinal ring [Supplemental Material Figure 3 (http://www.ehponline.org/members/2005/8052/supplemental.pdf)]. The other 20 abdominal testes were not found or sought by the hunter. In no case was a testis found within the inguinal canal. BCO deer lacked a scrotum.

For 2003–2004, we estimated testis size from photographs that included a scale.
In undescended testes, there was no spermatogenesis [as expected, because of higher abdominal temperature; Supplemental Material Figure 6 (http://www.ehponline.org/members/2005/8052/supplemental.pdf)]. The D格尔 in all abdominal testes was 100 (Table 1). Atypical germ cells characterized by abnormal nuclei and periodic acid–Schiff-positive (PAS-positive) cytoplasmic inclusions (glycogen) were found in two scrotal testes (Figure 3A) and four abdominal testes (Figure 3B). They were found individually or in small clusters (Figure 4A, B). At the electron microscopic level, they had ultrastructural features typical of CIS cells, namely, irregular nuclear contours, chromatin clumps, meandering nucleolus, marginalized nucleoli, swollen mitochondria, and unusual membranous profiles (Figure 4C, D). CIS cells are known to be a precursor of seminoma in humans, but as the fate of these atypical germ cells in SBTD is not known, hereafter they are termed CIS-like cells. Intratubular seminiferous-like lesions were found in two abdominal testes (Figure 5A).

Hyperplastic Sertoli cells, often arranged in rosettes and sometimes with neoplastic changes, were found in several abdominal testes [Supplemental Material Figure 6 (http://www.ehponline.org/members/2005/8052/supplemental.pdf)]. A proteinaceous material, sometimes including mineralized concretions, was found in association with these hyperplastic cells. Similar proteinaceous material, sometimes including mineralized concretions, was found in association with these hyperplastic cells. Similar proteinaceous material, sometimes including mineralized concretions, was found in association with these hyperplastic cells. Similar proteinaceous material, sometimes including mineralized concretions, was found in association with these hyperplastic cells.

Lesions characterized by hyperplasia, hypertrophy, and neoplasia were found in the excudant ducts. Carcinoma of the rete testis (Figure 6A), a rarely occurring lesion, was found in an abdominal testis. Adenoma of the rete testis (Figure 6B) was found in the same and another abdominal testis. Rete tubules containing concretions (Figure 6C) and cysts filled with proteinaceous material [Supplemental Material Figure 7A (http://www.ehponline.org/members/2005/8052/supplemental.pdf)] were occasionally found. Focal areas of adenomatous principal cells with PAS-positive secretions were found in the caput epididymidis of an NCO deer, and cysts filled with proteinaceous material [Supplemental Material Figure 7B (http://www.ehponline.org/members/2005/8052/supplemental.pdf)] were found in the epididymis of a BCO deer.

**Discussion**

The weight of evidence [Table 1, Figures 1–6; Supplemental Material Table 1, Figures 1–7 (http://www.ehponline.org/members/2005/8052/supplemental.pdf)] is that many male SBTD have multiple yet similar abnormalities and that the incidence of collective occurrence is not trivial. Hence, we concluded that something was affecting SBTD on the AP.

**Dilemma and hypothesis.** How do antler malformation, cryptorchidism, induction of abnormalities in all four primordial testicular cell types, and lack of hypospadias tie together? Leydig cells, stromal elements, and supporting cells (primordia of Sertoli cells) differentiate around primordial germ cells early in gonadogenesis; testosterone secretion follows. Bubenik (1990a) recognized that the antler pedicle primordia, obligatory for postnatal antler development, underwent differential growth early in gestation of male red deer (*Cervus elaphus*) coincident with initial production of testosterone. Testosterone was maximal at 24–29% of gestational length (233 days in red deer) and undetectable (<0.1 ng/g tests) after 45% of gestation (Lincoln 1973). The scrotal swelling was evident by 26% of gestation. The importance of Lincoln’s 1973

