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SHORT COMMUNICATIONS

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FEMALE PSEUDOHERMAPHRODITE POLAR BEARS AT SVALBARD

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ABSTRACT: During research on polar bears (*Ursus maritimus*) at Svalbard in April 1996, we captured two yearlings with a normal vaginal opening and a 20 mm penis containing a baculum. The penis was located caudal to the location in a normal male and was concealed within the vaginal opening by a single pair of labia. The urethral opening was situated laterally about 5 mm from the distal end of the penis. Neither of the yearlings showed signs of a Y chromosome, so both bears were regarded as female pseudohermaphrodites. On separate occasions in two bears, we recorded aberrant genitalia morphology with a high degree of clitoral hypertrophy in Svalbard, which we also classified as female pseudohermaphroditism. The observed rate of female pseudohermaphroditism in this area was 1.5% (4/269). Pseudohermaphroditism in this polar bear population could result from excessive androgen excretion by the mother caused by a tumor, or it could be a result of endocrine disruption from environmental pollutants.

Key words: Anatomy, female pseudohermaphrodite, polar bear, pollution, *Ursus maritimus*.

During research on polar bears (*Ursus maritimus*) at Svalbard (77°30′N, 19°E) in April 1996, we captured two yearling siblings accompanied by their 11-yr-old mother. During examination of the siblings, we noted their abnormal external genitalia (Fig. 1). Both yearlings had a normal vaginal opening with a 20 mm penis containing a baculum (determined by palpation). The penis was located caudal to the location in a normal male and was concealed within the vaginal opening by a single pair of labia. The urethral opening was situated laterally about 5 mm from the distal end of the penis. Thus, the siblings had both male and female external genitalia.

The genetic sex of the siblings was determined by testing for the presence of Y-chromosome DNA. Blood samples were collected from the femoral vein with vacutainers containing anticoagulant (heparin) and centrifuged within 4 hr of collection. The plasma and cells were frozen separately. We used methods established previously for detecting Y-chromosome-specific DNA in bears (Amstrup et al., 1993; Taberlet et al., 1993). Genomic DNA was extracted from the cellular fraction of the blood (which contains nucleated white blood cells) with standard methods involving incubation in SDS/proteinase-k and extraction with phenol/chloroform (Amstrup et al., 1993). The polymerase chain reaction (PCR) was then used to amplify DNA from the ZFY gene (Amstrup et al., 1993) and the SRY gene (Taberlet et al., 1993). DNA from these genes has been shown to occur in male bears, but not female bears, and indicates the presence of a Y-chromosome. Following PCR, the amplified ZFY DNA was digested with the *Hae*III restriction enzyme and the products were run on a 2% agarose gel with Tris-Borate-EDTA (TBE) buffer (Sambrook et al., 1989). The amplified SRY DNA was not digested with a restriction enzyme, and was run on 4% agarose gels with TBE buffer. Our analyses showed that the ZFY and SRY DNA bands were lacking in the polar bear siblings, but were present in other bears known to be males. Thus, we found no evidence of a Y-chromosome in the polar bear siblings. The PCR for the ZFY gene results in the co-amplification of the related ZFX gene that is on the X-chromosome, as well as the ZFY gene on the Y-

FIGURE 1. External genitalia of a yearling female pseudohermaphrodite polar bear from Svalbard. Bar = 20 mm.

FIGURE 2. Adult female pseudohermaphrodite polar bear from Svalbard with an enlarged clitoris with a glans-like distal end. Bar = 20 mm.

chromosome. The ZFX gene is differentiated from the ZFY gene by different DNA bands following digestion with *Hae*III (Amstrup et al., 1993). The polar bear siblings had the ZFX DNA, suggesting they each had one or more X-chromosomes, but not a Y-chromosome.

Polani (1981a) defined a pseudohermaphrodite as an individual in which ambiguity exists only in the external or internal genitalia, but not in gonads. An individual is classified as a female pseudohermaphrodite if ovaries are present and as a male pseudohermaphrodite when testides are present. Polani (1981b) pointed out that the extent of virilization of external genitalia in female pseudohermaphrodites can vary from simple clitoral hypertrophy to clitoral hypertrophy with various degrees of rugosity and even fusion of the labioscrotal folds.

Genetically, the yearlings were female but because we could not observe their internal gonads, we could not ascertain whether they were true hermaphrodites. Therefore, we have classified them as female pseudohermaphrodites.

Between 1988 and 1997, we examined 269 female polar bears at Svalbard for external genital development to assess breeding status. On two additional occasions, we recorded aberrant morphology in the form of excessive clitoral hypertrophy. In March 1990, a 15-yr-old female was observed with a red, approximately two cm long clitoris with a glans-like enlarged distal end. The female was classified as being in estrous based on labial enlargement. This female was equipped with a satellite transmitter. She was determined to be in a maternity den the next winter based on satellite transmitter information. In May 1997, we observed an adult female (15-yr-old) with an even more enlarged clitoris with a glans-like distal end (Fig. 2). This female was in estrous and was accompanied by an adult male. Milk could be extruded from the mammae suggesting that

she had recently weaned or lost her cubs. According to this description females examined in 1990 and 1997 might also be classified as female pseudohermaphrodites. However, from the observed behavior it seemed that the two were functionally females and reproductively sound. Therefore, the observed rate of female pseudohermaphroditism is 1.5% (4/269).

