Normal Morphology and Hormone Receptor Expression in the Male California Sea Lion (*Zalophus californianus*) Genital Tract

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ABSTRACT

Histomorphology and estrogen α (ER α), and progesterone receptor (PR) expression were evaluated in free-ranging stranded male California sea lions (*Zalophus californianus*). Hormone receptor expression was evaluated using an immunohistochemical technique with monoclonal antibodies. Estrogen and PRs were identified in the efferent ductules, prostate gland, corpus cavernosa, corpus spongiosium, penile urethra, and in the epithelium and stroma of both the penis and prepuce. In some tissues, ER α expression was more intense in the stroma, emphasizing the importance of the stroma in hormone-mediated growth and differentiation of reproductive organs. To our knowledge, this is the first study to localize ER α and PR to the epithelium of the glans penis. The results of this investigation add to the general knowledge of male California sea lion reproduction and suggest that estrogens could have a role in the function of the male reproductive tract. Anat Rec, 292:1818–1826, 2009. © 2009 Wiley-Liss, Inc.

Key words: California sea lion; estrogen receptor; progesterone receptor; male reproductive tract; urogenital cancer

Information on the normal features of the reproductive system in pinnipeds is important in evaluating causes of reproductive failure, diseases of the reproductive tract, and the potential effects of environmental contaminant exposure. Although much is known regarding reproduction in other carnivores, significantly less is understood about pinniped reproduction. Only a few studies have examined normal reproductive tract histomorphology in pinnipeds and these studies have primarily focused on female animals (Craig, 1964; Bigg and Fisher, 1974). Since the establishment of the Marine Mammal Protection Act in 1972, protecting marine mammals from exploitation, access to pinniped tissues is extremely limited in the United States (Young and Shapiro, 2001). Currently, opportunistic sampling of deceased stranded animals is the primary means of

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studying normal anatomy, histomorphology, and diseases of free-ranging animals (Gulland, 1999). Pinniped stranding is dependant on the species natural history. California sea lions (*Zalophus californianus*), like other pinnipeds, have a highly synchronized breeding system. Females give birth on rookeries on the Channel Islands, off of Southern California, USA, and islands off the coast of Baja California, Mexico. Peak birthing occurs by mid-June and estrus and sexual receptivity occur 3 to 4 weeks after birth. During the breeding season, males congregate on the island rookeries to establish and defend breeding territories. After the breeding season, males migrate widely to forge, ranging as far north as British Columbia, Canada (Reeves et al., 1992).

Male pinniped reproductive tract anatomy is similar to the general pattern observed in carnivores. In phocids, the paired inguinal testes are located between the deep blubber layers and the abdominal wall. In otariids, the testes are scrotal. All pinnipeds have an os penis, or baculum, the size of which has been shown to be consistent with body mass (Boyd et al., 1999). Histologic features of the testes have been reported in several species and most evaluations report seasonal cycles in spermatogenesis (Harrison, 1969; Griffiths, 1984). Similar to other carnivores, pinnipeds have a prostate gland but no other accessory sex glands have been reported (Boyd et al., 1999).

The role of estrogen in regulating male reproduction has only recently begun to be thoroughly explored. Estrogen receptors (ER) have been localized in the testes and efferent ductules in some species, but until recently few studies have examined the role of these receptors in male external genitalia (Cooke et al., 1991; Goyal et al., 1998; Atanassova et al., 2001; Nie et al., 2002; Tian et al., 2004). Studies in humans and rats have localized estrogen (ER) to the corpus cavernosa, corpus spongiosum, and penile urethral epithelium (Jesmin et al., 2002; Schultheiss et al., 2003; Dietrich et al., 2004; Mowa et al., 2006). The importance of estrogens in male reproduction was emphasized by the discovery that exposure to estrogenic compounds *in utero* or during early development could have effects on reproductive organ development and function. Prostate cancer, reduced sperm counts, and gonadal hypoplasia are a few examples of conditions associated with exposure to estrogenic substances in lab animals, wildlife, and humans (Colborn et al., 1993; Akingbemi, 2005). Effects of xenoestrogens can be attributed to binding to ERs. Goyal et al. (2007b) showed that neonatal exposure to the estrogenic chemical diethylstilbestrol (DES) in rats led to abnormal adipogenesis in the penis and upregulation of ER α. Hypospadism can develop in male offspring of females exposed to DES and it is hypothesized to result from endocrine disruption during development (Klip et al., 2002). Accordingly, recent work by Wang et al. (2007) showed upregulation of several estrogen responsive genes in patients with hypospadia.

A high prevalence of urogenital carcinomas has been documented in male and female California sea lions (*Zalophus californianus*) stranding along the California coast. In males, these tumors originate from epithelium of the penis, prepuce, and urethra (Gulland et al., 1996; Lipscomb et al., 2000). Multiple studies have documented high blubber burdens of potentially endocrine disruptive environmental contaminants in sea lions (Le Boeuf and Bonnell, 1971; Le Boeuf et al., 2002; Kannan et al., 2004) and exposure to these chemicals begins *in utero* (Greig et al., 2007). The potential reproductive and developmental effects of these contaminants are currently unknown; however, sea lions with cancer have been shown to have higher burdens of potentially endocrine disruptive organochlorines than animals without cancer (Ylitalo et al., 2005).