| Table 1. Histological characteristics of testes of NCO and cryptorchid SBTD. |
|----------------------------------------|
| **Male** (years) | **Antlers** | **Side** | **Location** | **Grade (%)** | **Testis** | **Special observations** |
|-----------------|-----------|--------|-------------|--------------|--------|------------------------|
| **03-01**       |           |        |             | 36           |         | | |
| 7.5             | N         | L      | S           | 27           | 24     | Multinucleate germ cells; focal foamy LC hypertrophy |
|                 |           |        |             | 10           | 0      | | |
|                 |           |        |             | 4            | 0      | | |
|                 |           |        |             | 6            | 7      | | |
| **03-10**       |           |        |             | 18           |         | | |
| 8.5             | N         | L      | S           | 64           | 26     | Two clusters of CIS-like cells; areas with spermatids sharing acrosome |
|                 |           |        |             | 10           | 0      | | |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 20           |         | | |
| **03-12**       |           |        |             | 68           |         | | |
| 6.5             | N         | L      | S           | 25           | 20     | Divergent quality in two loci; areas with spermatids sharing acrosome, microthilithasis |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 80           |         | | |
| **03-51**       |           |        |             | 28           |         | | |
| 2.5             | N         | L      | S           | 36           | 60     | No overt lesion |
|                 |           |        |             | 4            | 0      | | |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 37           |         | | |
| **03-02**       |           |        |             | 11           |         | | |
| 4.5             | N         | L      | A           | 74           | 24     | Hunter did not find testis |
|                 |           |        |             | 2            | 0      | | |
|                 |           |        |             | 0            | 0      | | |
| **03-09**       |           |        |             | 12           |         | | |
| 4.0             | N         | L      | S           | 67           | 31     | Normal except for few SCD tubules |
|                 |           |        |             | 2            | 0      | | |
|                 |           |        |             | 0            | 0      | | |
| **03-03**       |           |        |             | 100          |         | | |
| 8.5             | VA        | L      | A           | 0            | 100    | LC hypertrophy and tumor; ST replaced by microthilithasis and solid cords; concretions in IT |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 100          |         | | |
| **03-06**       |           |        |             | 100          |         | | |
| 9.0             | VA        | L      | A           | 0            | 100    | Stomatal tumor; SC tumor nodule; rete testis adenoma, cysts and microthilithasis |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 100          |         | | |
| **03-07**       |           |        |             | 100          |         | | |
| 1.5             | N         | L      | A           | 0            | 100    | Regressed LC clusters; rete testis hypertrophic |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 100          |         | | |
| **03-11**       |           |        |             | 100          |         | | |
| 5.5             | VA        | L      | A           | 0            | 100    | Nodular LC tumors; SC tumors; extensive microthilithasis; rete testis adenoma and carcinoma |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 100          |         | | |
| **03-13**       |           |        |             | 100          |         | | |
| 8.5             | VA        | L      | A           | 0            | 100    | CIS-like cells; beginning seminoma; nodular LC tumors around SCD tubules |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 100          |         | | |
| **03-14**       |           |        |             | 100          |         | | |
| 3.5             | N         | L      | A           | 0            | 100    | Clusters of CIS-like cells; seminoma |
|                 |           |        |             | 0            | 0      | | |
|                 |           |        |             | 100          |         | | |

Abbreviations: A, abdominal testis; D格尔, degree of germinal epithelial loss; IT, interstitial tissue; L, left; LC, Leydig cells; N, normal antlers; R, right; S, scrotal testis; SC, Sertoli cell; SCD, Sertoli cell only; ST, seminiferous tubule; VA, velvet-covered antlers. *Grade 5 tubules were not found except for a 1% incidence in deer 03-01.*

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observation that early development of primordial antler pedicles was initiated at approximately 26% of gestational length, shortly before the testes initiated their transabdominal descent (at ~ 34% of gestational length), apparently escaped earlier workers studying abnormal deer. Primordial antler pedicles are conspicuous in mule deer (*Odocoileus hemionus hemionus*) by 36% of gestation (Hudson and Browman 1959). Li and Suttie (2001) confirmed timing of the initial appearance of antler pedicle primordia in red deer.

Accepting this ontogenic scenario, the underlying cause of abnormalities in many SBTD could be a mutation(s) in an originator female/male, directly or indirectly causing all defects, with the mutation(s) transmitted to progeny over many generations (i.e., genetic).

