Letters to the Editor

Testis development, beef consumption and study methods

Sir,

We read with interest the paper by Swan et al. (2007). For reasons outlined below, we have concluded that the study might receive more credence than justified. Typically, pre-study reviews include: (i) is the question or goal important; and (ii) can the best answer to the question(s) posed be obtained via the planned methods. The unstated but described goal of this study was to non-invasively examine spermatogenesis in fertile adult men, to determine if beef consumption by their mother during pregnancy might have affected fetal development of their testes, as evidenced by seminal characteristics many years later. The underlying question, if meat might be an important vector to inadvertently deliver xenotoxins to pregnant mothers, and hence their fetuses, is important. Quantitative and qualitative aspects of spermatogenesis were probed via analysis of semen in a single masturbation sample from each male. Swan et al. (2007) did not mention why seminal volume was not recorded (this precluded calculation of total sperm per ejaculate), since it was recorded in their earlier studies (e.g. Swan et al., 2003). Gravimetric measurement of seminal volume adds little to total study cost. In our opinion, this omission precluded optimal study of the quantitative aspect of the question posed. This criticism is supported by data in Jørgensen et al. (2001) from which we calculated, using un-transformed data, that the coefficients of variation for seminal volume were 39–48%, for four populations of 207–349 fertile men. Setting out to non-invasively detect possibly subtle changes in spermatogenesis resulting from postulated abnormal fetal development of the testes is more demanding than asking if ‘semen quality’ is different between two or more populations. Adequate precision in measured outcomes for each male is essential. The appropriate measure to quantify spermatogenesis is number of sperm produced per day. Qualitative features of ejaculated sperm are a consequence of spermatogenesis as well as epididymal function, and selected attributes can be evaluated by appropriate methods, recognizing that studied attributes frequently are not independent. Abnormality in one facet of spermatogenesis is not inevitably accompanied by changes in the other.

The number of sperm produced daily by a man’s testes can be estimated from seminal data (Amann, 1981), but this requires information on both seminal volume and sperm concentration, so that total number of sperm ejaculated can be calculated, ideally for several samples per individual, each obtained after a known abstinence interval <6 days. Sperm concentration is insufficient because it is only one element in a three-element equation. Physiologically, sperm concentration depends on the number of sperm ejaculated (an imperfect estimate of number available in the cauda epididymidis or daily production; Amann, 1981) and their dilution by the volume of fluids from the caudae epididymides and accessory sex glands. Because volume and sperm concentration are what is measured, for each ejaculate the product of these two values must be calculated, divided by the hours of abstinence, and the resulting value multiplied by 24 to estimate daily production of sperm (essentially, total sperm number corrected for abstinence).

Andrologists anticipate male-to-male differences in seminal characteristics, but perhaps not the magnitude of their variation. Coefficients of variation for sperm concentration in semen from fertile men in four populations were 69–86%, and even higher for total sperm per ejaculate [75–91%; calculated from data in Jørgensen et al. (2001)]. More important with respect to our present concern, is unreliability of conclusions based on evaluation of a single sample of semen. Sherins (1995) stated that three samples are required to calculate a ‘stable value’. Swan et al. (2003) calculated total sperm ejaculated in each of two samples from 410 men, corrected for abstinence interval, but did not report the ranges in numerical or percentage difference between the two samples, or the intra-class correlation coefficient. However, using data for two samples from each of 654 healthy men, Gao et al. (2006) reported the intraclass correlation coefficients for seminal volume, sperm concentration and total sperm per ejaculate were 0.55, 0.71 and 0.60. This means that values for one sample explained only 30–50% of the values for the other sample from the same individual. These recent data substantiate the World Health Organization (1999) and standard text (e.g. Walsh, 2002) recommendations that even routine semen evaluations be based on at least two ejaculates. Obviously, an estimate of normalcy of daily sperm production, or total sperm per ejaculate, should be based on more than one sample per male. Swan et al. (2007) elected to examine a single ejaculate, perhaps convinced by unreported analyses from Swan et al. (2003) that analysing a second sample was unnecessary.