This study is part of a large investigation on the histomorphology and steroid receptor distribution in reproductive tract tissues of stranded California sea lions with and without urogenital cancer. The purpose of this investigation was to describe the morphologic features of and steroid hormone receptor distribution in the genital tract of male sea lions. Additionally, defining the hormone receptor distribution in tissues prone to cancer will aid in identifying potential factors, such as exposure to environmental endocrine disruptors that may play a role in urogenital cancer development in this species.

MATERIALS AND METHODS

Animals

Formalin-fixed whole reproductive tracts and archived paraffin-embedded tissues opportunistically collected from California sea lions that stranded live on the central California coast (37° 42′ N, 123° 05′ W to 35° 59′ N, 121° 30′ W) were examined. Adult ($N = 4$) and subadult male sea lions ($N = 2$) that died during rehabilitation at The Marine Mammal Center (TMMC) in Sausalito, CA, were evaluated. Causes of death included domoic acid toxicity ($N = 2$), leptospirosis ($N = 2$), trauma ($N = 1$), and pneumonia ($N = 1$). Animals selected for the study had no significant reproductive tract lesions based on gross and histologic examination. Age class was estimated by standard length (measured from nose tip to tail tip), weight, presence of a prominent sagittal crest (Reeves et al., 1992), and tooth dentin growth rings (Oosthuizen et al., 1998).

A gross necropsy was performed on all animals less than 24 hr after death. Routine fresh tissue samples, including the entire reproductive tract, were fixed in 10% neutral buffered formalin, processed routinely for paraffin-embedding, sectioned at 5 μm, and stained with hematoxylin and eosin for histologic examination. Sections examined histologically and via immunohistochemistry included cross sections of the glans penis and shaft of the penis ($N = 6$), transverse sections of the prepuce ($N = 6$), a cross section of the prostate gland and prostatic urethra ($N = 4$), and sagital sections of testis and head of the epididymis including the adjacent efferent ductules ($N = 6$). Autolysis was minimal in all six animals.

Immunohistochemistry

Tissues sections were deparaffinized using xylene and rehydrated using a graded ethanol series. Endogenous peroxidase activity was blocked by incubating sections in 0.2% H_2O_2 in methanol for 30 min. Antigen retrieval was accomplished for ER α and progesterone receptor (PR) by heating sections to 95°C for 30 min in Citrate buffer (pH = 6.2; Dako Cytomation, Carpinteria, CA) in a rice steamer. Sections were incubated in 3% normal goat serum for 30 min. Sections were incubated with a

TABLE 1. Immunohistochemical grading scores

Intensity score		Proportional score	
Score 0	No staining	Score 0	No staining
Score 1	Weak staining	Score 1	<1% positively stained nuclei
Score 2	Moderate staining	Score 2	1–9% positively stained nuclei
Score 3	Strong staining	Score 3	10–32% positively stained nuclei
		Score 4	33–65% positively stained nuclei
		Score 5	>65% positively stained nuclei

Total scores were calculated by adding the intensity and the proportional scores.

monoclonal antibody to human ER α (1:125; clone 1D5, Immunotech, Marseille, Cedex 9, France) and a monoclonal antibody to human PR (1:200; clone 10A9, Immunotech, Marseille, Cedex 9, France) overnight at 4°C in a moist chamber. Slides were incubated with a biotinylated anti-mouse link reagent (Biocare Medical, Concord, CA) for 10 min and then incubated with streptavidin horseradish peroxidase (Biocare Medical, Concord, CA) for 10 min. Positive staining was visualized using 3-amino-9-ethylcarbazole (AEC) chromogen (Zymed Labs, San Francisco, CA). After all steps sections were rinsed in phosphate buffered saline (PBS) spiked with polyoxyethylenesorbitan monolaurate (TWEEN® 20, Sigma-Aldrich, Inc, St. Louis MO). Sections of canine uterus known to be positive for ER α and PR were included in each procedure as positive controls. Antibodies utilized have been shown to be cross reactive to ER α and PR in a number of animal species (Vermeirsch et al., 2002; Martin de las Mulas et al., 2002; D'Haeseleer et al., 2006, 2007). Sections of the prostate gland from one male were incubated with a monoclonal antibody to smooth muscle actin (1:200; clone 1A4, BioGenex, San Ramon, CA) using a similar strepavidin–biotin-horseradish peroxidase procedure. Negative control sections were incubated with omission of the primary antibody.

Immunostaining of all sections was evaluated by a single person without prior knowledge of the animal from which the tissue was sampled. Expression of ER α and PR was scored using a semiquantitative grading system identical to the grading system used in canine studies (De Cock et al., 1997; Vermeirsch et al., 1999, 2002). For each tissue examined, the representative section was dividend into five regions of approximately equal surface area and randomly selected areas within those regions were evaluated. For each section 100 cells were evaluated for a total of 500 cells evaluated. For each tissue both a proportional and an intensity score was calculated. The proportional score corresponded to the percentage of 500 cell nuclei that stained positive. The intensity score reflected a subjective evaluation of the intensity of positive, brown - red nuclear staining in the area evaluated. For comparison of hormone expression between different tissues a total was calculated by the following formula TS = PS + IS, where TS is the total score, PS is the proportional score and IS is the intensity score (Table 1). Total scores ranged from 0 to 8 where, score 0 = (−); scores 2–5 = (+); and scores 6–8 = (++).