Alternatively, the cause could be continuing exposure of female deer and their developing fetuses to an environmental agent that affected the fetus(es). Regardless, female SBTD also might be affected phenotypically.

In our data, the abnormalities were found almost exclusively on the AP (68 of 76 BCO or UCO males) (Figure 1). However, occasional SBTD lacking obvious scrotal testes, some with abnormal antlers, have been shot in lowlands near the western end of Olga Bay (northwest of AP; Figure 1) or nearby Hepburn Peninsula in the mid-1990s and also on Shuyak Island (northernmost island in Figure 1) (Van Daele and McDonald 2001).

UCO or BCO males found elsewhere might have been descendants of the same originator animal or exposed to the same agent(s) at sites distant from the AP (e.g., low coastal area with similar vegetation, wash-up of oceanborne plants or matter, or contaminated fog). Alternatively, and regardless of cause, they or their dam might have migrated from the AP to the site where they were shot. Importantly, 66 of 76 cryptorchid males were BCOs and therefore could not sire offspring.

For reasons presented below, we do not favor transmission of a mutation(s) causing (a) cryptorchidism plus abnormal antlers and induced abnormalities in all four cell types in the testis; or (b) cryptorchidism, with abdominal location of testes (not the mutation per se) indirectly causing deformations of antlers and transformation of testicular cells. Rather, we hypothesize that male SBTD on the Kodiak Archipelago with cryptorchidism, antler malformation, and alteration of testicular cells were impacted by an endocrine disruptor while developing as a fetus during the first 40% of gestation. Arguably, the same agent(s) prevented descent of one or both testes into the potential scrotum and, in 70% of BCO males, altered the primordial antler pedicle to affect shape and/or retention of velvet. Alternatively, retention of velvet on 36% of BCO but on no NCO males might be an indirect consequence of changes induced in fetal Leydig cells (hamppering proliferation and/or postnatal function), thereby resulting in insufficient testosterone postnataally to promote shedding of velvet (Bubenik 1990a). This latter scenario could not explain altered antler shape. Concentration and/or timing of an agent(s) impinging on the early fetus could result in variable expression of multiple defects.

**Deer on AP are unique.** Cryptorchid deer represented 72% of the population shot on AP, with 9 times more BCO than UCO males. This is an unusually high prevalence of cryptorchidism and predominance of BCO over UCO (McEntee 1990). Further, 62% of BCO deer for which data were available had a shape defect of the antlers, 36% a velvet defect, and 29% both a shape and velvet defect.
defect. This combination of cryptoorchidism and antler malformation is different from earlier reports of deer with abnormal antlers or cryptoorchidism. Most reports of abnormal antlers (Carrasco et al. 1997; DeMartini and Connolly 1975; Robinette and Jones 1959; Taylor et al. 1964; Tiller et al. 1997) were for animals with atrophy of scrotal testes. Further, available evidence suggests that cryptoorchidism in deer typically is not accompanied by abnormal antlers [Supplemental Material (http://www.ehponline.org/members/2005/8052/supplemental.pdf)].

Testicular histopathology. The totality of lesions found in testes from both NCO and BCO or UCO deer on the AP is very unusual and is inconsistent with appearance of testicular tissue in naturally occurring cryptorchids of other species. A variety of antiandrogenic and estrogenic chemicals can induce cryptoorchidism (Gray et al. 2001; Toppa et al. 1996), but the nature of the lesions in the undescended testes depends on the agent. Typically, estrogenic chemicals induce mitogenic effects causing proliferative lesions, whereas antiandrogenic chemicals cause regressive changes.