Reports of abnormal sexual differentiation in mammals are most common in domestic and laboratory mammals and humans (Hunter, 1995). Abnormal sexual differentiation has been reported in few wild mammalian species. De Guis et al. (1994) reported a true hermaphrodite beluga whale (*Delphinapterus leucas*), and Tarpley et al. (1995) reported two instances of male pseudohermaphroditism in bowhead whales (*Balaena mysticetus*). Descriptions of female pseudohermaphrodites in wild mammals are even more scarce; however, Cattet (1988) described five such cases in four black bears (*U. americanus*) and one brown bear (*U. arctos*). On gross necropsy, he observed normal female genital tracts with accessory male external genitalia in the form of a penis-like structure and baculum. The external genitalia he described were very similar to what we observed. Cattet (1988) also reported one genital abnormality in a sample of 43 polar bears but did not describe that further.

Female pseudohermaphrodites with ambiguous or discrepant external genitalia almost always result from fetal exposure to androgenic substances, originating internally from the fetus itself or a maternal source, or from an external source operating via the mother (Polani, 1981b). Freemartinism is perhaps the most frequently studied sexual developmental anomaly (Benirschke, 1981). Freemartinism occurs when the placentas of multi-zygotic siblings fuse intimately and blood vessel connections of considerable magnitude are established between the fetal circulations (prenatal anastomosis). When a male fetus is so connected to a female sibling, the female reproductive organs are altered by androgenic influences which render the female sterile (Benirschke, 1981). Freemartinism has been described in cattle, sheep, goats, pigs, horses, and laboratory rodents, but in no other mammalian species (Benirschke, 1981).

The twin polar bear yearlings in this study were both females, as indicated by the absence of Y-chromosome DNA, so freemartinism was not possible, unless a third male sibling existed that we did not capture. Polar bears in some populations often have triplet litters (Ramsay and Stirling, 1988; Derocher and Stirling, 1995), however, the frequency of triplets in Svalbard is <5% (Larsen, 1985). When triplets do occur, the third cub is very small and typically dies early in life (Derocher and Stirling, 1996). A male sibling could have been part of this litter, but chorionic vascular anastomosis has not been described in bears (Cattet, 1988). The two adult females observed were reproductively sound. Therefore, we find it unlikely that the female pseudohermaphroditism observed here was due to a freemartin effect.

In bears, pseudohermaphroditism could be caused by excessive androgen secretion by the mother, originating from either an adrenal or ovarian tumor. These androgens then cross the placenta to influence the development of the fetal female genital tract (Cattet, 1988). Since the twins were similar in external genitalia this is a possible explanation. However, Cattet (1988) found the most likely explanation for the high occurrence of female pseudohermaphroditism to be due to exogenous induction caused by ingestion of compounds with androgenic activity by the mother during pregnancy. Recently, the endocrine disrupting effect of environmental organochlorine pollutants (OCs) have been studied. Laboratory experiments have demonstrated that exposure of fetuses to endocrine disrupting chemicals can profoundly disturb organ differentiation, because they can act as hormone agonists or antagonists (Colborn et al., 1993). Organs with receptors for gonadal hormones are at risk for

developmental abnormalities in offspring via maternal exposure.

Polar bears at Svalbard are highly polluted by OCs especially polychlorinated biphenyls (PCB's) (Bernhoft et al., 1997). There is a high significant correlation between PCB levels in subcutaneous fat, and blood cells and plasma (Bernhoft et al., 1997). The levels of PCB's in the mother and the twins were analyzed in blood plasma as described by Bernhoft et al. (1997). The sum of PCB levels given at a fat weight basis were found to be 1,856 ng/g, 6,288 ng/g, and 4,707 ng/g respectively. The % fat in plasma was 1.52, 1.81, and 1.87 respectively. The concentrations of PCB's in the blood plasma of the mother and the yearlings were within the mean ± 1 SEM of the mean levels at Svalbard (Bernhoft et al., 1997). Polar bears have a 3 yr reproductive cycle: females are impregnated in spring, have delayed implantation until autumn, bear their young by mid winter, and the young follow their mother until the spring of their third year of life (Ramsay and Stirling, 1988). Bernhoft et al. (1997) found that the PCB levels in adult females were higher in the year they get impregnated and when they have cubs of the year versus when they have yearlings. This is because the females transport PCB's by the blood to the fetuses during pregnancy and by milk to their young after birth (Bernhoft et al., 1997). Therefore, the PCB level in the mother might have been higher during her pregnancy when an endocrine disruption could have occurred. The high PCB level might affect the fetuses indirectly by a disturbance of the endocrine system of the mother, or directly by a disturbance of the endocrine system of the fetuses. We realize that the mechanisms involved are still unclear, but we do suggest that the female pseudohermaphrodites might be a result of endocrine disruption from environmental pollutants.

In conclusion, we believe that the pseudohermaphroditism observed could be (1) secondary to excessive androgen excretion by the mother caused by a tumor or (2) it could be a result of endocrine disruption from environmental pollutants.

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