Statistics

The Kruskal-Wallis, nonparametric test was used for analysis of the difference in total immunohistochemical scores between the penis and prepuce, proximal and distal urethra, and between the epithelium and stroma for the penis and prepuce. Other comparisons were not able to be performed due to low sample size. Statistical calculations were performed using Medcalc® statistical software, Version 9.1.0.1 1993 (Medcalc, Mariakerke, Belgium). A p-value < 0.05 was considered statistically significant.

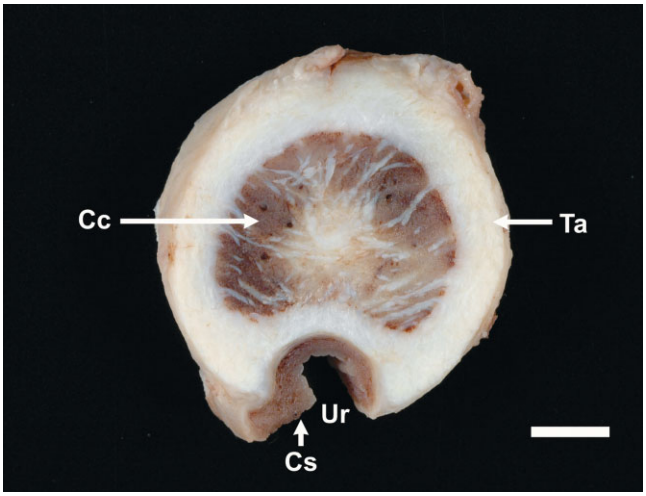

Fig. 1. Cross section of the shaft of penis of a male California sea lion (*Zalophus californianus*). The urethra has been opened during prosection. Formalin fixed. Cc, corpus cavernosa; Cs, corpus spongiosum; Ur, urethra; Ta, tunica albuginea. Bar = 1.0 cm.

RESULTS

Gross Morphology

Anatomic features of the reproductive tract of the male California sea lion are similar to other Otariids (Harrison, 1969; Boyd et al., 1999). Testes are enclosed in a hairless scrotum that is closely adhered to the ventral body wall in the inguinal region. The penis is retracted in the prepuce, which lies closely apposed to the ventral body wall between the umbilicus and the anus. The os penis or baculum extends to the tip of the glans penis and is surrounded at the distal end by frilled penile epithelium that extends just past the tip of the baculum. The distal opening of the urethra is enveloped in this epithelium. Along the length of the baculum, the urethra lies in a groove along the ventral surface just deep to the epithelium. There is no significant bulbus glandis. Anterior to the ischial arch the shaft of the penis is thick and contains the corpora cavernosa surrounded by a thick tunica albuginea. Ventral to the corpora cavernosa, the corpora spongiosum surrounds the penile urethra (Fig. 1). The prostate gland lies in the

TABLE 2. Summary of total ER α and PR immunohistochemical scores in different regions of the male California sea lion reproductive tract

	ER			PR		
Region	−	+	++	−	+	++
Glans penis ($n = 6$)						
Epithelium	2	2	2		3	3
Stroma	1	2	3		2	4
Prepuce ($n = 6$)						
Epithelium	3	3			4	2
Stroma		5	1		4	2
Corpora spongiosa ($n = 6$)	1	3	2	1	1	4
Corpora cavernosa ($n = 6$)	3	2	1		2	4
Distal urethra ($n = 5$)		2	3		4	1
Proximal urethra ($n = 4$)	1	1	2		3	1
Prostate gland ($n = 4$)						
Epithelium	1	1	2		3	1
Stroma	1		3		4	
Efferent ductules ($n = 6$)						
Epithelium	1	5			4	2
Stroma	1	3	2		2	4

Within a given region −, indicates number of animals with a score of zero; +, number of animals with a score of 2–5; and ++, number of animals with a score of 6–8.

pelvic canal surrounding the urethra just posterior to the trigone of the urinary bladder.

Histology and Hormone Receptor Localization

Testes, epididymus, and efferent ductules. Spermatogenesis, with spermatogonium, spermatocytes, spermatids, and numerous epididymal spermatozoa, was observed in two adult males that died during the month of June. Spermatogonium and few spermatocytes were observed in the seminiferous tubules in two males that died during the month of August. In the two remaining sea lions, which died in October and April, seminiferous tubule diameter was dramatically reduced and tubules were lined by Sertoli cells with few spermatogonium. In the testes of males dying in August, October, and April, clusters of Leydig cells adjacent to seminiferous tubules were small and cuboidal with small to moderate amounts of eosinophilic cytoplasm. In the two males with active spermatogenesis, Leydig cells were larger, polygonal and contained large amounts of granular eosinophilic cytoplasm with occasional brown lipofuscin pigment. The convoluted rete testis was centrally located within the mediastinal portion of the testes and lined by a single layer of cuboidal epithelium. Epithelial cells throughout the epididymis were tall columnar with stereocilia and cells lining the efferent ductules were columnar and occasionally ciliated.