For example, in rabbits, normal descent of testes into the scrotum can be blocked by in utero exposure to the antiandrogen flutamide, estrogenic octylphenol, or oestriol per se; by active immunization against gonadotropin-releasing hormone (ultimately reducing testosterone production); or by surgically (Veeramachaneni et al. 2001). The spectrum of lesions in the undescended testis was different depending on the etiological factor. CIS-like, atypical germ cells were found in the undescended testes of octylphenol- or oestriol-exposed animals but not in those of remaining groups. Similarly, in utero exposure to dibutylphthalate (Higuchi et al. 2003) resulted in CIS-like cells in the undescended testes, as did perinatal exposure to the insecticide \textit{p,p'-DDT} [1,1,1-trichloro-2,2-bis(\textit{p}-chlorophenyl) ethane] (some estrogenic action, predominantly antiandrogenic; Veeramachaneni 2000) or its metabolite \textit{p,p'-DDE} [1,1-dichloro-2,2-bis(\textit{p}-chlorophenyl) ethylene] (Veeramachaneni 2006). Thus, abnormal development of germ cells in males can result from direct in utero exposure to chemical pollutants during critical periods of gonadal differentiation. However, neonatal surgery preventing transabdominal descent of the testes, keeping them within the abdominal cavity thereafter, did not induce atypical cells within any of the four specialized cell types that form a testis (Veeramachaneni et al. 2001). Although a variety of genetic mutations or chemical agents can block normal testicular descent, not all cause germ cell transformation leading to CIS-like cells and development of tumors.

Testicular dysgenesis, an increasingly common developmental disorder, is an important cause of reproductive failure in men (Skakkebæk et al. 2001) and has been associated with a variety of environmental factors. Dysgenetic gonads are predisposed to the development of tumors or tumorlike proliferations of both germ cells and sex cord/stromal elements (Mostafi 1977) similar to those found in SBTD.

Exposure of pregnant mice to diethylstilbestrol resulted in adenocarcinoma of the rete testis of male offspring (Newbold et al. 1985) and interstitial cell tumors (Newbold et al. 1987) but not germ cell tumors. These proliferative lesions and tumors in the rete testis occur transgenerationally without further exposure of descendents (Newbold et al. 2000). Furthermore, microlithiasis in seminiferous tubules and adenomatous lesions in rete tubules similar to lesions found in these SBTD were found in South African eland \textit{(Tragelaphus oryx)} that had high body burdens of estrogenic nonylphenol (Bornman et al. 2004). The occurrence of extremely rare rete carcinoma or stromal cell tumors in 3 of 6 BCO SBTD, together with other testicular tumors, suggests exposures to estrogenic chemicals. Considering that increased susceptibility for diethylstilbestrol-induced lesions in the rete testis is transmitted transgenerationally (Newbold et al. 2000), could one or more pregnant SBTD have been exposed to an estrogenic agent causing similar transmission of a propensity for proliferative lesions and tumors to successive generations, perhaps eventually concentrated by inbreeding?

Antero genesis. The discussion of antero genesis presented in Supplemental Material (http://www.ehponline.org/members/2005/8052/supplemental.pdf) is the basis for our interpretation of the combination of testicular and antler dysgenesis. Descriptions and illustrations of sequelae of experimental hormonal manipulations (Bubenik 1990b; Goss 1990; Jaczewski 1990; Kolle et al. 1993) are not similar to atypical/bizarre-shaped antlers commonly found in SBTD on the AP. In deer, blockage of testosterone secretion by administration of cyproterone acetate can prolong growth and delay mineralization (Bubenik et al. 1975) and in some males result in antlers with sharp tips (Bubenik GA, personal communication).

However, it is not evident that a simple deficiency in secretion of testosterone, independent of any alteration of androgen receptors or other changes in the periosteum, caused elongated sharp antler tips in these SBTD. Serum from 7 of 12 SBTD with sharp antler tips had \( \geq 1.5 \) ng/mL testosterone, 5 had \( \geq 3.3 \) ng/mL, and all 12 averaged 1.2 ng/mL. Based on published reports (Bubenik 1990a; McMillin et al. 1974; Suttie et al. 1984), apparently approximately 1.5 ng/mL testosterone is sufficient for