Ideally, for each ejaculate total sperm number would be corrected for abstinence interval, as above. In Swan et al. (2007), only sperm concentration was available, and it is not clear if data were corrected for abstinence interval before log-transformation or use in regression and other calculations; adjustments incorporated into ‘adjusted mean sperm concentration’ are not explained. Total sperm per ejaculate also is influenced by ‘degree of sexual arousal’ during masturbation (Pound et al., 2002), but this would be hard to measure in a large study. However, this arousal effect might be reduced by averaging data across at least two samples.
Because planning decisions excluded calculation of total sperm per ejaculate, summary data on sperm concentration receive possibly unjustified importance. Did beef consumption account for a small or major proportion of total, or accounted for, variation in adjusted sperm concentration? Reporting the coefficient of determination, in addition to the regression coefficient, would have provided an estimate of the percentage of variation in adjusted sperm concentration associated with consumption of 0–21 meals per week including beef. Inclusion of a figure showing the 387 data points used to calculate the regression ($P = 0.04$) of log sperm concentration versus beef servings per week would have allowed readers to visualize the goodness of fit and ranges in values. If there were two or three variant values, they could have a huge effect on regression coefficients and statistical significance.

Qualitative features of ejaculated sperm reflect the normalcy of spermiogenesis and also maturation of sperm in the epididymis plus attributes of fluids from the accessory sex glands. Swan et al. (2007) reported percentage morphology normal sperm based on WHO 1987 criteria, apparently based on only 100 cells per sample and without consideration of the location or nature of defects observed. This presupposes that each type of morphological defect has an equal probability of rendering that cell incapable of fertilization, which is very unlikely. Number of cells examined and use of a single abnormal category precluded probing if specific types of abnormalities (e.g. vacuolated head, acrosome) might characterize sperm from individuals born to mothers consuming >7 or <7 beef servings/week; this might be expected if a given class of xenobiotic was involved. The second qualitative feature reported was percentage of motile sperm (Swan et al., 2007). Unfortunately, they included both stationary twitching sperm and highly progressive sperm, and all in between, in a single ‘motile’ category. This relegated the observations to a conclusion that a certain percentage of cells was ‘alive’, but begged the issue of the nature or quality of sperm motion, be it progressive or whatever. As evaluated, outcomes for sperm morphology or motility apparently were unrelated to mother’s beef consumption (Table 3 in Swan et al., 2007).

Taking data in Swan’s Table 3 at face value, it seems that weekly consumption of ethanol by the males whose semen was evaluated had a larger impact on adjusted concentration of sperm in their semen than consumption of beef by their mothers ($P < 0.000$ versus 0.041). Might this be explained by a dehydrating effect of ethanol, resulting in lower volumes of seminal fluid and, hence, less dilution of ejaculated sperm? We hope the popular press will not advocate consumption of ethanol to increase concentration of sperm in semen.

Swan et al. (2007) did not present data (apparently collected), on where the mothers resided while pregnant with the son studied, and they considered beef as a ‘uniform food’. However, when these women were pregnant, feedlot beef probably was more available in urban areas, especially on the east and west coasts, and the ‘cuts’ consumed would be influenced by many factors. With respect to cattle passing through a major feedlot, it probably is true that ‘most American beef during that time…’ was from cattle treated with growth promoters (Swan et al., 2007). However, a fair number of cattle slaughtered in the USA in 1950–1980 (and other eras) were older animals moved directly to slaughter from dairy or beef operations because of infertility, age or other reasons including availability of feed. Only rarely would such animals have been fed or implanted with growth stimulants, and they would have been an important source of stew meat, hamburger, hotdogs or processed meats. Further in that era, many families in rural areas ate local grass-raised beef. Cattle in feedlots or milking herds typically would have been exposed to pesticides, but this would be less likely for cull beef cows.

What impact did consumption of beef during pregnancy have on testes development in male fetuses, as reflected in spermatogenesis in sons when adults? From data provided, it is impossible to ascertain if number of sperm produced daily by his testes was affected by consumption of beef by his mother during pregnancy in 1, 2–6, or > 7 meals per week. Sperm production was not estimated. However, no effect on quality of sperm produced was detected. Further study of this question should include measurement of both seminal volume and sperm concentration, calculation of total sperm per ejaculate and conversion of that value to an estimate of daily sperm production by correcting for abstinence interval. Evaluation of 2–3 samples per male would greatly improve precision.

References


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doi:10.1093/humrep/dem228

Advance Access publication on July 17, 2007