Nuclear staining for ER α and PR was found in the nuclei of stromal cells surrounding the efferent ductules and in ductular epithelial nuclei in most of the males examined (Table 2). Stromal cells typically exhibited more intense immunostaining (Fig. 2). There was no difference in receptor expression in animals that died during different times of the reproductive cycle. Occasional epididymal epithelial and stromal cell nuclei were immunopositive for ER α and PR in a single animal. Leydig cells, Sertoli cells, germ cells, epididymal epithelium, and surrounding associated stromal cells were diffusely negative for both ER α and PR in the remaining animals.

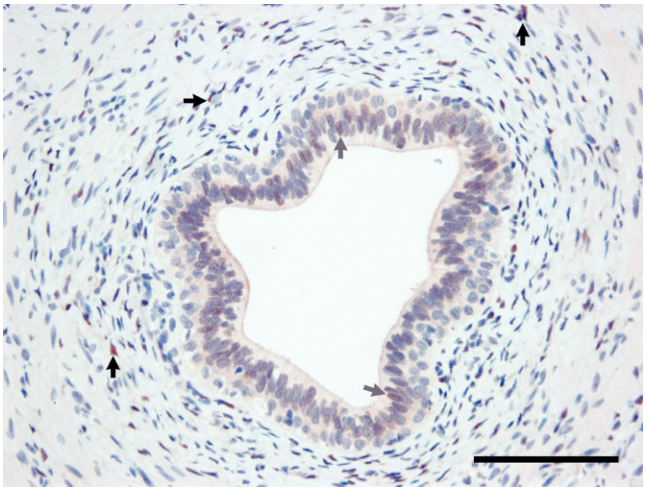

Fig. 2. Immunohistochemical localization of ER α in epithelial and stromal nuclei of the efferent ductules. Black arrows, strong immunostaining; Grey arrows, weak immunostaining. Bar = 100 μm.

Prostate gland. The prostate gland of the California sea lion is approximately spherical and consists of a thin disseminate portion in the wall and submucosa adjacent to the urethra and a moderately developed, poorly lobulated body that circumferentially surrounds the urethra and is thickest dorsally. The disseminate portion consists of a small number of acini. The tubuloacinar body is the major portion and is comprised of mucous and serous glands. Ducts are minimally branching and the acini are widely separated by large amounts of fibrous connective tissue and bands of smooth muscle (Fig. 3A). Ducts and glands were lined by cuboidal to low columnar epithelial cells and contained regionally variable amounts eosinophilic secretory material (Fig. 3B). The body of the prostate gland was surrounded by a thick capsule composed of fibrous connective tissue and smooth muscle.

There was weak to moderate staining of ER α in prostatic glandular epithelium and moderate to strong staining in surrounding stromal cells (Fig. 3C) in 3 of 4 males examined. PR expression was noted in both epithelium and stroma of the prostate gland. Both ER α and PR were expressed in the prostatic urethral epithelium similar to receptor expression in the urethra in the shaft of the penis. There was no difference in receptor expression in animals that died during different times of the reproductive cycle.

Glans penis. The penis was covered by a moderately thick layer of stratified squamous epithelium. At the distal tip of the glans penis, the epithelium was convoluted with deep pointed rete pegs and ranged between 6 and 14 cell layers thick. There was occasional mild keratinization. Along the more proximal aspect of the glans penis, epithelium had a similar thickness, ranging from 8 to 14 cells thick, with a flatter less convoluted surface than at the penis tip. Little keratinization was observed. Positive nuclear staining for both ER α and PR was present in the penile epithelium and underlying stroma