Figure 5. Testicular tumors. (A) Intratubular seminoma-like cells in BCO deer 03-14. Note considerable variation in size and shape of nuclei; such tumors have been designated as anaplastic. PAS and hematoxylin staining; differential interference contrast microscopy. (B) Intratubular, solid Sertoli cell tumor in the testis of BCO deer 03-6. Note regressed Leydig cells in the interstitium and compare with foamy, hypertrophic Leydig cells in association with the seminoma in A. Methylene blue and Safranin-O staining. (C) Leydig cell tumor (LCT) causing distortion of seminiferous tubules (ST) containing only Sertoli cells in the testis of BCO deer 03-03. (D) Stromal cell tumor in the testis of BCO deer 03-6. H&E staining. Scale bars: A and B = 25 µm; C and D = 100 µm.
hardening of antler bone. Accidental or experimental injury to an antler bud in otherwise normal males is followed by exuberant growth of the antlers, thereby resulting in structures (Figure 119 in Goss 1983) similar to many found in SBTD [Supplemental Material Figure 4 (http://www.ehponline.org/members/2005/8052/supplemental.pdf); Figures 4 and 5 in Bubenik et al. (2001)]. However, it is illogical that most BCO and no NCO males would have received injuries of a type causing bizarre antlers, as BCO deer are unlikely to be aggressive if they have low testosterone.

Consideration of a genetic cause of cryptorchidism. If there were one originator animal produced by a spontaneous mutation(s) of one or more genes, could the mutation be rapidly transmitted among deer on the AP, concomitant with sufficient inbreeding, to produce 72% cryptorchidism? Historically, there was disagreement whether cryptorchidism in a population was transmitted as a single or multiple dominant or recessive gene(s). However, a multigenetic recessive cause now is accepted (Nielen et al. 2001). For example, Rothschild et al. (1988) found that their pig data were consistent with recessive genes at two or more loci [Supplemental Material (http://www.ehponline.org/members/2005/8052/supplemental.pdf)].

Information on genes involved in testicular descent and limb-bud formation is evolving, especially for mice and humans (Klonisch et al. 2004). For humans, more than 20 different causes of cryptorchidism in humans have been traced to one or more specific loci of seven different chromosomes. In brief, a mutation in the Insl3, Great, or Hoxa-10/Hoxa-11 genes might block transabdominal testicular descent, and products of the Insl3, Great, or Hoxd gene families might be important for normal formation of the primordial antler pedicle, as with limb buds in mice. Depr probably could not not be the culprit, as it affects the inguinoscrotal phase of testicular descent rather than transabdominal descent. Dhh apparently has effects different from those we observed, although it is involved in differentiation of Leydig cells. Insl3 is produced in fetal Leydig cells at the time of transabdominal testicular descent. Male Insl3–/– mice are cryptorchid because of failure of the gubernaculum to pull the testes into the scrotum postnatally (Zimmermann et al. 1999). Importantly, tests in Insl3–/– males have normal spermatogenesis in most tubules if surgically placed into the scrotum postnatally (Nef and Parada 1999). Hence, although mutation of the Insl3 gene could block transabdominal descent of testes, it is unlikely that such a mutation could: a) induce CIS-like cells (transformed germ cells) and/or abnormal Leydig (mesenchymal) and Sertoli (epithelial) cells; and b) cause abnormal antler primordia. However, as discussed in “Testicular pathology,” transgerational transmission of a propensity for CIS-like cells has not been demonstrated, although in mice a propensity for tumors of the rete tests can be inherited. In the case of SBTD, such a change would have to be derived from a gene mutation(s) responsible for cryptorchidism.

In humans and mice (Ferlin et al. 2003; Gorlov et al. 2002), Insl3 peptide binds to a receptor transcribed by the Great gene. Gubernacular tissue contains an extraordinarily high concentration of this receptor. Hence, a mutation of either Insl3 or Great genes could cause cryptorchidism. However, among 82 cryptorchid men (43% BCOs), only 8.5% had a mutation or deletion of the Insl3 or Great genes (Ferlin et al. 2003).

Although populations of deer tend to retain heterozygosity (Chester and Smith 1987), the genetics of reproductive development or cryptorchidism in deer has not been studied. However, in a study based on 747 males born in 457 litters of boxer dogs, Nielen et al. (2001) found an 11% prevalence of cryptorchidism. When they assumed transmission of cryptorchidism via a polygenic recessive model, estimated heritability was 0.23. In families of related dogs, prevalence ranged from 0 to 20%. Penetrance was estimated at 85%–88% in heterozygotic or noncarrier males but approximately 23% in homozygous recessive males [Supplemental Material (http://www.ehponline.org/members/2005/8052/supplemental.pdf)]. If there were a hypothetical SBTD that underwent a spontaneous mutation of one specific gene (e.g.,

