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Pilot study for a sperm quality-based reproductive assessment scheme for deer☆

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ABSTRACT

Ecological risk assessments (ERAs) for mammals at chemically contaminated terrestrial sites conventionally apply a food-chain model to draw inferences about a population's reproductive condition. Very recently though, the ERA field was advanced beyond the desktop level with the introduction of Rodent Sperm Analysis (RSA), a direct health status assessment method for the actual chemically exposed site receptor. Here, the sperm parameters of rodents (count, motility, morphology) of contaminated sites and their habitat-matched noncontaminated reference locations are comparatively reviewed for a technically supported indication of reproductive capability, ERA's toxicological endpoint of greatest concern. With the extent to which sperm parameters need to be impaired in order to compromise reproduction being known, more definitive determinations are possible than with the food-chain model approach. We sought to adapt the RSA method to the white-tailed deer (*Odocoileus virginianus*), recognizing that this commonly evaluated mammalian species of ERAs is one of a very few species that avails itself to regularly being removed (through hunting) from the field. We conclude that the adaptation is viable, although sperm motility and a few other measures routinely compiled in RSA applications cannot be collected. In the pilot application, the deer population we assessed, with exposures to Superfund and other sites of known contamination, was not found to have compromised reproduction. This finding is consistent with RSA's supporting theory as well as population census information. The outcomes of so-called deer sperm analysis applications for herds exposed to one or more environmental stressors, have the potential to serve as reliable indicators of reproductive status. Such outcomes also provide further weight-of-evidence that suspected contamination is not associated with adverse ecological effects.

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1. Introduction

For many mammalian species including humans, the sperm parameters of count, motility, and morphology are recognized to be indicators, if not proven barometers of reproductive capability (Bucci and Meistrich, 1987; Chapin et al., 1997; Gray et al., 1992; Meistrich et al., 1994). For each parameter, it is understood that too great a shift in the direction of nonfavorability (i.e., a lessening of count or motility; an increase in the percentage of abnormally shaped cells) can account for lesser reproduction in such forms as fewer successful matings or smaller-sized litters (Chapin et al., 1997). Seemingly the laboratory rodent model has been the appropriate one to apply in gaining an understanding of the degree to which each parameter must be impaired in order to compromise reproduction. Rodents facilitate reproductive biology research because of their relatively small size and easily met housing requirements, achieving sexual maturity in as little as 6 weeks, having a gestation period of less than 1 month, and bearing sizeable litters. In 2003, definitive rodent-derived sperm parameter-based thresholds-for-effect were harnessed to craft Rodent Sperm Analysis (RSA), a reproductive assessment scheme for mammals at contaminated Superfund-type sites (Tannenbaum et al., 2003, 2007). In 2009, RSA, constituting the only direct health status assessment tool for ecological receptors at contaminated sites, and intended to add a field-verification element to the ecological risk assessment (ERA) process, was patented (U.S. Patent No. 7,627,434, Method for field-based ecological risk assessment using rodent sperm-analysis).

Where rodent populations inhabiting contaminated terrestrial sites bear no evidence of compromised sperm parameters (e.g., reduced count), as can be demonstrated with RSA's comparative assessment scheme, such information is used to conclude that the reproductive health of all other site mammals (e.g., *Sylvilagus floridanus*, *Vulpes vulpes*, *Neovison vison*, *Odocoileus virginianus*) is also sufficiently protected. Extrapolation from rodents to the larger and wider-ranging mammalian species that may be contacting the

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contaminated sites, and for whose protection soil remedial actions could realistically proceed follows from RSA theory. If rodents, assumed maximally exposed terrestrial receptors (owing to their having constant contact with soil and having highly limited home ranges that effectively bind them to contaminated sites), bear no signs of reproductive impairment, the same should be true for other site mammals that have a far lesser degree of contact with affected soils (Tannenbaum et al., 2003, 2007). The extrapolation scheme is also consistent with conventional ERA practice, where with almost no exception, laboratory rodent-derived chemical dose-response data are used in the desktop assessments for all other mammals (Kolluru, 1996; U.S. EPA, 1997; Sample et al., 1996).

Although RSA outcomes can be and have been used to assess the reproductive health of white-tailed deer (*O. virginianus*; SAIC, 2001), in theory this species should in itself be able to submit to a direct reproductive health status assessment scheme that is nearly isomorphic to RSA. With RSA, the strategic placement of baited live animal traps at both a Superfund/hazardous waste site and its habitat-matched, noncontaminated reference location, furnishes the requisite numbers of small rodents to be analyzed. Seemingly, requisite numbers of reproductive tissue specimens to allow for a valid comparison in deer can be secured via coordinated hunting programs. The subject study describes a fall 2009 pilot effort with two specific aims. First explored is the practicability of adapting the RSA method such that sperm quality in deer can serve as an indicator of that species' reproductive capability. Second, the reproductive capability of a white-tailed deer population with exposure to sites with industrial and other contamination inputs (and potentially bearing chemical residues, principally metals and explosives) is assessed.