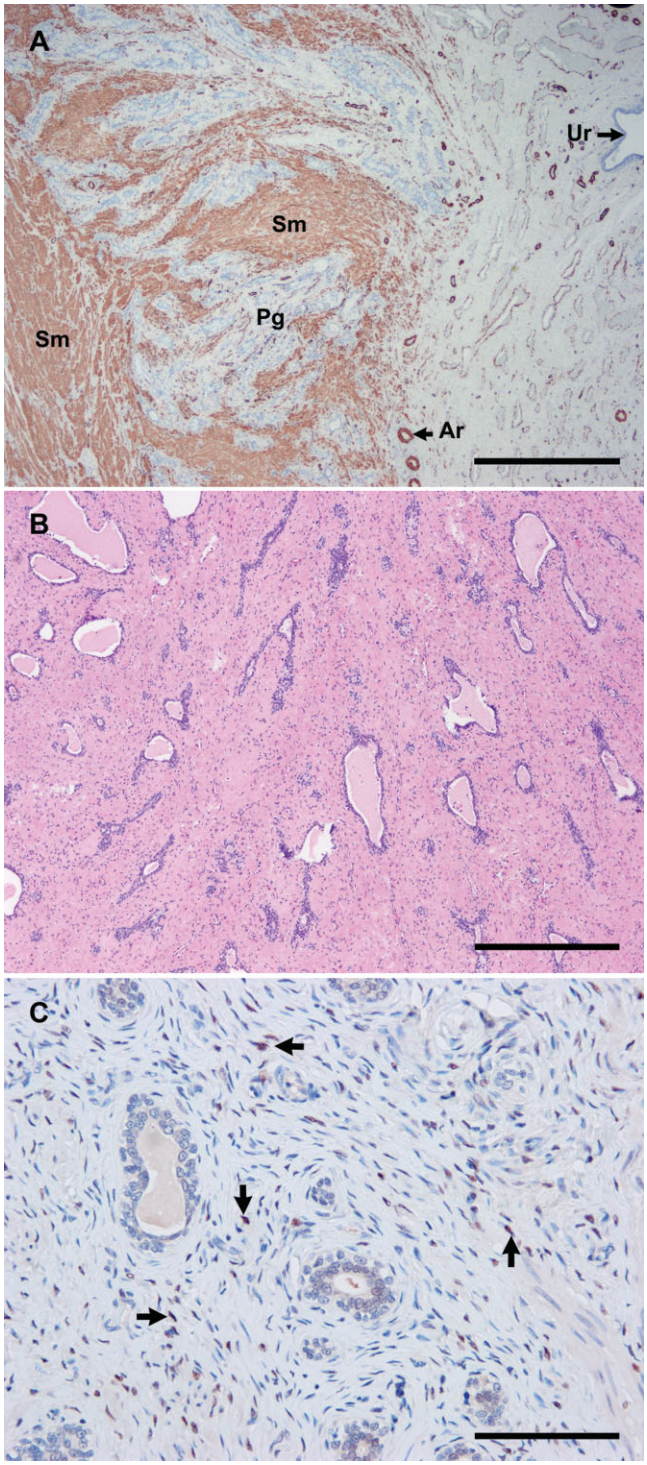

Fig. 3. **A–C:** Histologic and immunohistochemical features of the sea lion prostate gland. (A) Immunohistochemical staining for smooth muscle actin. Note the bundles of smooth muscle (Sm) dissecting between the prostatic glands and surrounding the arterioles (Ar). Ur, urethra; Pg, prostatic glands. Bar = 1.0 mm. (B) Prostatic gland. HE. Bar = 500 μm. (C) Immunohistochemical localization of ER α. Note the strong staining of stromal cell nuclei (black arrows). Bar = 100 μm.

in most animals examined (Table 2). Estrogen receptor α and PR positive nuclei were commonly observed in epithelial cells of the basal, parabasal, and deep to middle intermediate layers (Fig. 4). In one male in which the epithelium was negative, the underlying stromal cells expressed ER α. There was no statistically significant difference between immunohistochemical scores for the epithelium and stroma or in animals dying during different times of the reproductive cycle.

Shaft of the penis. Throughout the corpora cavernosa there were large clusters of thick walled blood vessels interspersed between bands of smooth muscle and nerve bundles. Blood vessels were dissected by thick collagen septae that extended to the tunica albugina. The urethra was lined by transitional urothelium ranging between 3 and 5 cells thick. The corpora spongiosa was comprised of a labyrinth of thin walled blood vessels surrounded by collagenous connective tissue and was thicker ventrally.

Both ER α and PR were diffusely expressed in the urethral urothelium, however, ER α staining was always more intense (Fig. 5). In the shaft of the penis, there was positive ER α immunostaining in smooth muscle and endothelium nuclei of the corpora spongiosa in 5 of 6 animals examined (Fig. 5). Staining was noted in the corpora cavernosa in 3 of 6 males, but was less intense. Total ER α scores were higher in the distal urethra than in the proximal urethra, however the difference was not statistically significant. There was no difference in receptor expression in animals that died during different times of the reproductive cycle.

Prepuce. The stratified squamous epithelium of the prepuce was slightly thicker than that of the penis, ranging from 8 to 20 cells thick. Epithelium lacked prominent rete pegs and the surface was smoothly convoluted. Estrogen receptor α expression was less frequent and generally weaker in prepuce epithelium than in the penis, however, stromal immunostaining was common. Positively stained nuclei were distributed throughout the entire thickness of the epithelium (Fig. 6A). Three animals had weak ER α immunostaining in the stroma, however, the epithelium was negative. In contrast, PR was expressed in 100% of the animals examined in both the prepucial epithelium and stroma (Fig. 6B). There was no statistically significant difference between immunohistochemical scores of the penis and prepuce or between the prepuce epithelium and underlying stroma. Immunohistochemical scores for the prepuce did not vary significantly in animals that died during different times of the reproductive cycle.