**Figure 6.** Neoplastic lesions and concretions in the rete testis. (A) Carcinoma, with proliferation of dysplastic epithelial cells (normally cuboidal) of the rete tests invading the stromal elements, in the testses of BCO deer 03-11. Methylene blue and Safranin-O staining. (B) Adenoma of rete tubules in the testis of BCO deer 03-6. Hypertrophic glandular epithelial cells with PAS-positive secretion are evident. PAS and hematoxylin staining. (C) Microthiasis within rete testis tubules in the testses of BCO deer 03-13. PAS and hematoxylin staining. Scale bars: A = 25 µm; B and C = 50 µm.
Cryptorchidism or antler deformation. The apparent incidence of cryptorchidism on the AP doubled within 5 years (1:4:1 in 1999 vs. 2.7:1 in 2003–2004). If concentration of a historic mutation via inbreeding was the cause of the problem of cryptorchidism on the AP, this would require virtually no movement of breeding animals onto or away from the affected region. Otherwise, the disparity in prevalence of cryptorchidism on the AP versus elsewhere in the archipelago (72% vs. 12%, considering all data) would not exist. In fact, however, SBTD migrated to populate the archipelago, including the AP. Furthermore, analyses of microsatellite DNA at 12 loci for 76 SBTD on the AP, northern Kodiak Island, or adjacent Uganiq Island revealed that deer in all three regions represented a single random-breeding population (Amann RP, Paetkau D, unpublished data). Together, these observations make a genetic cause of the cryptorchid problem on the AP unlikely.

Cryptorchidism as a cause of testicular neoplasia or antler deformation. It is generally accepted that cryptorchid testes are more prone to develop neoplasia, although such defects also occur in NCO males (McEntee 1990; Mostafi 1977). However, this does not establish cryptorchidism per se as the factor causing CIS-like cells or tumors and exclude an agent independently causing both cryptorchidism and testicular dysgenesis. The distinction is important. Indeed, emerging knowledge of gene defects in males (Klonisch et al. 2004) suggests a common cause for cryptorchidism and sensitization to a tumor-inducing agent. It is generally considered that retention of velvet on deer antlers after the normal time of shedding is a direct result of insufficient testosterone in blood (Bubnenk 1990a). Abdominal testes of other species apparently have reduced capability to secrete testosterone, although some reports are contradictory (Nef and Parada 1999; Sechell 1978). As described in “Antlerogenesis,” approximately 1.5 ng/mL testosterone is the threshold needed for final hardening of antlers and shedding velvet. Accepting the limitation of single blood samples for deer in our study, 18 of 45 (40%) BCOs had ≥ 1.5 ng/mL serum, as did 24 of 34 (71%) NCO deer (Figure 2). The 10 NCO deer with < 1.5 ng/mL testosterone did not have retained velvet, although each BCO deer with retained velvet had ≤ 1 ng/mL. Serum from BCO deer bearing normal antlers or abnormal antlers without velvet averaged (geometric mean) 3.8 and 1.9 ng/mL testosterone (Figure 2). The contribution, if any, from Leydig cell tumors (Table 1) to blood testosterone in BCO or NCO deer is unknown.

Figure 7. Timing during ontogeny of the testes, antler pedicles, and limbs in SBTD and postulated actions of an endocrine disruptor to transform testicular cells, alter the antler pedicle primordia, and disrupt transabdominal descent of the testes. Testicular testo: testosterone concentration in testicular tissue.

Insl3 or Hexa), such an originator might have produced carrier female and male descendants in numbers sufficient to produce the current prevalence of cryptorchidism over 15–30 years but could that cause all the abnormalities? Considering all SBTD whose serum testosterone was measured, 28 of 45 BCOs (62%) had polished antlers when shot. This suggests that relatively low production of testosterone by a BCO deer is not inconsistent with shedding of velvet. Indeed, scrutiny of available data for NCO red deer (Suttie and Odocoileus sp. (McMillin et al. 1974; West and Nordan 1976) led to the conclusion that the testosterone stimulation associated with hardening and then shedding of velvet represents an increase from typical values of < 0.3 ng/mL to > 2 ng/mL (as high as 10 ng/mL; Suttie et al. 1984). However, the critical question is not what concentration of testosterone is there but rather what is the minimal concentration of testosterone required to drive the response; it is difficult to determine the value of “enough” (see Figure 2 in Amann and Hammerstedt 1993). Demonstration of cause and effect, with respect to association of cryptorchidism and diminished testosterone production or diminished testosterone production and retention of velvet, is not provided by retention of velvet by 38% of BCO deer on the AP.