2. Materials and methods

2.1. Study areas

The Aberdeen Area of Aberdeen Proving Ground (APG-AA), the U.S. Army's oldest active proving ground located in southern Harford County, Maryland, and a Superfund site, (Fig. 1), constituted the contaminated site. APG-AA encompasses approximately 25,000 acres, and contains a number of operational ranges. Activities include various research and development testing, firing ranges, and open burn/open detonation of munitions components. Much of the peninsula is utilized for the testing of large and medium caliber munitions as well as some small caliber training and specialized equipment testing. Eastern Neck Wildlife Refuge (ENWR), situated approximately 45 km to the southwest of APG-AA in Rock Hall, Kent County, Maryland (Fig. 1) was selected as the study's noncontaminated reference location. In addition to ENWR fringing the Chesapeake Bay as does APG-AA, and the habitats of the two locations being similar (qualitatively) in terms of terrain and vegetation, their muzzleloader hunts perfectly coincided (discussed further in Section 4.1). En route to assembling the data to support the development of an RSA-parallel method for deer, the intent of the study was to harvest the scrotums of 10 bucks from each location. A sample size of 10 conforms both to the desired (minimum) number of adult male rodents to be collected from the trapping sites of RSA applications (Tannenbaum et al., 2003) and to the recommended number of animals of either sex in reproduction/developmental toxicity screening tests (USEPA, 2000).

2.2. Hunter participation

Hunters arriving at the APG-AA deer check-in station each day for the muzzleloader hunt were asked to comply with a deer reproduction study. Participating hunters were given a small ice cooler containing two frozen ice packs, a plastic ziplock bag, and a marking pen. An instruction sheet directed the hunters to remove the entire scrotum from a buck when field-dressing their kill, to place the scrotum in the bag, to record on the bag the date and time of kill and the buck's antler point number, and to place the bag between the ice packs inside the cooler (with the aid of rubber bands and foam stuffing). Hunters returning to the check-in station from the field were further instructed to give their samples to on-duty personnel who would transfer them to a −20 °C freezer located there. On-duty personnel recorded the field-dressed weights of the bucks and kept these records with each animal's uniquely assigned number that was also on the ziplock bag. Within a day or two of arrival at the check-in station, all the scrotum samples were transferred to a −80 °C freezer located at APG-Edgewood Area (APG-EA) and were kept in the deep freezer until analyzed.

Participants at ENWR's 1-day hunt were asked to call the refuge office on their cell phones as soon as they knew that they had taken a buck. A study coordinator then drove to the vicinity of the kill, usually arriving shortly after the time the hunter had located it. The scrotums were removed and kept on ice in the single large, ice-packed cooler that was driven to the different stands that called in with kill information that day. The scrotums were taken to APG-EA at the end of the day, placed in a −20 °C freezer, and transferred a few days later to the previously mentioned −80 °C freezer. The field-dressed weights for these bucks, linked to hunter-unique identification numbers and recorded by on-duty personnel at the ENWR check-in location, were provided to the study coordinator at the end of the 1-day hunt.

2.3. Sample analysis

Samples were processed several weeks later by Charles River Laboratories, Pathology Associates (Frederick, MD). The procedures for evaluating sperm counts and sperm morphology paralleled those used for rodents as described in Tannenbaum et al. (2007). Briefly, after each sample was completely thawed, the scrotal sac was cut, one testis was removed, and its epididymis was trimmed away. The caudal section of the epididymis was first weighed to four decimal places and homogenized in 40 milliliters (mL) of deionized water for 2 min using a Waring commercial blender (Model 51BL32), after which the suspension was transferred to a plastic test tube. After vortexing briefly, the sperm heads were stained (to assist with the automated counting of sperm) by pipetting a 100 microliters (μL) sample of the homogenate into a violet reaction vial containing a Hoechst dye (H33342) pellet that had first been dissolved with the addition of 100 μL of deionized water. A 9 μL sample of the stained sperm was then placed onto a 20-micron (μm) deep Cell-Vu glass slide which was loaded into a Hamilton Thorne Integrated Visual Optical System (IVOS) sperm analyzer programmed to read 20 fields along the slide. The recorded counts were adjusted for caudal epididymal weight (i.e., so that the count could be expressed as millions of sperm/gram (g) of cauda epididymis), as is routinely done in rodent work overall, and has been the standard practice for RSA applications.

For the sperm morphology measure, two eosin-stained slides were prepared for each animal following the procedure described in Tannenbaum et al. (2007). A minimum of 200 sperm cells per animal were examined for as many as four different abnormalities in both the head and the tail.

2.4. Statistical analysis

Standard *t*-tests were applied to support multiple-feature comparisons for the two deer populations. Specifically, population body weights, sperm counts, morphologically abnormal (sperm) cell