DISCUSSION

Although the morphology of the male sea lion reproductive tract was, in general, similar to that of other carnivores, there were several distinctive features. The sea lion testes are seasonally active. Active spermatogenesis was evident in two of the six males, both of which died during June, just prior to the when most breeding behavior is observed on rookeries (Odell, 1975). The seminiferous tubules of the males that died during August just after the peak of the breeding season, were in an intermediate stage between active spermatogenesis

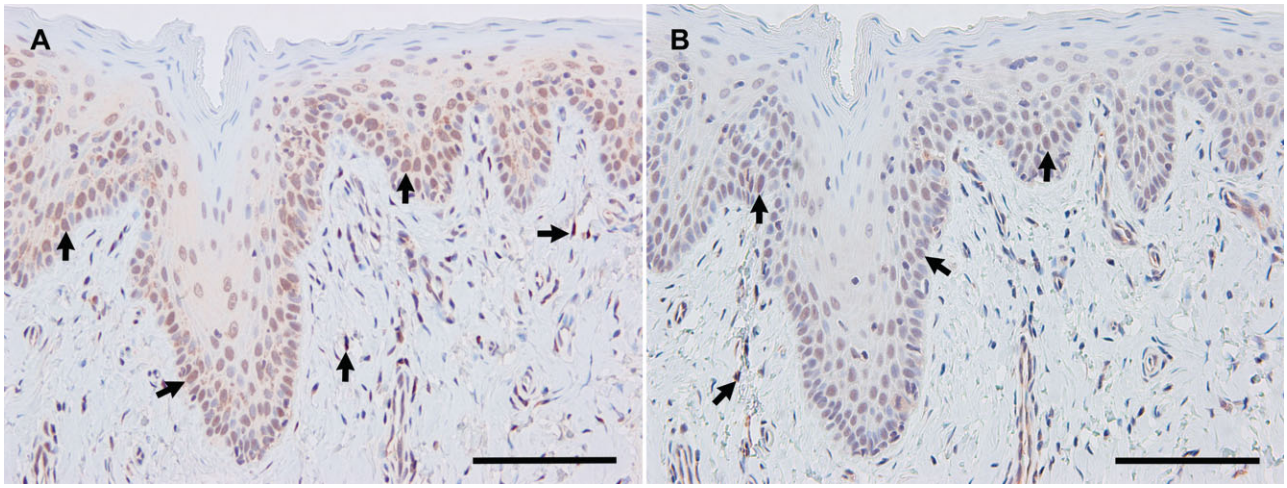

Fig. 4. **A**,**B:** Immunohistochemical localization of (A) ER α and (B) PR in epithelial and stromal nuclei (black arrows) in the glans penis. Bar = 100 μm.

and inactivity. The fluctuations in morphologic features of the seminiferous tubules and Leydig cells observed in this study were similar to those reported in other seasonal breeders, such as roe deer (*Capreolus capreolus*) (Klonisch et al., 2006). The prostate gland of sea lions is tubuloacinar and not as well developed as in some carnivores, such as the dog (McEntee, 1990). The body of the prostate gland contained a relatively smaller proportion of glands with large amounts of fibrous connective tissue and bundles of smooth muscle in comparison to descriptions in the dog. Glands often had a small diameter and the amount of protinaceous secretions was variable both regionally within individual animals and between animals. Similar to reports in other pinnipeds, the baculum of the male California sea lion is relatively longer than in other carnivores. A longer baculum is hypothesized to aid with insemination during copulation in shallow water or on the ice, as occurs in some phoids. In fur seals, the baculum grows in length with age and has been used historically in analyzing age in commercially hunted seals. In cryptorchid fur seals, the baculum is short and slender suggesting hormone-influences in growth (Harrison, 1969).

Similar to investigations in other species, we have found that ER α is expressed in the efferent ductules, prostate gland, urethra, the corpora cavernosa and corpora spongiosa (Goyal et al., 1998; Jesmin et al., 2002; Nie et al., 2002; Dietrich et al., 2004; Mowa et al., 2006). PR expression often paralleled ER α expression, consistent with estrogen and ER-mediated control of PR gene transcription (Critchley and Healy, 1998). The role of ER α in the testes is unclear and inconsistent results have been reported for testicular ER α expression in some species (Akingbemi, 2005). ER α immunohistochemical expression has been observed in Leydig cells of the dog and cat, but not in humans, goats, common marmosts (*Callthrix jacchus*), or stump-tailed macaques (*Macaca arctoides*). In contrast, ER β has been shown to have a more widespread distribution, with expression observed in Sertoli cells, Leydig cells, spermatogonia, and peritubular stormal cells in multiple species (Goyal et al., 1997; Saunders et al., 2001; Nie et al., 2002). In this study, testicular ER α immunostaining was not observed in any of the six males examined, however, ER β will need to be examined in order to completely evaluate the role of ERs in regulation of testicular function in pinnipeds.

Estrogen receptors have consistently been reported in the efferent ductules in a number of species (Cooke et al., 1991; Goyal et al., 1997; Saunders et al., 2001; Nie et al., 2002). Estrogens and efferent ductular ER α have recently been shown to play a key role in male fertility. Efferent ductules function to transport newly formed spermatozoa and rete fluid to the epididymal duct. Rete fluid is absorbed in the ductules concentrating the spermatozoa (Senger, 2005). Zhou et al. (2001) showed that in the efferent ductules, estrogen regulates sodium transport and fluid absorption through the Na+/H+ exchanger – 3 (NHE3). Abnormalities observed in the ductules of estrogen receptor knockout mice included epithelial ultrastructural abnormalities, decreased expression of NHE3, and fluid accumulation. Five of six of the male sea lions examined had ER α expression in efferent ductule epithelium and surrounding stroma, further supporting the importance of estrogen in efferent ductule function across species. The significance of the PR expression noted in the efferent ductules is uncertain at this time. Little is known about the function of PR in the male reproductive tract and few studies have examined PR expression in males. PR has been localized to the epididymis in cynomolgus macaques (*Macaca fascicularis*), common marmosets, and in humans, however, the efferent ductules were not examined (Luetjens et al., 2006).