Consideration of an environmental agent. For reasons given above, we cannot rationalize a spontaneous mutation(s) in one originator animal, followed by increased prevalence due to inbreeding, as the cause of cryptorchidism plus transformed testicular cells and abnormally shaped antlers. If a hypothetical genetic mutation was the cause of all defects, with only 27% NCO males on the AP in 2003–2004, most males and females must carry the altered gene(s). Therefore, in a few years, deer should be absent from the Peninsula because virtually all males will be BCOs. Time will tell. The argument that local extinction would be precluded by inward movement from distant areas of deer lacking the hypothetical mutation argues equally well against the notion of an inbreeding problem. Emigration or transplantation of only a few animals lacking a genetic defect can rapidly eliminate a problem (Keller and Weller 2002).

Alternatively, it could be hypothesized that concurrent expression of two or three independent causes (gene mutations) was the cause of all defects. Of 94 deer shot on the AP, 61 were BCOs, of which 43 had abnormally shaped antlers (70%), and 6 of 6 BCOs studied had detected testicular tumors and/or CIS-like cells (100%). Laws of chance make independent causes very unlikely.

By elimination, but especially because of what is known about endocrine disruptors and ontology of the reproductive system and antlers, one must consider that a common causative agent(s) acted on male fetuses to cause all three defects. This hypothesis is illustrated in Figure 7 and is supported by observations for other species. Although we hypothesize that the endocrine disruptor(s) cause damage during a window narrower than 25–40% of gestation, we suspect that it is present in the female deer throughout.
mature of gestation (i.e., winter), if not year-round. Alternatively, the observed dysgenesis might result from an epigenetic effect transmitted over generations (Anway et al. 2005).

Numerous studies have reported sequence of in utero exposure of male fetuses to a multiplicity of agents including estrogenic and antiandrogenic chemicals (Anway et al. 2005; Colborn et al. 1993; Gray et al. 2001; Higuchi et al. 2003; McMahon et al. 1999; Nef et al. 2000; Newbold et al. 1985, 1987, 2000; Safe et al. 2001; Toppari et al. 1996; Veeramachaneni 2000; Wilson et al. 2004). Because hypospadia, a long-known symptom of decreased action of androgens during the development of reproductive system, was not encountered in SBTD, we speculate that the putative endocrine disruptor is estrogenic rather than antiandrogenic or with some other action. Supporting this hypothesis is the fact that lesions observed in the testes and excurrent ducts of affected SBTD are proliferative and dysplastic (typical of estrogenic stimulation) and not regressive (typical of antiandrogens).

Certain estrogenic molecules can down-regulate the Insl3 gene and disrupt normal testicular descent (Emmen et al. 2000; Nef et al. 2000), so testes remain abdominal [Supplemental Material Figure 3 (http://www.ehponline.org/members/2005/8052/supplemental.pdf)] and also cause dysplastic and/or neoplastic lesions in the male reproduc-tive tract similar to those seen in SBTD [Figures 3–6; Supplemental Figures 6, 7 (http://www.ehponline.org/members/2005/8052/supplemental.pdf)]. Additionally, products of the Insl3 gene could be among factors affecting formation of the primordial antler pedicle (as for limb buds; Kloisch et al. 2004).

We suspect that the postulated estrogenic endocrine disruptor molecule affecting SBTD on the AP is associated with contaminated fog (Chernyak et al. 1996), washed-up ocean matter deposited on deer browse, or brush (Kolen, T. von Saal FS, Som AM. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101:378–384.

We hypothesize that testis–antler dysgenesis results from exposure of pregnant females to an estrogenic environmental agent(s) affecting epigenetically to transform testicular cells and also to alter the primordial antler pedicles and affect expression of the Insl3 or other genes to block transabdominal descent of fetal testes in most animals. Only the latter is evident at birth.

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