In addition to androgens, estrogen plays a role in growth and differentiation of the prostate gland. Estrogen receptors α and β and PR have been localized to glandular epithelial cells in normal, hyperplastic, and neoplastic epithelium and stroma in humans, dogs, and lab animals (Cooke et al., 1991; Leav et al., 2001; Grieco et al., 2006; Luetjens et al., 2006; Gallardo et al., 2007). Similarly, ER α immunostaining was common in prostatic epithelial and stroma cell nuclei in this study. Epithelial-stromal interaction is thought to play a major role in prostate gland development, differentiation,

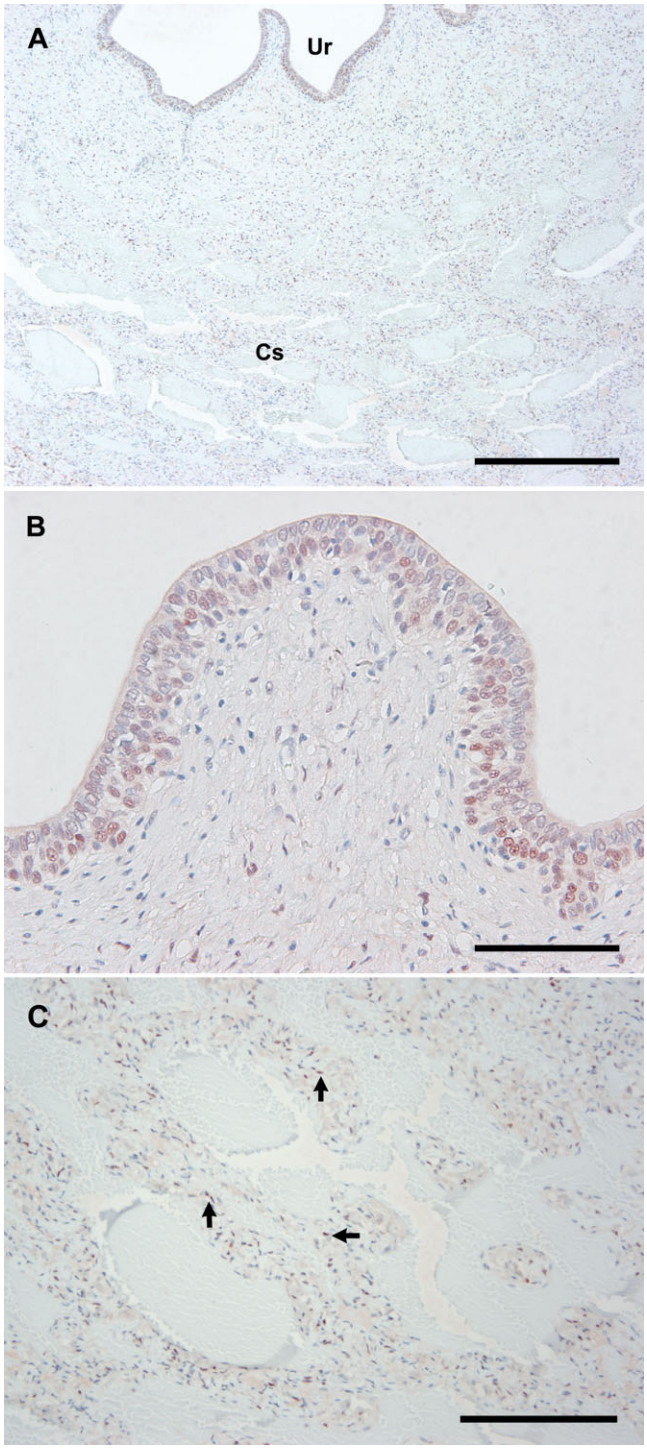

Fig. 5. **A–C:** Immunohistochemical localization of ER α in the (A) urethra (Ur) and corpus spongiosum (Cs). Bar = 500 μm. (B) Urethral epithelium. Bar = 100 μm. (C) Corpus spongiosum. Bar = 200 μm.

growth, and carcinogenesis and both androgen and estrogen receptors are typically found throughout the prostate stroma. In humans, estrogen receptor α is predominately expressed in the stroma in the normal prostate gland, however, both stromal and epithelial receptors are responsible for estrogen-mediated squamous metaplasia in mouse models (Leav et al., 2001; Cunha et al., 2004). In this study, ER α immunostaining was often more intense in the stroma than in gland epithelia suggesting that in sea lions the stroma also plays an important role in mediating prostate gland estrogenic responses.

The widespread immunostaining of epithelial cell nuclei with both ER α and PR throughout the penis and prepuce was an unexpected finding. Previous investigations localized ER α to smooth muscle, mesenchymal, and endothelial nuclei in the corpora cavernosa and corpora spongiosa and urethra penile urothelium in several species including humans, dogs, and rats, however, there was no reference to squamous epithelium in any of these studies (Schulze and Barrack, 1987; Jesmin et al., 2002; Schultheiss et al., 2003; Dietrich et al., 2004). Hausmann et al. (1996) found ER α expression in human prepucial epithelial cells, however, to our knowledge, this is the first study to localize ER α and PR to the epithelium of the glans penis. The role of estrogen in mediating normal development and function of male external genitial is unclear. Although dihydrotestosterone (DHT), a testosterone metabolite, is critical for the development and function of the penis, the finding of aromatase, which converts androgen to estrogen, and ERs throughout the penis suggests that estrogens may also be important (Jesmin et al., 2004). Estrogen receptor α can mediate vasodilation through activation of endothelial nitric oxide synthase (eNOS) and releasing NO (Chen et al., 1999). The expression of ER α in erectile tissue suggests that ERs could play a role in vascular processes in the penis and Shirai et al. (2003) showed downregulation of ER α in aged rats with erectile dysfunction. Additionally, exposure to estrogenic substances during development in the rat can lead to morphological abnormalities in the penis, and this effect was dependent on a functional ER α (Goyal et al., 2007b). Exposure to estrogenic substances during development can also cause upregulation of ER α in penile stromal cells and upregulation of stromal PR in the seminal vesicles, epididymis, vas deferens and prostate gland (Williams et al., 2000, 2001; Goyal et al., 2004, 2007a). Whether the sex hormone receptor expression found in the sea lion penile epithelium in this study is normal is currently unknown. California sea lions have high blubber burdens of potentially estrogenic environmental contaminants and can be exposed *in utero*, during early life through lactational transfer, and throughout life through consumption of fish (Le Boeuf and Bonnell, 1971; O'Shea and Brownell, 1998; Greig et al., 2007). Unfortunately, assessing the potential effects of endocrine disruptive contaminants is difficult as contaminant burdens in free-ranging sea lions can be highly variable, are affected by body condition, and may not be entirely reflective of past or early-life exposure.

Although the sample number was low in this study making statistical relevance difficult to assess, some general trends are apparent. Similar to results observed in other species, ERs are expressed in different segments of the male reproductive tract, suggesting that estrogens may play a role in normal male reproductive function. Evaluation of a greater number of normal sea lions and potentially other pinnipeds species may be needed to determine whether the sex hormone distribution observed in this study is normal in pinnipeds or is a

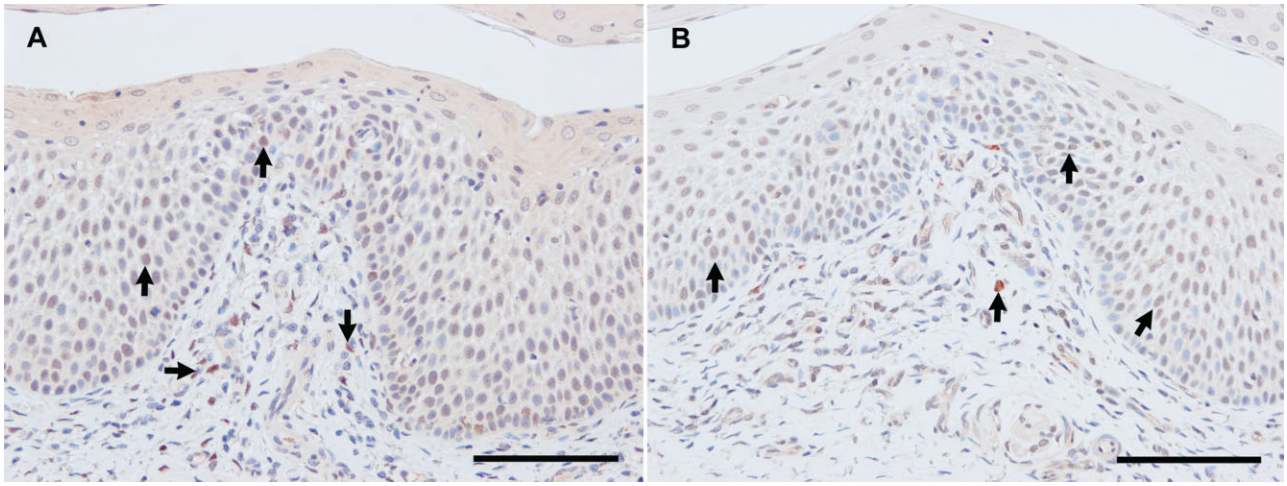

Fig. 6. **A**,**B:** Immunohistochemical localization of (A) ER α and (B) PR in epithelial and stromal nuclei (black arrows) in the prepuce. Bar = 100 μm.

sequella of exposure to endocrine disrupting chemicals. Additionally, evaluation of androgen receptor and aromatase in male reproductive tracts could be beneficial.

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